Institut de Génétique et de Biologie Moléculaire et Cellulaire

UMR CNRS 7104/INSERM U596/ULP/Collège de France

Thèse

présentée à
l'URF des Sciences de la Vie et de la Santé
de l'Université Louis PASTEUR - Strasbourg I.

Discipline Biologie Moléculaire

pour obtenir le titre de
Docteur de l'Université Louis PASTEUR de Strasbourg

par

Eduardo Castañeda Saucedo

Role of the Retinoid X Receptor α (RXRα) and its Heterodimeric Partners during Skin Carcinogenesis

Directeur**:** Daniel Metzger
Co-directeur**:** Pierre Chambon

Date de soutenance 1 Juillet 2004

Jury: Prof. Pierre Chambon
Dr. Daniel Metzger
Prof. Johan Auwerx
Prof. Peter Angel
Dr. Lluis Fajas

Acknowledgments

To Prof. Pierre Chambon and Dr. Daniel Metzger for giving me the opportunity to prepare my PhD. thesis in their laboratory, for their scientific and personal support, for their critical comments and for all what I have learned from them.

I would like to thank Prof. Johan Auwerx, Prof. Peter Angel and Dr. Lluis Fajas for accepting to be part of my Ph.D. jury.

To Dr. Patricio Gariglio, for all his support before and during my Ph.D., for his critical comments, and most important for his friendship.

To Arup Indra, my colleague, collaborator and friend, with whom I have worked during my Ph.D., for his advice, critical discussions and for all the good time we spent together.

To Mei Li, for her scientific support, suggestions and critical comments, and in particular for her nice friendship.

Special thanks to Monique Duval, for all her inconditional support with animal work.

To all the current and former members of the laboratory, Jean-Marc, Takeshi, Meng, Ming, Philipp, Reiko, Yasunari, Nathalie, Celine, Isabelle, Romain, Pierre, Michael, Michal, Ali, Bernadette, Christelle, Jean-François, Monica, Martha, Wojtek, Emilie, Valèrie, (and all those whom I may forget) for their critical comments, their support, and their friendship.

To all people from the IGBMC common facilities (histology service, oligonucleotide synthesis service, animal facility, photographic service, informatics service). Special thanks to Kristina, Nadia and Bernard, for their technical support. To all other people who make our work possible at the IGBMC.

Special thanks to Collete Kutschis, for all her support and friendship.

To all my friends and colleagues, current and former members of the IGBMC, thanks to whom my time at the IGBMC has been so pleasent.

Special thanks to the "***Ligue Contre le Cancer, comité du Bas-Rhin***" for the financial support during my thesis.

Finally, thanks to Lourdes, my wife and my friend, for being there all the time, in the god and the not so good times, for her motivation, her support and her love. **Thank you**.

Abreviations

AF-1	*activation function 1*
AF-2	*activation function 2*
AK	*actinic keratosis*
AP-1	*activator protein 1*
BCC	*basal cell carcinoma*
BM	*basement membrane*
CE	*cornified envelope*
DBD	*DNA binding domain*
DMBA	*7-12-dimethylben(a)anthracene*
ECM	*extra cellular matrix*
ER	*estrogen receptor*
FC	*focal carcinoma*
GM-CSF	*granulocyte macrophage colony stimulator factor*
HAT	*histone acetyl transferase*
HDAC	*histone deacetylase.*
HMT	*histone methyl transferase*
IHC	*immunohistochemistry*
IL-1	*interleukin 1*
IRS	*inner root sheath*
ISC	*in situ carcinoma*
ISH	*in situ hybridisation*
KGF	*keratinocyte growth factor*
K13	*keratin 13*
K14	*keratin 14*
LBD	*ligand binding domain*
LXR	*liver X receptor*
MG's	*melanocytic growths*
NSAID	*non steroidal anti-inflammatory drugs*
NR	*nuclear receptor*
ORS	*outer root sheath*
PMD	*post mitotic differentiating*
PPAR	*peroxisome proliferator activated receptor*
RA	*retinoic acid*
RAR	*retinoic acid receptor*
RT-PCR	*reverse transription coupled polymerase chain reaction*
rtTA	*reverse tetracycline-controlled transactivator*
RXR	*retinoic X receptor*
SCC	*squamous cell carcinoma*
SCF	*stem cell factor*
Sp-1	*specificity protein-1*
SpCC	*spindle cell carcinoma*
TA	*transit amplifying*
Tam	*tamoxifen*
Tet	*tetracyclin*
TPA	*12-O-tetradecanoyl-phorbol-13-acetate*
UV	*ultraviolet*
VD_3	*vitamin D_3*
VDR	*vitamin D receptor*

Table list.

Figure list.

Résumé
La peau des Mammifères comporte, sur sa face externe, l'épiderme, tissu épithélial pavimenteux (squameux) stratifié et kératinisé (corné), composé de kératinocytes (espèce majoritaire), de cellules pigmentaires (mélanocytes), de cellules immunitaires (cellules de Langerhans) et de mécanorécepteurs (cellules de Merkel) (Kanitakis, 2002). L'épiderme est ancré, via une lame basale, à du tissu conjonctif dense fibroélastique, le derme (Watt, 2002). Ce tissu largement vascularisé, composé de fibroblastes et de cellules immunitaires (mastocytes et histiocytes), repose sur l'hypoderme, association de tissu conjonctif lâche et de tissu adipeux (Kanitakis, 2002). Des invaginations épidermiques dans les tissus sous-jacents constituent des crêtes épidermiques ou forment les annexes épidermiques telles que les poils, les glandes sébacées (holocrines), ou les glandes sudoriférantes (apocrines) (Fuchs, 1998; Larsen, 1993; Mackenzie, 1975). La peau joue des fonctions très diverses, elle a des fonctions métaboliques, sensorielles, thermorégulatrices, immunitaires, et elle représente une barrière contre la déshydratation et les agressions externes (radiations UV, friction, parasites . . .)
Les rétinoïdes, métabolites actifs de la vitamine A, ont des effets pléïotropes sur l'homéostasie et le développement des Vertébrés. La peau est un des tissus cibles des retinoïdes. En effet, une carence ou un excès systémique en vitamine A entraîne une augmentation du nombre de couches cornéocytaires de l'épiderme (hyperkératose) (Look et al., 1995; Tsambaos et al., 1980; Tsambaos et al., 1985). Au niveau moléculaire, les rétinoïdes se lient à deux classes de facteurs de transcription dont l'activité est contrôlée par des ligands spécifiques, les récepteurs de l'acide rétinoique (RARα, β et γ) et les récepteurs X des retinoïdes (RXRα, β et γ) (Chambon, 1994; Chambon, 1996). Les RXRs sont des partenaires de dimérisation pour d'autres récepteurs nucléaires tels que le RARs, le récepteur de la vitamine D (VDR), les récepteurs activés par les prolifférateurs des péroxisomes (PPARs) et les "Liver X receptor" (LXRs). RXRα est le RXR le plus fortement exprimé dans l'épiderme, où il joue un rôle clef dans l'homéostasie de la peau (Li 2000,2001).
De nombreuses études montrent que, les RARs, le VDR, les PPARs et les LXRs, sont également impliqués dans l'homéostasie de la peau. La vitamine D et des ligands spécifiques pour les PPARs et les LXRs induisent la différentiation des kératinocytes de l'épiderme in vivo et améliorent la récupération de la barrière de perméabilité après une disruption mécanique (Fisher and Voorhees, 1996; Gibbs et al., 1996; Hanley et al., 1998; Hanley et al., 1999; Hanley et al., 2000b; Komuves et al., 2002; Milde et al., 1991; Sakai and Demay, 2000; Sorensen et al., 1997). De plus, les souris portant des mutations nulles du gène PPARα (souris

PPARα$^{-/-}$) ou PPARβ (souris PPARβ$^{-/-}$), présentent des retards de la cicatrisation des blessures cutanées (Michalik et al., 2001), et les souris portant une mutation nulle du gène VDR (souris VDR$^{-/-}$) développent une alopécie sévère (Yoshizawa et al., 1997).

Des altérations des récepteurs RARs, RXRs et VDR sont associées à la transformation cellulaire et la formation de tumeurs (Boudjelal et al., 2002; Darwiche et al., 1995; Issing and Wustrow, 1996; Kumar et al., 1994; Reichrath et al., 1995). En effet, une diminution de l'expression des RARs et des RXRs dans l'épiderme a été observée au cours de la cancérogenèse cutanée chez l'homme et la souris (Xu et al., 2001). Il a été également montré que l'expression de VDR est diminuée dans l'épitheliomes basocellulaires chez l'homme (Reichrath et al., 1999a), et que la susceptibilité au développement de mélanomes chez l'homme est associé à des polymorphismes du gène VDR (Hutchinson et al., 2000a). L'application éctopique des rétinoïdes ou de la vitamine D, ainsi que de cartains ligands de PPARα, peuvent diminuer la formation de papillomes, induites par un protocole de cancérogenèse chimique, chez la souris (Kensler et al., 2000; Thuillier et al., 2000; Yu et al., 1995Ê). D'autre part, il a été montré que des ligands de PPARγ inhibent la croissance de cellules de mélanome en culture (Mossner et al., 2002). Ces données suggèrent donc une action concertée des récepteurs VDR et PPARs avec RXRα dans la genèse des cancers cutanés.

Une stratégie de mutagenèse somatique conditionnelle, qui repose sur l'activité de la recombinase Cre, a été développée au laboratoire. La recombinase Cre est une recombinase de la superfamille des intégrases, qui reconnaît des sites spécifiques appelés loxP (Austin et al., 1981). Les sites loxP sont des séquences de 34 paires de bases comprenant une région de 8 paires de bases, flanqué de deux régions symétriques organisées en palindromes; la région centrale donne une orientation au site de recombinaison. La position et l'orientation des sites loxP déterminent la nature des produits de recombinaison. Si ces sites sont positionnés sur la même molécule d'ADN et s'ils possèdent la même orientation, le segment d'ADN défini par ces sites (floxé) peut être excisé. Pour obtenir l'invalidation d'un gène chez la souris, dans un tissu donné et à un moment choisi par l'expérimentateur il faut donc disposer, d'une part, d'animaux dont le gène est "floxé" et d'autre part exercer un contrôle spatio-temporel sur l'activité de la recombinase Cre dans ces animaux. Songeant à contrôler l'activité de la recombinase Cre chez la souris, des protéines de fusion de la recombinase Cre avec des domaines de liaison au ligand (LBD) de récepteurs nucléaires insensibles à leurs ligands naturels ont alors été réalisées au laboratoire (Metzger et al., 1995). Dans un premier temps,

une protéine de fusion de la recombinase Cre avec le LBD du récepteur ER humain portant une mutation ponctuelle, qui excise une séquence génomique floxée en présence de œstrogènes synthétiques (tamoxifèné) et ayant une activité nulle en présence d'œstradiol (E2), (Cre-ER^{T}) a été crée au laboratoire (Feil et al., 1996).

Une lignée transgénique pour la protéine Cre-ER^{T}, dont la synthèse est contrôlée par le promoteur réduit de la kératine K5 bovine (lignée K5-Cre-ER^{T}), ce que limite l'expression de la protéine de fusion aux épithélia de la peau et de la langue, montrent que l'excision d'un fragment génomique dans tous les kératinocytes de l'épiderme est induite par injection du ligand synthétique (tamoxifène). Une deuxième protéine de fusion, portant deux mutations ponctuelles dans le LBD du récepteur ER humain (Cre-ER^{T2}) a été généré au laboratoire. Chez les animaux transgéniques pour la protéine de fusion Cre-ER^{T2}, placée sous le contrôle du promoteur réduit de la kératine K5 bovine (Indra et al., 1999), l'activité recombinase est induite par le tamoxifèné de la même façon que chez les animaux K5-Cre-ER^{T}, mais pour des quantités dix fois moindres de ligand synthétique. Une excision d'un fragment génomique dans tous les kératinocytes épidermiques peut aussi être induite chez des animaux K14-Cre-ER^{T2} (Li et al., 2000), l'expression de la recombinase chimérique étant dirigée par un promoteur dont l'activité, dans l'épiderme, est identique à celle du promoteur de la kératine 5. Pour ces lignées, l'activité recombinase peut être contrôlée par un ligand synthétique, chez l'adulte comme au cours du développement. Toutefois, la lignée de souris trangéniques pour la recombinase Cre constitutive, placée sous le contrôle du promoteur de la kératine K14 (lignée K14-Cre), est un outil plus pratique si l'on souhaite invalider un gène pendant la mise en place de l'épiderme.

Afin d'élucider le rôle éventuel de RXRα et des certains de ses partenaires de dimérisation dans la genèse des cancers cutanés, nous avons utilisé des lignées des souris portant des mutations des récepteurs nucléaires, soit sélectivement dans l'épiderme ou dans la lignée germinale, que nous avons soumis à un protocole de cancérogenèse chimique. Dans un premier temps, nous avons utilisé une lignée de souris transgénique bi-génique, établie au laboratoire, qui exprime la recombinase chimérique Cre-ER^{T2} sous le contrôle du promoteur de la kératine K14, dans la couche basale de l'épiderme, et dont l'activité est induite par le tamoxifène, et qui porte des allèles RXRα conditionnels (Li et al., 2001b; Li et al., 2000), ce qui permet d'invalider sélectivement RXRα dans les kératinocytes de l'épiderme à un moment choisi. Nous avons généré des souris RXRα$^{ep-/-}$, chez lesquelles RXRα a été invalidé sélectivement dans les kératinocytes de l'épiderme à l'âge adulte, et nous avons induit des

tumeurs cutanées chez ces souris en utilisant un protocole de cancérogenèse chimique en deux étapes (Yuspa et al., 1996). Dans un premier temps, l'*initiation* tumorale est induite par l'application locale d'un cancérigène chimique, le *7-12-dimethylben(a)anthracene* (DMBA), lequel induit notamment une mutation de l'oncogène *ras* (Finch et al., 1996; Quintanilla et al., 1986a). Dans un deuxième temps, l'application locale deux fois par semaine de *12-O-tetradecanoyl-phorbol-13-acetate* (TPA) stimule la croissance des cellules initiées et induit la formation de tumeurs bénignes d'origine épithéliale (papillomes) qui peuvent se transformer en carcinomes en poursuivant le traitement au TPA pendant 30-35 semaines (Yuspa, 1998; Yuspa et al., 1990).

Nous avons observé que les souris RXR$\alpha^{ep-/-}$ traitées au DMBA et au TPA, développent deux à trois fois plus de tumeurs que les souris contrôles, que la taille des tumeurs est augmentée, et que ces tumeurs progresent fréquemment en carcinomes, alors que celles des souris contrôles ne présentent que rarement une transformation cancéreuse. Nous avons également montré que les souris RXR$\alpha^{ep-/-}$ ainsi traitées développent des lésions pigmentées bénignes (nævus), lesquelles se transforment à forte fréquence en mélanomes, alors que les souris contrôles traitées dans les mêmes conditions ne présentent que très peu de nævus. Ces résultats montrent un rôle clef de RXRα dans la formation des tumeurs cutanées (carcinomes et mélanomes) induites par un protocole de cancérogenèse chimique. Ces donnés indiquent également que les kératinocytes mutés stimulent la formation de mélanomes, probablement par des mécanismes paracrines.

Nous avons également traité des souris PPAR$\gamma^{ep-/-}$, chez lesquelles PPARγ est invalidé sélectivement dans les kératinocytes de l'épiderme à l'âge adulte, et des souris knock-out PPAR$\alpha^{-/-}$ avec du DMBA et du TPA. Nous avons montré que les souris PPAR$\alpha^{-/-}$ et PPAR$\gamma^{ep-/-}$ développent, comme les souris RXR$\alpha^{ep-/-}$, deux à trois fois plus des tumeurs que les souris contrôles. Contrairement aux souris RXR$\alpha^{ep-/-}$, les souris PPAR$\alpha^{-/-}$ et PPAR$\gamma^{ep-/-}$ développent un nombre similaire de nævi que les souris contrôles et ne développent pas des mélanomes. Nous avons également montré que les souris VDR$^{-/-}$ et les souris contrôles traitées avec de DMBA/TPA développent un nombre similaire de papillomes, alors que le nombre des nævi est augmenté chez les souris VDR$^{-/-}$. Cependant, les nævi des souris VDR$^{-/-}$, contrairement à ceux des souris RXR$\alpha^{ep-/-}$, ne se transforment pas en mélanomes. Ces résultats suggèrent que les hétérodimèrs RXRα/PPARα et RXRα/PPARγ des kératinocytes, mais ne pas les hétérodimères RXRα/VDR, jouent un rôle de suppresseur de tumeurs

d'origine épithéliale induites par application de DMBA/TPA. Par contre, les hétérodimères RXRα/VDR seront impliqués dans le control de la proliferation des mélanocytes par les kératinocytes, et peut-être dans la formation des mélanomes.

Pour mieux comprendre les mécanismes par lesquelles RXRα contrôle la cancérogenèse cutanée, nous avons analysé les altérations de l'expression de gènes impliqués dans le contrôle de la prolifération et la différenciation cellulaire, de l'apoptose, et de la migration et de la communication cellulaire. Nous avons observé une diminution de l'expression du gène Pten dans la peau des souris RXRα$^{ep-/-}$ et des souris PPARγ$^{ep-/-}$. Nous avons également observé une augmentation de l'expression des intégrines α6 et β4 dans les couches suprabasales de l'épiderme chez les souris RXRα$^{ep-/-}$ et PPARγ$^{ep-/-}$. Par contre, les niveaux d'expression de Pten et des intégrines α6 et β4 ne sont pas altérés dans la peau des souris VDR$^{-/-}$. Il a été montré que la diminution de Pten est liée au développement de cancers chez l'homme (Teng et al., 1997), et que l'invalidation sélective de Pten dans les kératinocytes de l'épiderme entraîne le développement d'épithéliomes (Suzuki et al., 2003). Il a été également montré que la surexpression des integrines α6β4 dans les couches suprabasales de l'épiderme chez la souris, augmente la susceptibilité des souris au développement d'épithéliomes (Owens et al., 2003). Ces donnés suggèrent donc que les hétérodimères RXRα/PPARγ dans les kératinocytes, sont impliqués dans le contrôle de l'expression de Pten et des intégrines α6 et β4, et que la diminution de Pten et la surexpression des intégrines dans les couches suprabasales de l'épiderme, résultant de l'invalidation de ces hétérodimères, seraient à l'origine de l'augmentation de la susceptibilité au développement d'épithéliomes chez les souris RXRα$^{ep-/-}$ et les souris PPARγ$^{ep-/-}$.

D'autre part, nous avons montré que les niveaux d'expression de la e-cadhérine, une protéine qui participe à l'adhésion cellulaire et qui est impliquée dans la communication kératinocytes-mélanocytes (Jamal and Schneider, 2002), sont diminués chez les souris RXRα$^{ep-/-}$ et les souris VDR$^{-/-}$, mais pas chez les souris PPARα$^{-/-}$ ou les souris PPARγ$^{ep-/-}$. Ces données suggèrent que les hétérodimères RXRα/VDR sont peut-être impliqués dans le contrôle de l'expression de la e-cadherine, et dans la communication kératinocyte-mélanocyte.

Les oncogènes *n-ras, h-ras et k-ras* sont les gènes les plus souvent mutés dans les cancers humains (Bos, 1989). Chez la souris, l'application ectopique de DMBA induit, entre autre, des mutations de l'oncogène *h-ras* dans les kératinocytes de l'épiderme et de l'oncogène *n-ras* dans les mélanocytes (Balmain et al., 1988). De plus, il a été montré que les souris chez

lesquelles l'oncogène *ras* est invalidé sont partiellement résistantes à l'induction chimique des tumeurs cutanées (Ise et al., 2000). Ces données suggèrent donc un rôle clef de l'oncogène *ras* dans le développement des cancers cutanés.

Afin d'approfondir l'étude du rôle de RXRα dans la cancérogenèse cutanée, et en particulier les possibles interactions entre les voies de signalisation *ras* et RXRα, nous avons utilisé un modèle de souris génétiquement initiées, portant l'oncogène activé *h-ras* (souris transgéniques Tg.AC) chez lesquelles des tumeurs epitheliales peuvent être induites par blessure cutanée, exposition aux rayons UV ou application locale de TPA (Cannon et al., 1997; Leder et al., 1990; Trempus et al., 1998). Nous avons croisé les souris Tg.AC avec des souris K14-Cre/RXRα$^{L2/L2}$, chez lesquelles RXRα est invalidé dans les kératinocytes de l'épiderme au cours du développement embryonnaire (RXRα$^{ep-/-(c)}$), et avec des souris K14-Cre-ER^{T2}/RXRα$^{L2/L2}$, chez lesquelles RXRα peut être invalidé à l'âge adulte par injection de tamoxifène (RXRα$^{ep-/-}$). Nos résultats montrent que l'invalidation de RXRα au cours du développement chez les souris Tg.AC (Tg.AC/RXRα$^{ep-/-(c)}$) est suffisante pour induire la formation de papillomes. Par contre, lorsque RXRα est invalidé chez les souris Tg.AC adultes (Tg.AC/RXRα$^{ep-/-}$) ces souris ne développent pas des tumeurs spontanées, mais développent deux fois plus de tumeurs que les souris contrôles en réponse au traitement au TPA. Des analyses biochimiques et histologiques ont montré que la plupart des tumeurs spontanées chez les souris Tg.AC/RXRα$^{ep-/-(c)}$, présentent des caractéristiques pré-cancéreuses, et que certaines des ces tumeurs se transforment en carcinomes. Enfin, ni les souris Tg.AC/RXRα$^{ep-/-(c)}$, ni les souris Tg.AC/RXRα$^{ep-/-}$ ne développent des mélanomes. Ainsi, des mutations induites par l'application de DMBA, notamment des mutations de l'oncogène ras dans les mélanocytes, sont probablement nécessaires pour la formation des mélanomes chez les souris RXRα$^{ep-/-}$.

L'approche génétique que nous avons utilisé à donc permis de montrer le rôle de suppresseur de tumeurs de RXRα dans la peau, fonction que serait importante principalement pendant l'étape de promotion tumorale et médiée probablement par des hétérodimères RXRα/PPARα ou RXRα/PPARγ pour les tumeurs d'origine épithéliale. De plus, RXRα dans les kératinocytes, probablement sous forme d'hétérodimère avec VDR et d'autres récepteurs nucléaires, contrôle également la formation de nævus et leur transformation en mélanomes. Enfin, nos résultats suggèrent que RXRα joue son rôle anti-tumoral en contrôlant l'expression

de gènes impliqués dans la régulation de la prolifération des kératinocytes et la communication cellulaire, tels que Pten, la e-cadhérine et les intégrines $\alpha 6$ et $\beta 4$.

Summary

Nuclear Receptors (**NRs)** are ligand-dependent transcription factors that play important roles during embryonic development and homoeostasis. Several NRs, including the retinoic acid receptors (RARα, β and γ), the retinoic X receptors (RXRα, β and γ), the vitamin D receptor (VDR), the peroxisome proliferator activated receptors (PPARα, β and γ) and the liver X receptors (LXRα and β), play key roles in the morphogenesis and homeostasis of the skin. RXRα is the predominant retinoid receptor in the epidermis and a obligatory heterodimeric partner for other non-steroidal NRs. Aberrant expression and function of several of these NRs have been found during skin carcinogenesis, both in mouse and human. Natural or synthetic ligands for RXRs, RARs, VDR and PPARs exhibit protective effects against tumor formation in human and mouse. To investigate the possible role of keratinocytic RXRα in skin caricnogenesis, we have subjected adult mice, in which RXRα was selectively ablated in keratinocytes ($RXR\alpha^{ep-/-}$ mice), to the chemical two-step tumorigenesis protocol. Upon DMBA/TPA treatment $RXR\alpha^{ep-/-}$ mice exhibited an increased tumor number, growth rate and a much higher frequency to malignant carcinoma, than control mice. DMBA/TPA-treated $RXR\alpha^{ep-/-}$ mice also develop melanocytic growths, which degenerate into melanoma. Furthermore, mice in which PPARγ was selectively ablated in keratinocytes ($PPAR\gamma^{ep-/-}$), and mice bearing a PPARα-null mutation are also more susceptible to DMBA/TPA-induced epithelial tumors, than control mice. However, $PPAR\gamma^{ep-/-}$ and PPARα-null mice did not develop melanocytic growth or melanoma upon DMBA/TPA treatment. We also showed that the susceptibility to epithelial tumors induced by DMBA/TPA is not affected in mice bearing a null mutation of the VDR gene. However, VDR null mice developed a large number of MGs in response to DMBA/TPA treatment, but in contrast to the MGs from $RXR\alpha^{ep-/-}$ mice, these MGs did not progress to malignant melanoma. Thus, keratinocytic RXRα/PPARγ and RXRα/PPARα heterodimers may function as suppressor of epithelial tumors, whereas RXRα/VDR heterodimers may play a role in the formation of melanocytic growth. Further studies are required to identify the heterodimerc partner(s) of RXRα involved in the suppression of malignant melanoma.

We also used Tg.AC mice as an alternative model to study the role of RXRα during skin carcinogenesis. Tg.AC transgenic mice carry an activated v-Ha-ras oncogene under the

control of a partial ζ-globin promoter and are highly sensitive to tumor formation in response to TPA treatment. Surprisingly, Tg.AC/RXRα$^{ep-/-(c)}$ mice, carrying the Ha-ras oncogene and in which the selective ablation of the RXRα alleles occurred in epidermal keratinocytes during fetal epidermal morphogenesis, developed skin papillomas in the absence of any promotion agent, some of which progressed to carcinoma. Moreover, in response to a single topical application of DMBA, RXRα$^{ep-/-(c)}$ mice, develop epithelial tumors. Thus, ablation of RXRα in keratinocytes during epidermal morphogenesis, is sufficient to promote the formation of Ha-ras initiated skin tumors.

All together our results demonstrate that keratinocytic RXRα is a tumor suppressor of epithelial carcinogenesis (most probably within a heterodimer with PPARα and PPARγ) and malignant melanoma, (most probably whitin a heterodimer with VDR and other NRs), via paracrine mechanisms.

Index

1. Introduction

One of the main challenges of modern biological and medical research is to develop suitable models to study complex human diseases such as diabetes, obesity and cancer. The study of such multifactorial and multigenic diseases requires appropriate models which recapitulate the natural history, pathobiology and biochemistry of the human disease, including biological, genetic, etiologic and therapeutic related aspects such as a histological features, mutations, chromosomal changes, and gene expression profiles (Arch, 2002; Boelsterli, 2003; Hann and Balmain, 2001; Kulkarni and Zisman, 2003; Liu et al., 2003b).

The mouse has proved immensely valuable as a model system to better understand various aspects of human cancer. Indeed, studies of mouse models helped to define the nature of cancer as a genetic disease, and demonstrated the causal role of genetic events found in tumors (Hann and Balmain, 2001). Mouse models allow the isolation of all tumor stages, as well as normal tissue, which are then amenable to pathological, genetic and biochemical analyses and, hence have been instrumental in investigating cancer-related genes and their role in carcinogenesis (Herzig and Christofori, 2002). Moreover, mouse models are valuable tools for the identification of early diagnostic markers and for testing new therapeutic agents. The most important criteria that should be taken in account to validate the "ideal" murine tumor model have been recently reviewed (Hann and Balmain, 2001).

The development of powerful molecular biology and genetic engineering techniques, allowing manipulating the mouse genome, contributed to the development of better mouse models of human cancer. Genetic alterations observed in human cancers can be reproduced in the mouse by the introduction of transgenes or by introducing null mutations in the germ line (Hann and Balmain, 2001; Herzig and Christofori, 2002; Wu and Pandolfi, 2001). Moreover, the recently developed strategy to introduce somatic mutations in the mouse genome by spatio-temporally controlled targeted somatic mutagenesis, gives an additional advantage for introducing genetic alterations in a given tissue and in specific stages of cancer development.

Taking advantage of established mouse models of skin cancer, and of genetically modified mice allowing the introduction of somatic mutations in the mouse epidermis in a tissue-specific and temporally- controlled manner, we initiated an ambitious project to investigate the role of some members of the hormone nuclear receptor superfamily in the development of skin cancer.

1.1 Structure and Function of the Skin

The skin is the largest organ of the body accounting for about 10-15% of total body weight in adult humans. The skin not only gives us our appearance and shape, it also serves other important functions such as protection against mechanical, thermic, and environmental impacts, regulation of body temperature, and is a very important sensorial organ. The skin is composed of an association of tissues from various origins organized in three layers: epidermis, dermis, and hypodermis. In addition, the skin contains a number of specialized structures, including hairs, sebaceous glands and sweat glands (**figure 1**).

Embryologically, the epidermis and its appendages are of ectodermal origin, whereas the dermis and hypodermis originate from the mesoderm (Byrne et al., 1994; Sengel, 1985). The morphogenesis of skin epithelia requires complex interactions between the embryonic ectoderm and mesoderm (Sengel, 1985). In humans, in the fourth week of embryonic development, the surface ectoderm proliferates to form a new outer layer of a simple squamous epithelium called the periderm. The underlying layer of proliferating cells is called the basal layer. Epidermal differentiation begins in the 11th week, and by the end of the fifth month the epidermis acquires its definitive stratified epithelia features (Larsen, 1993).

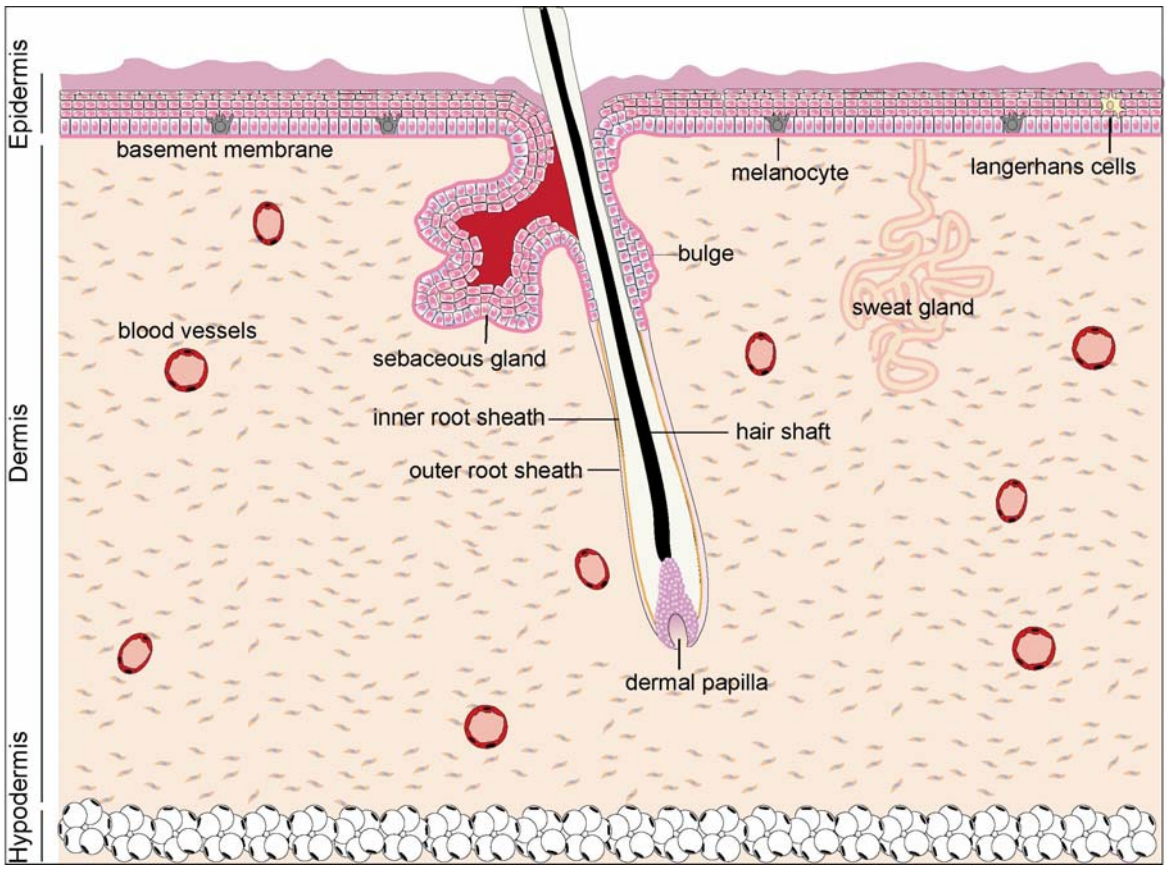

Figure 1. Structure of human skin. Adapted from Fuchs and Raghavan (2002).

In the mouse, the onset of differentiation within the ectoderm begins at 9.5 days of embryonic development. At this step, skin epidermis is composed of a single cell layer and it

starts to differentiate into a stratified epithelia around 10.5-11 days of development (Byrne et al., 1994; Kopan and Fuchs, 1989; McGowan and Coulombe, 1998). Between days 11 and 17, the epidermis becomes fully differentiated, and acquires adult-like barrier properties (Byrne et al., 1994; Hardman et al., 1998). Formation of the hair follicle begins during embryonic development. In humans mature follicles are formed by the 6th month of development, whereas in mice they develop postnatally (Larsen, 1993; Panteleyev et al., 2001; Panteleyev et al., 1997).

1.1.1 Epidermis

Epidermis is a stratified epithelium that renews itself continuously. It is made of various cell types, the majority of which (90-95%) are keratinocytes. The remaining cells include Langerhans cells, melanocytes and Merkel cells (Grousson and Peguet-Navarro, 1998; Haake and Scott, 1991; Hirobe, 1995; Kurosumi et al., 1979; Mackenzie, 1975). Epidermal keratinocytes are arranged in four layers representing different stages of keratinocyte differentiation: the basal, spinous, granular and cornified layers. Keratinocytes in the basal layer are undifferentiated proliferating cells, that are tightly attached to a multiproteic structure that serves as an adherent connection between the epidermis and the dermis; the basement membrane (BM) (Brakebusch et al., 2000; Chan, 1997; Hertle et al., 1991; Sonnenberg et al., 1991). Adhesion of basal keratinocytes to the BM is mediated by transmembrane heterodimeric receptors called integrins. $\alpha 6\beta 4$ integrins are the main heterodimers involved in the attachment of basal keratinocytes to the BM (DiPersio et al., 2000; Georges-Labouesse et al., 1996). In contrast to other integrins that are distributed over the basal, apical, and lateral surfaces of basal cells, $\alpha 6\beta 4$ integrins are primarily located at the basal surface, and are structural components of hemidesmosomes (Hertle et al., 1991; Sonnenberg et al., 1991; Watt, 2002).

In response to not well defined signals, keratinocytes in the basal layer detach from the BM, and initiate a terminal differentiation program. Keratinocytes migrate to the spinous layer were they undergo morphological and biochemical changes, including decrease in the nuclear to cytoplasmic ratio, formation of new cell-to-cell adherent junctions (desmosomes), and loss of $\alpha 6$ and $\beta 1$ integrins expression. Moreover, major changes in keratinocyte cytoskeleton occur at this stage. The cytoskeleton of basal keratinocytes is composed of two main intermediate filaments, the type II keratin 5 (K5) and the type I keratin K14 (K14) (Byrne et al., 1994; Chu and Weiss, 2002; Fuchs, 1995). In the spinous layer expression of K5 and K14 is lost, and expression of keratins 1 (K1) and 10 (K10) is activated (Kim et al., 1984; Kopan and Fuchs, 1989; Loening et al., 1982; Moll et al., 1984).

Following the terminal differentiation program, keratinocytes migrate upwards to the granular layer were they switch again gene expression programs to initiate the formation of the protective permeability barrier. The formation of the permeability barrier requires coordinated activation of a complex set of events including keratinization, formation of the

cornified envelope, formation of intracellular lamellar bodies, and induction of cell death. Keratinization results from the accumulation and cross-linking of cytoplasmic keratins, which results in the formation of a compact keratin network. In parallel, the expression of several proteins that are structural components of the cornified envelope (CE) is activated (Marshall et al., 2001), including involucrin, loricrin, filaggrin and the small proline-rich proteins (Steinert and Marekov, 1995). This proteins are sequentially incorporated, via action of epidermal transglutaminases (Greenberg et al., 1991; Peterson and Wuepper, 1984; Steinert and Marekov, 1999; Wilhelm et al., 1996), to an extremely tough protein polymeric structure located just below the cytoplasmic membrane. CE proteins are cross-linked to membrane anchored proteins and to the keratins complex in the cytoplasm (Steinert and Marekov, 1997). In addition, several lipid species, synthetized by keratinocytes in specialized structures called lamellar bodies (LB) (Weaver et al., 2002), are delivered selectively to the intercellular spaces at the stratum granulosum - stratum corneum interface (Elias et al., 1998), were they accumulate forming the epidermal permeability barrier *(Eckert et al., 1997).* Finally, cell death is induced in keratinocytes at late stages of differentiation (Gandarillas et al., 1999; Maruoka et al., 1997). Thus, the tight structure formed by interactions between proteic and lipidic components of the cornified layer serves as a mechanical protection and permeability barrier for the organism.

In addition to keratinocytes, human epidermis contains melanocytes, Langerhans cells and Merkel cells. Melanocytes originate from the neural crest and migrate into the epidermis during embryonic development (Haake and Scott, 1991; Holbrook et al., 1989). In humans, melanocytes are distributed regularly among basal keratinocytes and in the hair follicle, whereas in mouse they are restricted to the hair follicle (Haake and Scott, 1991; Silver et al., 1977). Melanocytes produce melanin, the main natural pigment of the skin, and they transfer it to the keratinocytes. Melanin not only gives the color to the skin, but protects the deeper layers of the skin from solar radiation (Bessou-Touya et al., 1998). Langerhans cells are mobile dendritic, antigen presenting cells present in all stratified epithelia. They represent 3-6% of all cells in the epidermis, where they are considered as immature, becoming mature after contact with antigens. Langerhans cells arise from the bone marrow. They first appear in the epidermis during development, and continue to migrate into the dermis throughout life. Merkel cells are pressure-detecting mechanoreceptors. In humans they lay at the base of the epidermis in the thick skin of palmar and plantar regions, and are associated with underlying nervous endings in the dermis (Larsen, 1993).

1.1.2 Dermis.

Dermis is a supportive and elastic tissue, which consists of cells and fibrous molecules. In addition to its protective and supportive role, the dermis also contains blood vessels and nerves. It undergoes continuous turnover, regulated by mechanisms controlling the synthesis and degradation of its protein components. Fibroblasts (FB), the main cell population in the dermis are responsible for the synthesis of all the proteic components of

the dermis (Booth et al., 1980; Prockop, 1982; Tajima and Pinnell, 1981). A large majority of dermal fibers are made of interstitial collagens, which accounts for 98% of the total mass of dried dermis that are responsible for the mechanical resistance of the skin.

1.1.3 Hypodermis.

It is a fatty tissue representing the deepest part of the skin, separating it from the underlying body constituents. The hypodermis is composed mainly of adipocytes arranged in lobules separated by connective tissue. It plays an important role in thermoregulation, insulation, provision of energy, and protection from mechanical injuries (Hayward and Keatinge, 1981; Nunneley et al., 1985).

1.1.4 Hair Follicle

The hair follicle is an appendage of the epidermis. It develops from the embryonic epidermis as an epithelial extension. This epithelial extension differentiates into three enclosed epithelial cylinders. The central most cylinder forms the hair shaft. The outermost cylinder forms the outer root sheath (ORS) that separates the whole structure from the dermis. The middle cylinder, the inner root sheath (IRS), guides the shaft in its passage outward.

The hair cycles through periods of growth (anagen), regression (catagen) and rest (telogen). During anagen, the follicle grows deep into the hypodermis of the skin and the hair grows in length. During catagen, cells in the lower part of the follicle undergo a massive wave of apoptosis (Weedon and Strutton, 1984), whereas the regressing follicle maintains its attachment to the hair shaft through both telogen and the induction of the next anagen phase (Lavker et al., 1998). The hair follicle continues to cycle during almost all life, but hair synchronization is lost after the second cycle in mice (Bernot et al., 2002). At the bottom of the follicle is located the hair bulb, a terminal bell-like extremity containing matrix cells responsible for hair growth. The hair bulb forms an invagination that envelops the follicular papilla, in which papillary fibroblast important for the regulation of hair growth are located (Jahoda and Reynolds, 1996; Paus, 1998). Sebaceous glands are located in the upper part of the follicle and made of an outer layer of basal cells, and of several layers of mature, lipid-laden cells. They produce the oily sebum that lubricates the skin and the hair. Another important structure located in the upper part of the follicles is the bulge (Kanitakis, 2002). The bulge is a special compartment, which is part of the ORS, were the hair follicle stem cells are located (Akiyama et al., 2000; Taylor et al., 2000).

1.1.5 Epidermal Stem Cells

The epidermis is a continuously regenerating tissue. Throughout adult life there is a requirement for the production of new interfollicular keratinocytes to replace the cells that

are continually being shed from the surface of the skin. In response to skin injury, such as skin wounds, production of new keratinocytes is also required to repopulate the epidermis. Furthermore, during each anagen stage of the hair cycle new keratinocytes are produced to form the ORS of the growing follicle. Thus, a population of pluripotencial stem cells must exist in the epidermis to accomplish all this regenerative tasks. Several experimental approaches demonstrated that the bulge of the hair follicle contains a population of putative keratinocyte stem cells (Cotsarelis et al., 1990; Morris and Potten, 1999). It has been shown that the progeny of the bulge stem cells can migrate up or down to populate the interfollicular epidermis and the hair follicle, respectively (Cotsarelis et al., 1999; Liu et al., 2003a; Taylor et al., 2000), suggesting that bulge keratinocytes represent the pluripotential keratinocyte stem cell population. However, keratinocytes with stem cell properties have been also identified in the interfollicular epidermis (Ghazizadeh and Taichman, 2001; Jones et al., 1995), which may be responsible for the maintenance of the interfollicular epidermis keratinocyte population. Moreover, it has been shown that stem cells from the interfollicular epidermis can differentiate to form a normal hair (Ferraris et al., 1997; Lavker and Sun, 2000). These observations suggest that some plasticity may exist in the epidermal keratinocyte population. Thus, differences between the bulge and the interfollicular epidermis microenvironment may play a crucial role in the determination of keratinocyte identity in vivo.

The basal layer of the epidermis contains a heterogeneous population of keratinocytes in which three cell types are distinguished: epidermal stem cells, transit amplifying (TA) cells, and post mitotic differentiating (PMD) cells (Kaur and Li, 2000; Lavker and Sun, 1982; Pavlovitch et al., 1989). Interfollicular epidermal stem cells were first identified by their ability to retain radioactive labels for long periods of time on pulse-chase experiments (Bickenbach and Holbrook, 1987; Potten et al., 1982), and it is generally accepted that they represent 2-10% of all basal keratinocytes (Alonso and Fuchs, 2003; Potten and Morris, 1988). They are slow dividing cells with unlimited self-renewal capacity characterized by high expression levels of integrins α6 and β1 (Morris and Potten, 1994; Watt, 1988). When cultured *in vitro*, stem cells adhere rapidly to the substrate and form colonies with great reproductive capacity, that can be used to form a structurally complete epidermis in organotypic cultures (Barrandon and Green, 1987; Bickenbach and Chism, 1998). Asymmetric division of epidermal stem cells gives rise to a daughter stem cell, identical to the dividing stem cell, and to a TA cell (Jones et al., 1995; Savill, 2003; Savill and Sherratt, 2003; Seery and Watt, 2000). In contrast to stem cells, TA cells divide rapidly, they have limited proliferation capacity, exhibit lower levels of integrins α6 and β1 expression, and in culture take a longer time to adhere to the substrate (Adams and Watt, 1990; Jones et al., 1995; Kaur and Li, 2000; Tennenbaum et al., 1996). After few rounds of proliferation, TA cells stop dividing and initiate the terminal differentiation program, becoming PMD cells (Adams and Watt, 1990; Jaken and Yuspa, 1988; Jensen et al., 1985; Skerrow, 1978).

p63, a homologue of the tumor suppressor p53, has been identified as a putative marker for keratinocyte stem cell (Mills et al., 1999; Pellegrini et al., 2001). Interestingly, in mouse

epidermis p63 expression was detected in a small population of basal keratinocytes with very low proliferation rate (the stem cells), and its expression was gradually reduced in the more proliferative TA cells (Koster et al., 2002; Mills et al., 1999; Parsa et al., 1999; Pellegrini et al., 2001; Tsujita-Kyutoku et al., 2003). Experimental data obtained from cell culture and a wide range of in vivo studies lead to the establishment of a model to explain the structural and functional organization of epidermal stem cells and their progeny in the epidermis (**figure 2**). Basal keratinocytes are organized in units in which the slow dividing stem cells are located in the center of the unit, surrounded by the highly proliferative TA cells and the PMD cells which are committed to terminal differentiation (Ghazizadeh and Taichman, 2001; Jensen et al., 1999; Pellegrini et al., 2001). The progeny of each stem cell migrate upwards, forming clonal units of differentiating keratinocytes (Jensen et al., 1999; Mackenzie, 1997; Poumay et al., 1993; Savill and Sherratt, 2003).

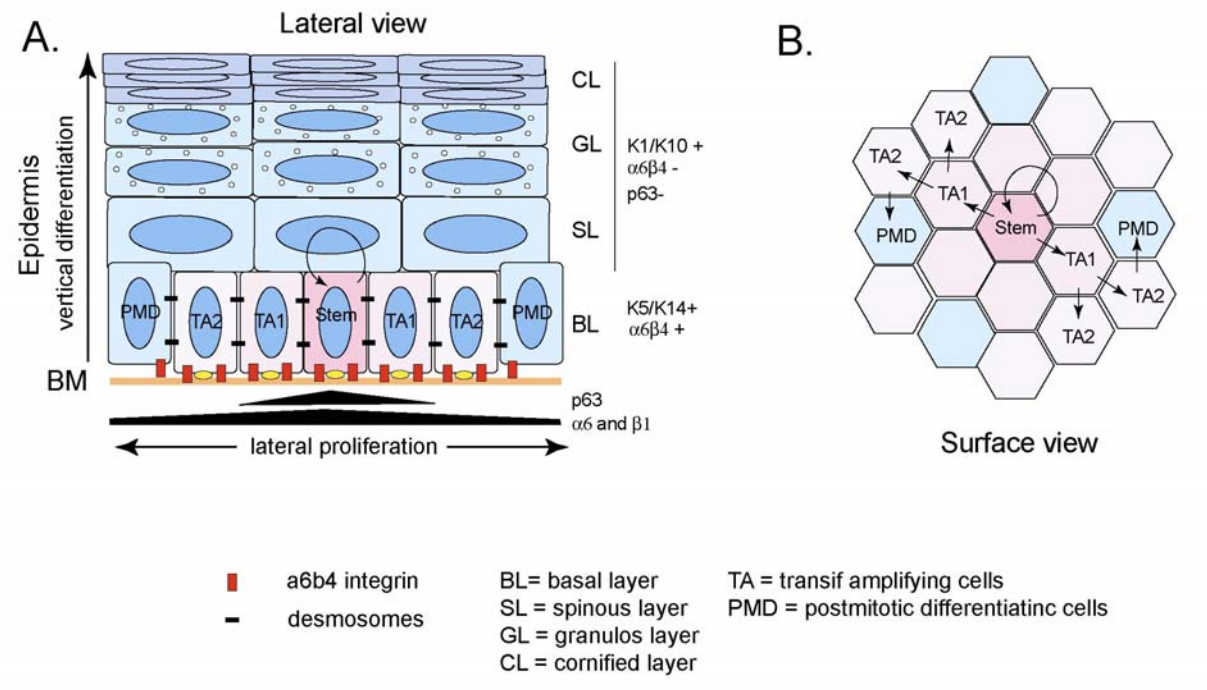

Figure 2. Keratinocyte proliferation unit. Adapted from Potten and Booth (2002).

1.1.6 Skin homeostasis

In healthy skin, basal keratinocytes proliferate slowly, and in response to not well defined signals, some of the basal cells initiate the terminal differentiation program, migrate upwards, and are finally shed from the skin. However, in response to epidermal injury or under certain pathological conditions, keratinocytes become activated. Activated keratinocytes are hyperproliferative, migratory, they change their cytoskeleton, augment the levels of membrane receptors, and produce components of the extracellular matrix (ECM) (Freedberg et al., 2001). Activated keratinocytes produce signals to alert the surrounding cells, including other keratinocytes, FB, melanocytes, and lymphocytes. The affected cells types in turn produce their own paracrine and autocrine signals to potentiate the response. Once the damage has been repaired, de-activating signals are produced, promoting the reversal of the basal phenotype. Fine balance between keratinocyte proliferation and differentiation, and the capacity of keratinocytes to become activated in response to injury depends on complex interactions between different cells types present in all three compartments of the skin. Such interactions involve cell-cell contacts, production of autocrine and paracrine factors, and ECM components.

Probably the most studied interaction between two cell types in the skin is that of keratinocytes and fibroblasts. It is well known that epidermal-mesenchymal interactions play a key role not only in skin morphogenesis, but also in the maintenance of skin homeostasis. In the epidermis, the BM physically separates keratinocytes and fibroblasts, therefore interactions between the two cell types depends on the production of secreted factors that act in a paracrine manner. Several factors involved in the FB-keratinocyte communication have been identified. Keratinocyte growth factor (KGF) (Marchese et al., 1990; Rubin et al., 1989) and the granulocyte macrophage colony stimulation factor (GM-CSF) (Braunstein et al., 1994) for example, are fibroblast secreted factors that affect keratinocyte proliferation. On the other hand, keratinocytes produce and secrete interleukin 1 (IL-1) which, in addition to its autocrine effect on keratinocytes (Kupper et al., 1988), induces expression of KGF and GM-CSF in fibroblasts (Maas-Szabowski et al., 1999). Using organotropic cultures, Szabowski and co-workers demonstrated that in the presence of fibroblast impaired in the production of KGF and GM-CSF, keratinocyte proliferation and differentiation is strikingly affected (Szabowski et al., 2000). Thus, keratinocyte-fibroblast communication plays a major role in the maintenance of skin homeostasis.

Another well known interaction between two different cell types in the skin is that of keratinocytes and melanocytes. Keratinocyte-melanocyte interactions have been largely documented in terms of skin pigmentation. Melanocytes contain highly specialized organelles called melanosomes in which melanin is produced. Melanosomes undergo a complex maturation process, and are transferred to keratinocytes through melanocyte dendrites which are in direct contact with keratinocytes (Bessou-Touya et al., 1998; Cario-Andre et al., 1999; Seiberg et al., 2000). The relationship between keratinocytes and melanocytes is not limited to the melanin transfer process. Studies using keratinocytes and

melanocytes in culture, reconstituted epidermis, and animal models demonstrated that epidermal keratinocytes are involved in the regulation of melanocyte growth and differentiation, migration and dendricity (Archambault et al., 1995; De Luca et al., 1988; Hirobe, 1994; Nakazawa et al., 1995; Scott and Haake, 1991; Tenchini et al., 1995; Valyi-Nagy and Herlyn, 1991; Valyi-Nagy et al., 1993; Valyi-Nagy et al., 1990). The mechanism by which keratinocytes control melanocytes function appears very complex. It involves cell-to-cell contacts (Hara et al., 1995; Hsu et al., 2000; Tang et al., 1994; Valyi-Nagy et al., 1993), keratinocyte-secreted factors (Donatien et al., 1993; Imokawa et al., 1992; Tenchini et al., 1995), and ECM components (Nakazawa et al., 1995).

In contrast to keratinocytes, FB and melanocytes, the role of adipocytes in the skin, and its interaction with other cell types, has been poorly investigated. For a long time, adipocytes were considered as "passive cells" in the skin. However, recent experimental data demonstrate that adipocytes can affect the growth and differentiation of other cells types in the skin, suggesting that they play an active role in the maintenance of skin homeostasis (Frank et al., 2000; Misago et al., 1998; Miyashita et al., 1992; Stallmeyer et al., 2001; Sugihara et al., 2001; Tokuda et al., 1999). Thus, maintenance of skin homeostasis requires an orchestrated action of the different cells types on the three skin compartments. Located in the outer most layer of the skin, keratinocytes seem to play a central role in the maintenance of skin homeostasis.

1.2 Skin cancer: an alteration of skin homeostasis

The skin is daily exposed to a variety of insults that disturb homeostasis. In normal conditions, skin homeostasis is reestablished by mechanisms involving the orchestrated action of several cell types in the three compartments of the skin. Genetic alterations affecting the proliferation and/or differentiation of the cells in the skin can lead to permanent loss of homeostasis, resulting in pathological states such as psoriasis or cancer.

Exposure to ultraviolet (UV) radiations is considered as the main risk factor for the development of skin cancer in human (Armstrong and Kricker, 2001; de Gruijl, 1999; Schreiber et al., 1984). UV radiations causes photodamage in the DNA by inducing the formation of dimers between adjacent pyrimidine residues (Johnson, 1978; Regan et al., 1978; Snellman et al., 2003). The cell can respond to the UV-induced damage by repairing the DNA to avoid harmful mutations (D'Ambrosio et al., 1981; Muramatsu et al., 1992; Runger et al., 1997), or if the damage is too great, by inducing apoptosis to remove potential cancer cells from the population (Iwasaki et al., 1996; Kulms and Schwarz, 2000). Failure of these pathways can results in UV-induced mutations that accumulate with time. If these mutations affect genes involved in the control of cell proliferation, differentiation and apoptosis, the result can be the formation of tumors. Mutations in several genes involved in the control of proliferation, differentiation and apoptosis have been identified in human skin cancer including p53 (Einspahr et al., 1999; Ouhtit et al., 1998), patched (Aszterbaum et al., 1998; Bodak et al., 1999), b-raf (Kumar et al., 2003; Kumar et al., 2004), N-ras (Jiveskog et al., 1998; Reifenberger et al., 2004), and the INK4a-ARF locus (Kreimer-Erlacher et al., 2003).

Cancers of the skin can be originated from different cell types such as keratinocytes, melanocytes, fibroblast, and Merkel cells (**Table 1**) (Hsu et al., 2002; Smith and Skelton, 2003). Depending on their origin, skin cancers are classified in two main groups; melanoma and non-melanoma skin cancer. Melanoma is a malignancy originated from melanocytes, whereas non-melanoma skin cancer includes cancers originated from cells other than melanocytes. The most frequent skin cancers are originated from melanocytes and keratinocytes.

1.2.1 Melanoma

Melanoma is a malignancy originated from the melanocytes, and is considered the most dangerous skin cancer. Long-term clinical and histopatological observations have led to the definition of five major subtypes of tumor progression (Clark, 1991).

Table 1. Common Neoplasm of the Skin

	Benign	Malignant
Epidermis	Acanthosis nigricans	Basal cell carcinoma
	Seborrheic keratosis	Squamous cell carcinoma
	Keratoacanthoma	
	Actinic keratosis	
	HPV-induced premalignant papules (epidermodysplasia verruciformis, bowenoid papulosis)	
Mucosa	Leukoplakia	Squamous cell carcinoma
Dermal connective tissue	Neurofibroma	Dermatofibrosarcoma protuberans
	Dermatofibroma	Merkel cell tumor
Appendage tumors	Trichilemmoma (in Cowden syndrome)	
	Sebaceoma (in Muir-Torre syndrome)	
	Nevus sebaceus	
Melanocyte	Melanocytic naevus	Malignant Melanoma

Modified from Cancer Medicine 5th Edition.

The *Nevus* represents the earliest hyperplasic melanocytic lesion. The d*ysphasic nevus* shows an increased level of cytologic and architectural atypia, and is thought to be a candidate precursor for cutaneous melanoma (Kraemer and Greene, 1985). The *radial growth phase primary melanoma* is the first recognizable malignant stage. The *vertical growth phase melanoma* cells infiltrate as an expanding mass into the dermis and subcutaneous tissue. Finally, the *metastatic melanoma* represents the most advanced step of tumor progression (Hsu et al., 2002). Development of cutaneous malignant melanoma is often associated with mutations in two genes involved in the control of cell proliferation, n-ras (Jiveskog et al., 1998) and b-raf (Uribe et al., 2003).

1.2.2 Non-melanoma skin cancer

The term non-melanoma skin cancer (NMSC) defines a number of malignant tumors originated from skin cells other than melanocytes. The most common NMSC are, Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), which originate from epidermal keratinocytes.

Basal Cell Carcinoma

BCC is a malignant tumor that develops from the basal, undifferentiated cells of the epidermis, it is usually slow growing and rarely metastasizes

(Saldanha et al., 2003; Wong et al., 2003). The tumor appears as proliferating basaloid cells forming cords and islands, invading into the dermis (Saldanha et al., 2003). The peripheral cells have characteristic palisading arrangement. A connection between the tumor island and the epidermis can be demonstrated in most of the cases. The tumor may have focal areas of ulceration, with an accompanying chronic inflammatory infiltrate (Cancer Medicine 5th edition). Mutations in genes such as p53 (Bolshakov et al., 2003; Ouhtit et al., 1998), patched (Bodak et al., 1999; de Gruijl et al., 2001; Lacour, 2002) and Fas (Boldrini et al., 2003) are frequently observed in human BCC.

Squamous Cell Carcinoma

Squamous **C**ell **C**arcinoma (**SCC**) is a malignant tumor arising from epidermal keratinocytes, which grows in a destructive way and metastasizes mainly via the lymphatic system. Actinic keratosis represents an early stage of SCC. It is characterized by atypical keratinocyte proliferation involving the suprabasal layers of the epidermis (Cockerell, 2000). However, the architectural organization of keratinocytes is not affected. In a more advanced stage, the so called *in situ* SCC*,* the epidermal keratinocyte organization is lost, keratinocytes are arranged haphazardly with a "windblown" appearance. The border between epidermis and dermis remains sharp throughout the lesion. There is no tendency for keratinocytes to flatten out and mature, as they migrate from the basal layer upward, as is seen in normal epidermis. There is usually a moderate amount of chronic inflammatory infiltrate in the upper dermis. Bowen's disease is an *in situ* SCC that occurs on non-exposed skin. In SCC the tumor cells are seen to proliferate downward in cords and single cells into the dermis. The degree of differentiation is determined by the extent of the tumor trying to recapitulate normal keratinizing epithelium, with the Spindle-Cell Squamous Carcinoma (SpCC) representing the least differentiated form (Cancer Medicine, 5th Edition). SCC present high frequency of mutations in the p53 gene (Bolshakov et al., 2003; Einspahr et al., 1999) and in the INK4a-ARF locus (Kreimer-Erlacher et al., 2003). In addition, low frequency of H-Ras mutations have also been detected in AK and SCC lesions (Spencer et al., 1995; van der Schroeff et al., 1990).

1.3 Mouse models of skin carcinogenesis

A number of mouse models have been validated to study cancers originated from several organs including lung, mammary gland, nervous system, prostate and skin (MMHC NCI database). Mouse models for both melanoma and non-melanoma skin cancer have been generated based on three main approaches: 1) induction of skin tumors by chemical treatment or exposure to UV-radiations; 2) generation of transgenic mice expressing oncogenic proteins under the control of tissue specific promoters; and 3) generation of knockout mice with genetic alterations that are observed in human familial cancers. Some examples of mouse models of melanoma and non-melanoma skin cancer are summarized in **table 2**. In this section I will describe the two models that we have used in our study, the two-step chemical carcinogenesis (DMBA/TPA) model, and the genetically pre-initiated (Tg.AC) mouse model.

1.3.1 The chemical skin carcinogenesis model

The two-step chemical skin carcinogenesis protocol has been used for over 50 years. In this model, skin tumors are induced by a single topical application of a chemical carcinogen into mouse skin, followed by repeated applications of a tumor promoter. The treatment gives rise to a series of phenotypical changes in the skin, including epidermal hyperplasia, formation of benign squamous tumors (papillomas) and the progression of some squamous papillomas to malignant squamous carcinoma, with eventual metastasis of the cancer cells to distal organs (**figure 3**). The phenotypic evolution from normal keratinocytes through squamous papilloma into squamous carcinoma is associated with genetic and epigenetic reproducible changes that characterize each stage of the process (Hennings et al., 1993).

Initiation

The term initiation defines a genetic event which consists on the introduction of a permanent mutation in the DNA of the cell, generally as a result of a DNA damage induced by genotoxic agents such as UV radiation or chemical substances. In the two-step chemical carcinogenesis protocol, initiation is induced by topical application of the potent carcinogen 7,12-dimethylbenz(a)anthrancene (DMBA). Topical application of DMBA into mouse skin induces activation mutations in the H-ras, N-ras, and Ki-ras oncogenes, which results in the expression of constitutively active Ras oncoproteins (Pazzaglia et al., 2001; Quintanilla et al., 1986a). Mutation and activation of Ha-ras oncogene are critical events for the formation of skin tumors. Indeed, Ha-ras null mice develop significantly less tumors than wild-type mice in response to a DMBA/TPA protocol (Ise et al., 2000), and the tumors induced in Ha-ras null mice exhibit mutations in the K-ras gene, suggesting that activation of the K-ras gene can replace the Ha-ras activation at the initiation step (Ise et al., 2000). The DMBA induced

mutation in the Ha-ras oncogene is an irreversible event. However, the mutation is silent, and additional stimuli (promoter) are required to induce the formation of tumors.

Table 2. Mouse models of skin cancer

Cancer Type	Model	Phenotype	Reference
Melanoma	***Tyr-SV40E***	Predominantly ocular, but also cutaneous melanomas with a high rate of metastasis	(Bradl et al., 1991)
	Tpras	Cutaneous melanomas develop after topical DMBA application	(Broome Powell et al., 1999)
	K14-stem cell factor	Hyperpigmentation with increased melanin production and increased epidermal melanocytes	(Kunisada et al., 1998)
	Ink4a$^{\Delta 2/3}$	Cutaneous melanomas develop after DMBA treatment with occasional metastasis	(Krimpenfort et al., 2001)
	Tyr-H-ras Ink4a/Arf$^{\Delta 2/3}$	Cutaneous and ocular melanomas without evidence of metastasis	(Chin et al., 1997)
	Pten+/- Ink4a/Arf$^{\Delta 2/+}$	Cutaneous melanomas develop spontaneously at a low rate	(You et al., 2002)
Non-melanoma skin cancer (NMSC)	***DMBA/TPA***	Squamous papilloma formation with progression to invasive carcinoma	(Yuspa, 1998)
	Ultraviolet radiation	Squamous cell carcinomas and spindle cell squamous carcinoma	(Jiang et al., 1999)
	K14-HPV16 early region	Squamous hyperplasia with atypia with progression to invasive carcinoma of varying degrees	(Arbeit et al., 1994)
	K5-Gli	Development of spontaneous BCC	(Nilsson et al., 2000)
	Tg.AC	Spontaneous squamous papillomas in old animals. Squamous papillomas with progression to carcinoma after TPA treatment or exposure to UV	(Leder et al., 1990)

Promotion

Promotion is accomplished by repeated applications of the tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA). Experiments in vitro and in vivo demonstrated that TPA stimulates differentiation of normal keratinocytes (Wei et al., 2003), induces basal cell hyperproliferation in the epidermis (Heyden et al., 1994), and a strong inflammatory reaction in the skin (Aldaz et al., 1985) of adult mice. In contrast, initiated cells have impaired ability

to respond to differentiation signals (Kulesz-Martin et al., 1991), and in response to TPA treatment, their proliferation to differentiation ratio may be increased, which results in the clonal expansion of the initiated population and the formation of squamous papillomas (Ghosh et al., 2004; Karen et al., 1999).

Malignant conversion

Conversion of a benign tumor to malignant carcinoma is characterized by an increased autonomy from both the environment and the host. Papillomas induced by repeated application of TPA may progress to malignancy in the absence of any additional exogenous exposure, although the rate of progression is slow and the frequency of malignant conversion is low (Hennings et al., 1993). In general 25-35 weeks of promotion are required to induce malignant conversion, and only few of the tumors may become malignant. The tumor incidence, multiplicity and frequency of malignant conversion depend in great extent on the genetic background of the animals (Naito and DiGiovanni, 1989). It has been shown that a subset of papillomas that have a greater risk for progression into malignancy (high-risk papillomas) can be identified at early stages during tumor progression (Glick et al., 1993). High-risk papillomas appear earlier and are characterized by an upregulation of $\alpha 6\beta 4$ integrins expression in the keratinocytes of the suprabasal layers (Tennenbaum et al., 1993), and reactivation of the keratin 13 (K13) expression (Rundhaug et al., 1997).

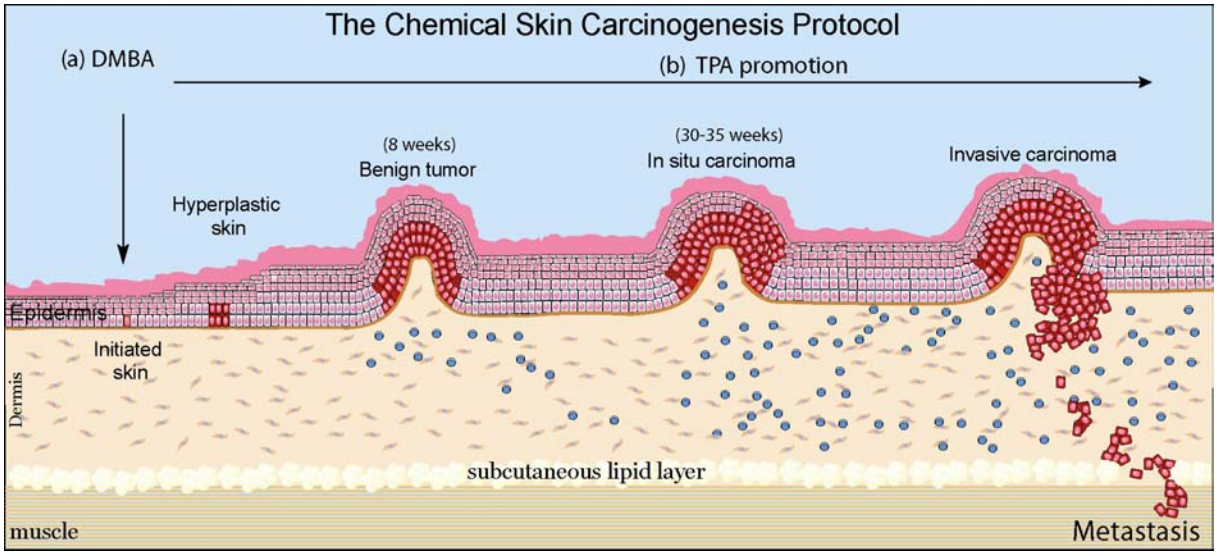

Figure 3. The chemical skin carcinogenesis model. (a) Initiation is induced by a single topical application of the potent carcinogen 7,12-dimethylbenz(a)anthrancene (DMBA). (b) Promotion is achieved by repeated applications of 12-0-tetradecanoylphorbol-13-acetate (TPA). TPA treatment induces progressive changes in the skin, including epidermal hyperplasia, formation of benign papillomas, progression of the papillomas to carcinoma and the eventual invasion of the malignant cells to the dermis and distal organs.

Malignant conversion is characterized by invasion through the basement membrane and migration into the underlying stroma. In situ carcinoma is an early stage of malignant progression, and is characterized by the presence of disorganized basal-like keratinocytes through all the epidermal layers. In a more advanced stage (focal carcinoma), the basement membrane separating the epidermis from the dermis is disrupted and the epidermal cells invade the underlying dermal compartment. Finally, in the most advanced stages of malignant conversion, the squamous cell carcinoma, epidermal keratinocytes are seen as nests or islands of cells invading the dermis. Malignant conversion involves important changes in cell-cell and cell-stoma interactions (Kerkela et al., 2000; Kerkela et al., 2001), and is often associated with mutations in p53 and upregulation of the AP-1 family of transcription factors (Domann et al., 1994; Watts et al., 1995). Targeted expression of a mutant p53 protein to the epidermis for example, results in accelerated chemically-induced tumorigenesis and malignant transformation (Wang et al., 1998), whereas mice carrying a null mutation of the c-fos gene developed papillomas but not carcinomas in response to DMBA/TPA treatment (Saez et al., 1995), suggesting that p53 and c-fos play key roles during the progression stage.

1.3.2 The TG.AC model

In 1990, Leder and co-workers generated a transgenic mouse strain (Tg.AC) that carries the activated v-Ha-ras oncogene under the control of the mouse embryonic α-like, zeta-globin gene promoter (Leder et al., 1990). They found that Tg.AC mice spontaneously developed skin papillomas at areas of epidermal abrasion, and that in response to topical application of TPA, Tg.AC mice developed multiple papillomas, some of which progressed to squamous cell carcinomas.

Further studies on Tg.AC mice demonstrated that development of skin papillomas can also be induced by application of other known tumor promoters (Spalding et al., 1993), by full thickness wounding of the skin (Cannon et al., 1997), and by exposure to UV-radiations (Trempus et al., 1998). It has been shown that transcriptional activation of the H-ras transgene in Tg.AC mice is a key event for the development of skin tumors. The expression of the Ha-ras transgene is undetectable in the skin of unchallenged Tg.AC mice, but is expressed in epidermal keratinocytes after 10 weeks of topical application of TPA (Battalora et al., 2001), and at higher levels in all induced skin tumors (Cannon et al., 1997; Hansen and Tennant, 1994a; Hansen et al., 1996; Leder et al., 1990). The Tg.AC mouse has been extensively used as a model for evaluating the carcinogenic potential of chemicals and pharmaceuticals (Sistare et al., 2002; Tennant et al., 1998), and for the study of genes involved in skin carcinogenesis. It has been shown for example, that double transgenic mice, bearing the Keratin 6-ornithine decarboxylase and v-Ha-ras transgenes, developed spontaneous skin tumors (Smith et al., 1998). Introduction of a null mutation of the kinase suppressor of ras (KSR) gene into Tg.AC mice results in a complete abrogation of papilloma formation (Lozano et al., 2003). Thus, genetically pre-initiated Tg.AC mice represent an

interesting model to study skin carcinogenesis, in which epidermal squamous papillomas, that with time may progress to squamous carcinomas, can be rapidly induced by TPA treatment (**figure 4**).

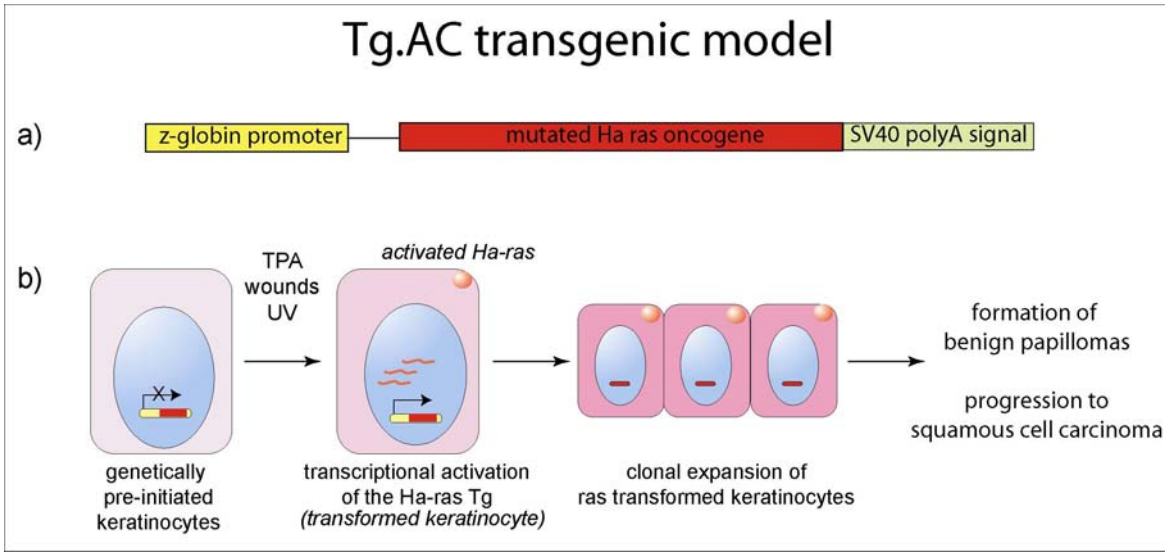

Figure 4. The Tg.AC model. (a) structure of the ζ-globin-Ha-ras transgene (Tg). (b) Events during tumor formation in the Tg.AC mice. The Ha-ras Tg is transcriptionally silent, but in response to stimuli such as TPA, UV, or skin wounds, the Ha-ras Tg is activated in kerationcytes, producing an activated Ras oncoprotein. The Ha-ras transformed kertinocytes expand clonally to form squamous papillomas, which can degenerate to malignant carcinoma.

1.4 Tissue-specific temporally-controlled somatic mutagenesis in the mouse

Functional inactivation of genes by introduction of null mutations in the mouse germ line has yield remarkable advances in understanding the roles played by specific gene products in vivo. However, this strategy has some inherent limitations. For example, invalidation of genes coding for proteins that play essential functions during development can result in early lethality. The effect of a germ line mutation may also be compensated during development. Furthermore, invalidation of genes that play important functions in different cell types may result in complex phenotypes. Thus, a strategy to introduce somatic mutations in a given tissue and in a temporally controlled manner is required to overcome this limitations. The discovery in the early 1980's, by Austin and co-workers, of the Cre-loxP site-specific homologous recombination system from the bacteriophage P1 (Austin et al., 1981), provided the basis for the development of such technology.

The cre-loxP system consists on a site-specific recombinase, the recombinase Cre, which recognizes specific DNA sequences called "lox P" sites and induces homologous recombination between two loxP sites. The loxP site is a 34 bp sequence composed of two 13 bp palindromic sequences separated by 8 bp. The 8 bp between the two palindromes confers an orientation to the loxP site. When two loxP sites are placed in head-to-head orientation, the Cre recombinase will induce an inversion of the sequence located between the two loxP sites (floxed sequence), whereas recombination between two loxP sites placed in head-to-tail orientation results in the excision of the floxed sequence (Austin et al., 1981) (**figure 5**). The purification and characterization of the Cre recombinase by Abremski and Hoess in 1984 (Abremski and Hoess, 1984), and the demonstration, a few years later, by Sauer and Henderson that the Cre recombinase can efficiently induce the excision of a floxed DNA fragment in mammalian cells in culture (Sauer and Henderson, 1989), provided the experimental basis for the development of a system to induce site-specific mutations in the mammalian genome.

The first reports showing that Cre-mediated excision of DNA fragments can be induced in higher organisms were published in the early 1990's (Odell et al., 1990; Orban et al., 1992). In these reports the Cre recombinase was introduced as a transgene into Tobacco plants (Odell et al., 1990) or into transgenic mice (Orban et al., 1992) carrying a floxed DNA reporter gene. Excision of the floxed DNA fragment was efficiently induced in both transgenic tobacco plants and transgenic mice. Later, using tissue-specific promoters to drive the expression of the cre recombinase, several laboratories demonstrated the efficacy of the cre recombinase system to introduce mutations in a given gene in a tissue-specific manner (Kulkarni et al., 1999; Ray et al., 1999; Tarutani et al., 1997).

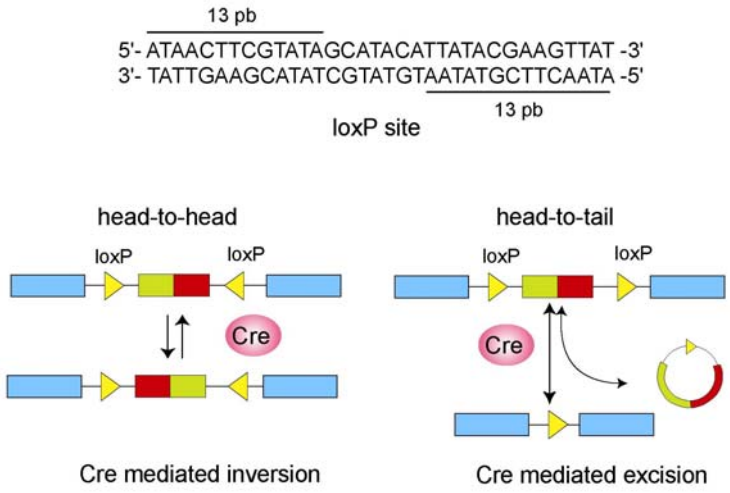

Figure 5. The Cre-loxP system of homologous recombination.

On the other hand, two strategies have been used to achieve the temporal control of the Cre-mediated recombination. One strategy was based in the control of the Cre expression at the transcriptional level. For this purpose, double transgenic mice carrying the cre recombinase under the control of a tetracycline (Tet)- dependent promoter and a reverse tetracycline-controlled transactivator (rtTA) transgene, must be generated. The rtTA protein is constitutively expressed, but it remains inactive. Exogenous administration of tetracycline will activate the rtTA which can now bind to the Tet-dependent promoter to activate expression of the cre recombinase (St-Onge et al., 1996; Utomo et al., 1999). The other strategy is based on the principle that the activity of some proteins can be modulated by the presence of a specific ligand. Metzger and co-workers generated a fusion protein between the Cre recombinase and the ligand binding domain (LBD) of the human Estrogen Receptor α (hERα), resulting in a Cre recombinase which activity is controlled by estrogens (Cre-ER). They established a cell line in which the Cre-ER is constitutively expressed and they demonstrated that estradiol or the estrogen agonist/antagonist 4-hydroxytamoxifen efficiently induced the recombinase activity, whereas no activity was detected in the absence of ligand or in the presence of a synthetic antiestrogen (Metzger et al., 1995). However, introduction of the Cre-ER into transgenic mice may result in the constitutive activation of the recombinase by endogenous estrogens. To avoid the activation by endogenous estrogens, a new fusion protein was generated in which the Cre recombinase was fused to a mutated LBD of the hERα, resulting in a chimeric recombinase, Cre-ER^T, which is activated by tamoxifen, but not by natural estrogens (**figure 6**) (Feil et al., 1996). Using this Cre-ER^T chimeric recombinase, Feil and co-workers generated transgenic mice in which the Cre-ER^T is expressed under the control of the cytomegalovirus promoter, and they demonstrated that excision of a floxed DNA sequence was efficiently induced in several tissues of Cre-ER^T transgenic mice, after tamoxifen administration (Feil et al., 1996).

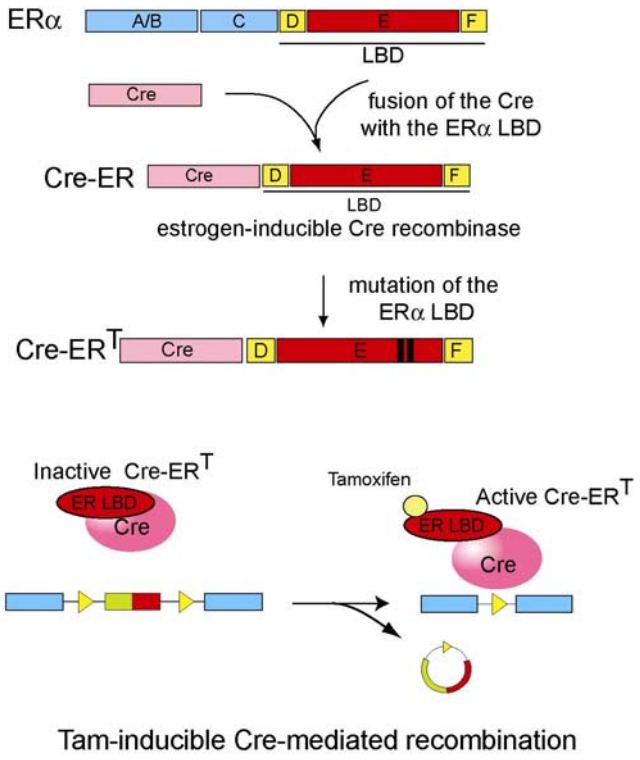

Figure 6. Strategy to generate a tamoxifen-induceble Cre recombinase. Adapted from Metzger (2001)

However, the doses of tamoxifen required to induce the Cre-ER^T activity in these transgenic mice were high, which may result in side effects due to the estrogen agonist/antagonist action of tamoxifen. The problem was circumvented by the introduction of a second mutation in the LBD of the Cre-ER^T recombinase, resulting in a recombinase (Cre-ER^{T2}) which was approximately 10-fold more efficiently induced by tamoxifen than the Cre-ER^T (Feil et al., 1997). Indra and co-workers generated transgenic mice in which the expression of the Cre-ER^{T2} recombinases is driven by the bovine keratin 5 (K5) promoter to the basal layer of the epidermis, and demonstrated that excision of a floxed DNA can be efficiently induced in the basal layer of the epidermis of adult K5-Cre-ER^{T2} mice by administration of 10-fold lower doses of tamoxifen than those required to activate the Cre-ER^T (Indra et al., 1999). Similar results were obtained in transgenic mice in which the Cre-ER^{T2} recombinase is expressed under the control of the keratin 14 (K14) promoter (Indra et al., 2000; Indra et al., 1999; Vasioukhin et al., 1999). The mechanism of tamoxifen-induced Cre-ER^{T2}-mediated mutagenesis in keratinocytes is represented in **figure 7**. On the other hand, the introduction of Cre mediated mutations selectively in epidermal keratinocytes during mouse embryonic development was achieved in transgenic mice in which a constitutively active Cre recombinase is expressed under the control of the K14 promoter (K14-Cre) (Indra et al., 2000). Thus, K5-Cre-ER^{T2} or K14-Cre-ER^{T2} and K14-Cre transgenic mice represent powerful tools to introduce somatic mutations in adult epidermis and during embryonic development, respectively.

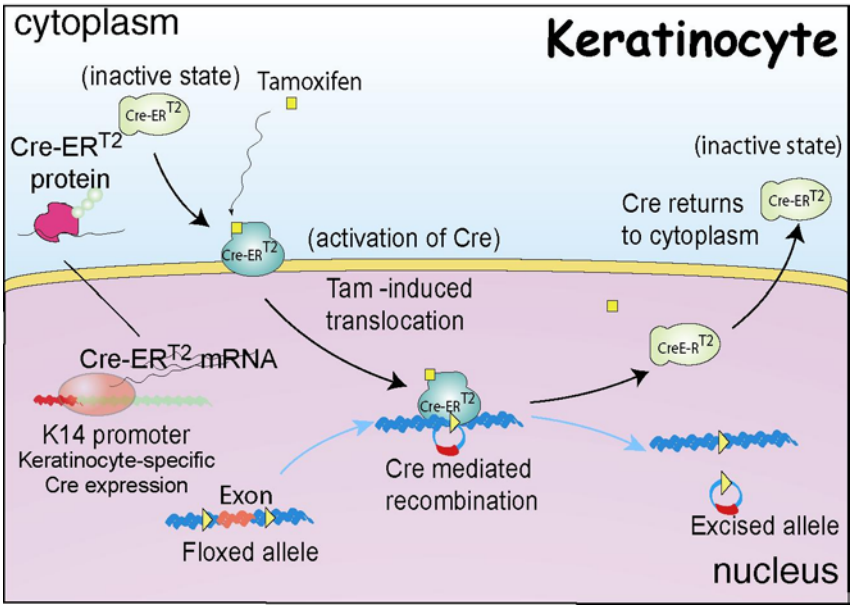

Figure 7. Model of the mechanism of Cre-ER^{T2}-mediated recombination in keratinocytes.

1.5 The Nuclear Receptor Superfamily

Higher organisms are complex biological entities, in which the function of many different cell types, tissues and organs has to be controlled in an orchestrated manner. During evolution, higher organisms have developed sophisticated signaling networks to control the function of all cells (tissues and organs) in the body. As part of this sophisticated networks, the organism produces a large number of chemical substances (hormones) that controls and regulates the activity of certain cells or organs. Hormones can act either locally, affecting the cell or organ in which they are produced (autocrine action), or they can be secreted and transported through the circulation to affect other distant cells and organs (paracrine action). They are essential for every activity of daily living, including the processes of digestion, metabolism, growth, reproduction, and mood control. Many hormones are active in more than one physiological process. Production and secretion of hormones as well as the ability of target cells to recognize and transduce the hormonal signal are crucial for the maintenance of body homeostasis.

Steroid and thyroid hormones, the active metabolites of vitamin A (retinoic acid), and vitamin D (vitamin D3) are structurally related hormones, that play important roles in growth, differentiation, metabolism, reproduction and morphogenesis of higher organisms, including humans. Progesterone for example, prepares the lining of the uterus for implantation of an ovum, and is also essential for the maintenance of pregnancy (Bagchi et al., 2003; Conneely et al., 2002; Giudice, 1999; Rosario et al., 2003); androgens and estrogens are involved in the development of secondary sex characteristics (Gooren and Kruijver, 2002; Hughes et al., 2001); glucocorticoids are important for glucose and lipid metabolism (Andrews and Walker, 1999; Birt et al., 1999; Brindley, 1995; Fernandez-Mejia et al., 1999; Klein et al., 1997; Sivitz and Pasley, 1995); vitamin D plays a key role in calcium metabolism and in the formation and maintenance of bones (Brown et al., 1999; van den Bemd et al., 1995; Yoshizawa et al., 1997), and vitamin A is essential for visual function, reproduction, growth, immunity, and epithelial function (Chambon, 1994; Gerster, 1997; Halliday et al., 1992; Livera et al., 2002; Zile, 2001).

Higher organisms have all the enzymatic machinery required to produce steroid and thyroid hormones or vitamin D, but not vitamin A, which has to be taken from the diet in the form of retinyl esters or as provitamin A carotenoids (**table 3**) (Gerster, 1997)**.** The five major classes of *steroid hormones:* progestagens, glucocorticoids, mineralocorticoids, androgens and estrogens, as well as vitamin D, are derived from cholesterol. Cholesterol is either obtained from the diet or synthesized from acetate by a Co A reductase enzyme (Koga et al., 1990; Russell, 1992). Cholesterol metabolism and steroid hormone synthesis takes place mainly in the adrenal cortex and in the gonads (Ben-Zimra et al., 2002; Carr et al., 1983; Hall, 1991; Rouiller et al., 1990; Sanders and Stouffer, 1997; Wisner and Gomes, 1975), whereas vitamin D is synthesized mainly by the keratinocytes in the epidermis (Bikle et al., 1986; Holick, 2003; Nemanic et al., 1983).

Table 3. Synthesis of the classical lipophilic hormones.

Hormone	Precursor	Main site of synthesis
Glucocorticoids	Cholesterol	Adrenal gland
Progesterone	Cholesterol	Adrenal gland
Androgens	Cholesterol	Adrenal gland
Estrogens	Cholesterol	Ovaries
Vitamin D	Cholesterol	Skin
Thyroid Hormones	Iodine/Tyrosine	Thyroid gland
Vitamin A	Taken as preformed vitamin A retinyl esters or pro-vitamin A carotenoids	

Thyroid hormone is produced in the thyroid gland by incorporation of iodine, a microelement taken from the diet, to the aminoacid tyrosine (Whitehead, 2001). Independently of their origin, steroid/thyroid hormones, vitamin D3, and retinoic acid share a common feature. They are small lypophilic molecules that, in contrast to other water soluble hormones that require interaction with receptors on the surface of the membrane of target cells and intracellular signaling cascades to exert their activity (Boonstra et al., 1995; Levitzki, 1988; Pessin and Okada, 1999), act by directly interacting with intracellular proteins (**Figure 8**).

1.5.1 Discovery and Characterization of the NRs

Biochemical studies published during the second half of the 60's demonstrated that estrogens and androgens bind with high affinity, and in a specific manner to cellular proteins (Liao and Fang, 1969; Toft and Gorski, 1966), suggesting that the action of such hormones was mediated by interaction with specific cellular receptors. Moreover, it was demonstrated that hormone binding proteins for glucocorticoids and androgens were located not only in the cytoplasm but also in the nucleus of the cells (Brecher and Wotiz, 1969; Fang et al., 1969), and that nuclear localization of the androgen specific binding protein was dependent on the presence of androgens (Fang et al., 1969; Liao and Fang, 1969). These reports marked the beginning of a very active period of investigation in the search for steroid nuclear receptor proteins. A number of studies based in biochemical approaches confirmed the presence of specific nuclear receptors for estrogens (Mester and Baulieu, 1972) and androgens (Keenan et al., 1975; Smith et al., 1975), and demonstrated the presence of specific nuclear receptors with high affinity for several hormones including glucocorticoids (Middlebrook et al., 1975; Shyamala, 1975), progesterone (Feil and Bardin, 1975), vitamin D_3 (Brumbaugh and Haussler, 1975), and thyroid hormone (Charles et al., 1975; Spindler et al., 1975). The observation that thyroid receptor (TR) and glucocorticoid receptor (GR) interact directly with DNA (Charles et al., 1975; Rousseau et al., 1975), and the demonstration that TR was associated with chromatin regions containing endogenous RNA polymerase activity (Charles et al., 1975) provided the first evidences to establish a direct link between hormone nuclear receptors and transcriptional regulation.

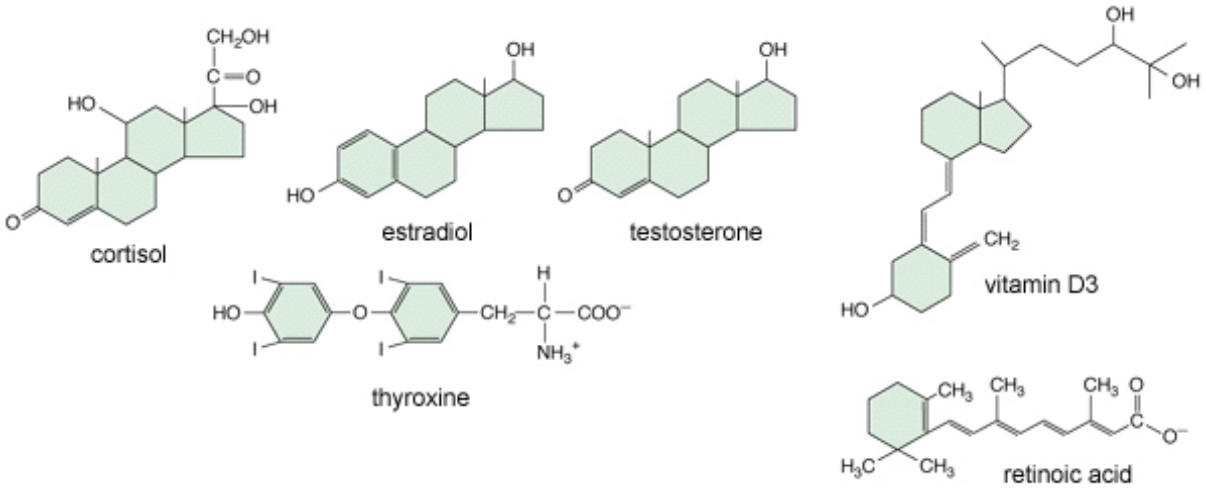

Figure 8. Structure of the major lipophylic hormones. From Molecular Biology of the Cell. 3rd Edition.

The development of genetics and molecular biology in the 80's, contributed to the identification and characterization of the genes for several nuclear receptors. The characterization of the GR (Govindan et al., 1985; Miesfeld et al., 1984) and the Estrogen Receptor α (ERα) genes (Green et al., 1986) in the early 1980's marked the beginning of a cascade of discoveries leading to the cloning and characterization of several NRs involved in hormonal signaling (Willson and Moore, 2002). Using a variety of genetic, biochemical, and molecular biology techniques, a number of NR genes were cloned and characterized, including the chicken progesterone receptor gene (Gronemeyer et al., 1987), the genes for several human and mouse retinoic acid receptors (Brand et al., 1988; Giguere et al., 1987; Krust et al., 1989b; Petkovich et al., 1987; Zelent et al., 1989b), the human androgen receptor (AR) gene (Lubahn et al., 1988; Trapman et al., 1988) and the TR gene (Chang et al., 1989).

In 1978, Wrange and Gustafson had already demonstrated, by biochemical approaches, that the GR has two functional domains: a DNA binding domain (DBD) and a ligand binding domain (LDB) (Wrange and Gustafsson, 1978). Comparison of the newly emerging nuclear receptors give rise to the exciting observation that significant regional homology existed between the steroid hormone receptors as well as the cellular receptors for thyroid hormone and retinoic acid (Krust et al., 1986; Owen and Zelent, 2000). NRs exhibit a modular structure consisting of an NH2 terminal region (A/B), a conserved DNA-binding domain (DBD) or region C, a linker region D, and a conserved region E that contains the ligand binding domain (LBD) (Freedman and Luisi, 1993; Kumar and Thompson, 1999; Laudet et al., 1992) (**figure 9**).

The A/B domain is the most variable region both in size and sequence, and it contains a transcriptional activation domain (AF-1) that shows promoter and cell-specific activity, and is a target for phosphorylation mediated by various signaling pathways which can modulate the receptor activity (Ali et al., 1993; Berry et al., 1990; Metzger et al., 1992; Rochette-Egly, 2003; Tora et al., 1989). The DBD, the most conserved domain of NRs, confers the ability to recognize specific DNA target sequences and activate gene transcription. The DBD contains two zinc fingers, one of which is involved in the recognition of core DNA motifs and the other is involved in dimerization (Hard et al., 1990; Katahira et al., 1992; Lee et al., 1993; Mader et al., 1993; Zechel et al., 1994). The D domain, that is not well conserved among NRs, serves as a hinge between the DBD and the LBD, allowing the rotation of the DBD, and in many cases harbors nuclear localization signals (Guiochon-Mantel et al., 1989; Lee and Mahdavi, 1993; Picard et al., 1990; Picard et al., 1988; Picard and Yamamoto, 1987; Shyamala, 1975). The LBD is a multifunctional domain that, in addition to the binding of ligand, mediates homo- and heterodimerization (Fawell et al., 1990; Forman et al., 1989; O'Donnell and Koenig, 1990; Spanjaard et al., 1991). The LBD contains an activation function (AF-2) motif that participates in the recruitment of NRs associated proteins and is responsible for the ligand-dependent transcriptional activation (Durand et al., 1994; Le Douarin et al., 1996; vom Baur et al., 1996). The availability of the sequences of NRs genes and the crystal structure of the NRs proteins together with the development of powerful computer software and sequence databases gave rise to the new concept of "reverse endocrinology" in which the characterization of the receptor precedes the study of its physiological function, leading to the discovery of new hormonal responses (Aranda and Pascual, 2001; Banner et al., 1993; Herz et al., 1997; Hirose et al., 1994; Moras and Gronemeyer, 1998). The subfamilies of NRs from human and mouse are shown in **table 4** (**NUREBASE**).

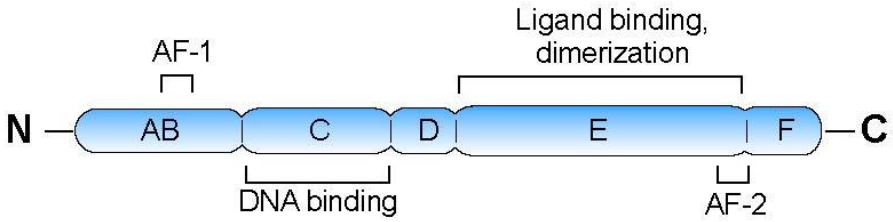

Figure 9. Modular structure of the NRs. Adapted from Aranda and Pascual 2001.

Table 4. Human and Mouse nuclear receptors

1.5.2 Mechanism of Nuclear Receptors Action.

NRs interact with DNA through recognition of specific sequences in the promoter region of target genes, called response elements, which are composed of a core hexameric motif. Two consensus motifs have been identified: the sequence *AGAACA* that is preferentially recognized by steroid class III receptors (see **table 4**), whereas *AGG/TTCA* served as a recognition motif for the receptors from the other classes (Beato et al., 1995). Although some monomeric receptors can bind to a single hexameric motif, most receptors bind as homo- or heterodimers to elements composed by two core hexameric motifs (Glass, 1994). For dimeric response elements, the half sites can be configured as palindromes, inverted repeats, or direct repeats. Steroid hormone receptors typically bind to palindromic elements as homodimers whereas non-steroidal receptors can bind as heterodimers to elements in different configurations. In that case, the arrangement and the spacing between motifs will determine the selectivity and specificity (Glass, 1994). Direct repeats with a spacing of 3, 4 and 5 base pairs were observed to specify preferentially transcriptional responses to vitamin D, thyroid hormone and retinoic acid, respectively, whereas palindromic and inverted palindromic elements appear to be regulated by several classes of receptors (**figure 10**) (Mangelsdorf et al., 1991; Naar et al., 1991; Schrader et al., 1994).

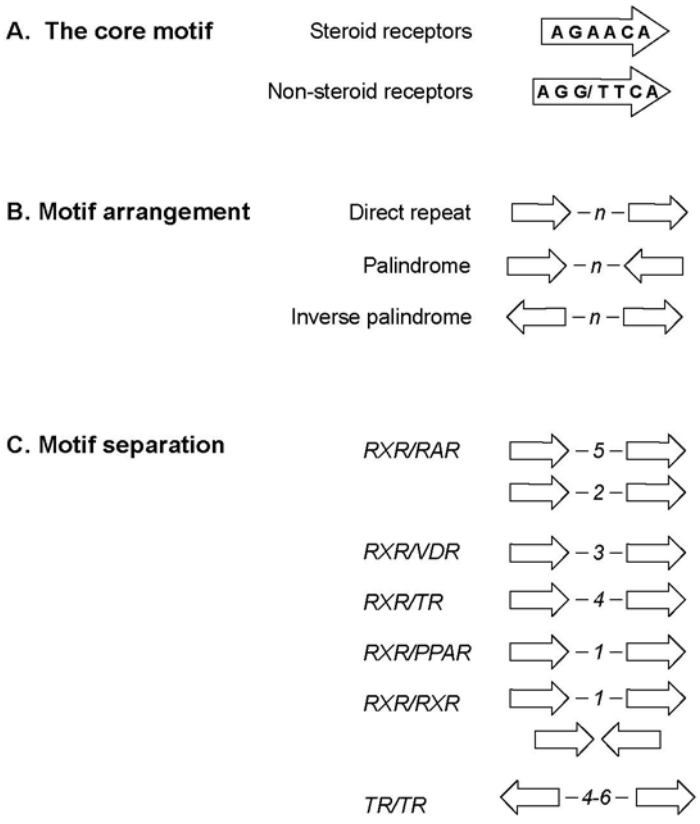

Figure 10. Nuclear receptors response elements. Adapted from Glass 1994.

Moreover, it has been shown that dimerisation between receptors enhances their affinity for they response elements (Jiang et al., 1997; Perlmann et al., 1996). Interestingly, the three Retinoic X Receptors (RXRα, RXRβ, and RXRγ), that bind specifically to 9-cis retinoic acid, can form heterodimers with a number of non-steroidal nuclear receptors such as the Retinoic Acid Receptors (RARs), vitamin D_3 receptor (VDR), TR, and the peroxisome proliferator activated receptors (PPARs), enhancing their DNA-binding to specific response elements and their transcriptional activity (Bardot et al., 1993; Issemann et al., 1993; Kliewer et al., 1992; Leid et al., 1992; Mangelsdorf and Evans, 1995; Schulman et al., 1998; Yu et al., 1991; Zhang et al., 1992). Thus, as common heterodimeric partners for non-steroidal nuclear receptors, RXRs are central regulators of several hormonal signaling pathways.

1.5.3 Coactivators and corepressors.

Another important aspect of the mechanism of NR action is how this DNA-bound nuclear receptors interact and modulate the activity of the transcriptional machinery. Using both genetic and biochemical approaches, several NR interacting proteins were identified (Beato et al., 1995; Butt and Walfish, 1996; Chakravarti et al., 1996; Sande and Privalsky, 1996). Identification and characterization of these NR interacting proteins lead to the observation that NRs act in conjunction with two classes of cofactors to regulate transcription in the context of chromatin: corepressors and coactivators (Kraus and Wong, 2002; Lemon and Freedman, 1999). The identification and characterization of accessory proteins that interact with unliganded NRs (corepressors) (Chen and Evans, 1995; Horlein et al., 1995), demonstrated that NR mediated transcriptional repression occurs by two main mechanisms. NR/corepressors complexes can repress transcription either by directly interacting with the transcriptional machinery (Fondell et al., 1996; Fondell et al., 1993; Muscat et al., 1998; Wong and Privalsky, 1998) or indirectly by modifying chromatin structure (Hu and Lazar, 2000; Huang et al., 2000; Ito et al., 2000; Jenster, 1998; Nagy et al., 1997; Townson et al., 2004). Chromatin modifying factors with histone deacetylase (HDAC) activity are recruited to the NR/corepressor complex through direct interaction with the corepressor (Hu and Lazar, 2000; Huang et al., 2000; Ito et al., 2000; Jenster, 1998; Nagy et al., 1997; Townson et al., 2004) or mediated by other intermediate factors such as mSin3A (Hu and Lazar, 2000; Huang et al., 2000; Ito et al., 2000; Jenster, 1998; Nagy et al., 1997; Townson et al., 2004). The recruitment of HDAC factors to the NR complex results in deacetylation and condensation of the chromatin, which renders the promoter inaccessible for the transcriptional machinery. Upon binding of the ligand, conformational changes in the NR complex lead to exclusion of the corepressors from the complex and the subsequent recruitment of coactivators (Driscoll et al., 1996; Egner et al., 2001; Leng et al., 1993; Luck et al., 2000; Muller et al., 1984; Yen et al., 1993) (**Figure 11**).

Three classes of coactivators have been described: histone acetyl transferases (HATs) (Blanco et al., 1998; Chen et al., 1997; Kraus et al., 1999; Li et al., 1999), histone methyl

transferases (HMTs) (Strahl et al., 2001; Xu et al., 2004), and histone kinases (Banks et al., 2001; Bhattacharjee et al., 2001). In general, coactivators play one or more of the following roles in nuclear receptor transcriptional activity: (1) they function as bridging factors to recruit cofactors to the DNA-bound NRs (Yao et al., 1996), (2) they acetylate nucleosomal histones and various transcription factors at the promoters of target genes (Blanco et al., 1998; Chen et al., 1997), (3) they function as bridging factors between NRs and the basal transcriptional machinery (TRAP/DRIP) (Lee and Lee Kraus, 2001; Muscat et al., 1998; Takeshita et al., 1996; Treuter et al., 1999).

Recruitment of coactivators or of components of the transcription machinery by the NR complexes is also modulated by phosphorylation events which, in cooperation with the ligand, modulate the transcriptional activity of the NRs (Rochette-Egly, 2003). NRs can be phosphorylated by kinases that are associated with general transcription factors (Chen et al., 2000; Rochette-Egly et al., 1997), or that are activated in response to a variety of extracellular signals (Adam-Stitah et al., 1999; Hoeck et al., 1989; Hsieh et al., 1991; Jurutka et al., 1996; Kato et al., 1992; Lee et al., 2000; Pekarsky et al., 2001; Rochette-Egly et al., 1997).

In addition, NRs can also modulate transcription by interacting with other general transcription factors. It has been shown that several NRs exhibit a potent antagonistic effect on the AP-1 family of transcription factors (Saatcioglu et al., 1997; Salbert et al., 1993). However, in some cases NRs have positive effects on AP-1 transcriptional activity (Hanley et al., 2000b; Huang et al., 2003; Tseng et al., 2003). The precise mechanism of NRs anti AP-1 activity is not clear, however two main mechanisms have been suggested. The first one involves the inhibition of AP-1 binding to its DNA response elements either by physical interaction between NRs and AP-1 complexes (Aslam et al., 1999; Delerive et al., 1999; Fanjul et al., 1994; Kuroki et al., 1995; Salbert et al., 1993), or by blocking the JNK-dependent AP-1 activation (Bruna et al., 2003; Caelles et al., 1997; Gonzalez et al., 2000) (Caelles et al., 1997; Zhou et al., 1999).

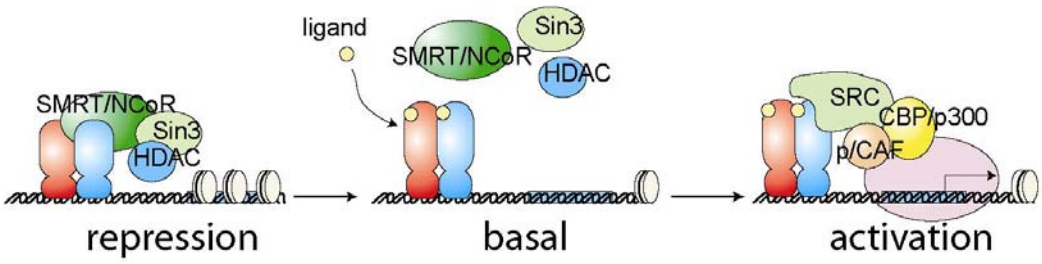

Figure 11. Mechanism of NR transcription regulation. Adapted from Chen (2000).

The second mechanism involves the competition for common cofactors required for both NRs and AP1 transcriptional activation such as CBP (Benkoussa et al., 2002; DiSepio et al., 1999; Horvai et al., 1997). NRs have been also shown to interact with the general transcription factor Sp1. NR/Sp1 interaction can have negative or positive effect on Sp1 transcriptional activity. For example ER, VDR, and PPARs can synergistically activate Sp1 mediated transcription (Husmann et al., 2000; Krey et al., 1995; Krishnan et al., 1994; Liu and Freedman, 1994; Porter et al., 1996), whereas TR inhibits Sp1-dependent transcriptional activation (Xu et al., 1993). Thus, NRs are ligand-activated transcription factors involved in a sophisticated and extremely complex hormonal signaling network, not only as hormone signal transducers, but also as integrators of multiple signaling pathways.

1.6 Nuclear Receptors in Skin Homeostasis and Skin Carcinogenesis.

Skin structure and homeostasis is affected by a number of hormonal signals such as sex hormones and vitamins. Differences between skin from man and woman for example are determined by the levels of estrogens and androgens (Rosenfield and Deplewski, 1995; Thornton, 2002; Tur, 1997). Vitamin A or vitamin D deficiency results in alterations of skin structure and function (Fisher and Voorhees, 1996; Pavlovitch et al., 1984). For several years, major efforts have been done to understand the mechanisms by which hormones affect skin homeostasis. Different experimental approaches such as in situ hybridization (ISH), reverse-transcription coupled polymerase chain reaction (RT-PCR), and immunohistochemistry (IHC), demonstrated the presence of various members of the NR superfamily in the skin (**table 5**). In this section, I will discuss some relevant in vitro and in vivo studies demonstrating that NRs play important roles in the maintenance of skin homeostasis. Because RXRs are heterodimeric partners for several non-steroidal nuclear receptors, and RXRα is the predominant RXR in the skin, I will focus on known functions of RXRα and its heterodimeric partners in skin homeostasis.

1.6.1 The Retinoic Acid Receptors

Retinoids and Skin Homeostasis

The observation that animals maintained in a vitamin A deficient diet exhibit epithelial alterations provided the first experimental evidence for the role of vitamin A in the maintenance of epithelial homeostasis (Bo, 1956; Bo, 1957; Hayes et al., 1970; Reiter, 1965). Clinical and experimental data have shown that vitamin A deficiency affects epithelial homeostasis of several organs including the eye (Pfister and Renner, 1978; Tseng et al., 1984), the cervix (Gijbels et al., 1992), the trachea (Lancillotti et al., 1992). Moreover, administration of exogenous vitamin A to experimental animals also results in alterations of epidermal homeostasis. Exogenous vitamin A stimulates proliferation of both adult and embryonic epidermis (Tsambaos et al., 1980; Tsambaos et al., 1985), induces expression of keratins commonly expressed in hyperproliferative epidermis such as K6, K16 and K17 (Eichner et al., 1992; Tsambaos et al., 1980; Tsambaos et al., 1985), and inhibits keratinocyte terminal differentiation and the formation of the cornified layer (Brown et al., 1985; Eichner, 1986; Eichner et al., 1992; Gibbs et al., 1996).

Table 5. Expression of NRs in the skin.

		Epidermal Keratinocyte	Dermal Fibroblasts	HF Ker	Dermal papilla	Melanocyte	Langerhans	References
RAR	α	+	+	+	+	NK	NK	(Billoni et al., 1997; Boehm et al., 2004; Krust et al., 1989a; Reichrath et al., 1997; Reichrath et al., 1995; Torma et al., 1993; Zelent et al., 1989a)
	β	-	+	+	+	+	NK	
	γ	+	+	+	+	NK	NK	
RXR	α	+	+	+	+	+	+	(Billoni et al., 1997; Krust et al., 1989a; Reichrath et al., 1997; Reichrath et al., 1995; Torma et al., 1993; Tsou et al., 1997; Yuspa, 1998; Zelent et al., 1989a)
	β	+	+	+	NK	NK	NK	
	γ	+	NK	+	NK	NK	NK	
VDR		+	NK	+	+	+	+	(Milde et al., 1991; Reichrath et al., 1995; Reichrath et al., 1994)
PPAR	α	+	+	+	NK	+	NK	(Billoni et al., 2000a; Kang et al., 2004; Rosenfield et al., 2000)
	β	+	NK	+	NK	+	NK	
	γ	+	NK	+	NK	+	NK	
LXR	α	+	NK	NK	NK	NK	NK	(Fowler et al., 2003; Kitajima et al., 1992; Wirth et al., 1987)
	β	+	NK	NK	NK	NK	NK	
ER	α	+	+	NK	NK	NK	NK	(Brandenberger et al., 1997; Jee et al., 1994)
	β	+	+	NK	NK	NK	NK	
AR		+	+	NK	NK	NK	NK	(Blauer et al., 1991; Keenan et al., 1975; Liang et al., 1993; Yuspa, 1998)
TR		+	+	+	NK	NK	NK	(Billoni et al., 2000b; Ichikawa et al., 1987; Torma et al., 2000; Torma et al., 1993)

NK=no known; (+) = expressed; (-) = not expressed.

In agreement with these results, RA stimulates proliferation and inhibits terminal differentiation of keratinocytes in culture (Gibbs et al., 1996; Varani et al., 1989), indicating that RA affects epidermal homeostasis, at least in part, by directly modulating keratinocyte proliferation and differentiation.

The Retinoic Acid Receptors in the Skin

Expression of retinoic acid receptors in the skin was first demonstrated by northern blot and RT-PCR analysis. These experiments demonstrated the presence of RARα, RARγ and RXRα mRNAs in both normal skin and kertinocytes in culture (Elder et al., 1992; Torma et al., 1993). It was also shown that the most abundant transcripts in normal skin are those for RXRα and RARγ (Torma et al., 1993). In agreement with data obtained from RNA analysis, western blot and immunohistochemical (IHC) analysis demonstrated that RARα, RARγ and RXRα proteins are present in the skin, and that RXRα and RARγ are the predominant isotypes (Fisher et al., 1994). In addition, it was shown that RXRβ and RXRγ are also expressed in normal skin (Reichrath et al., 1997; Sherman and Partridge, 1997). Different experimental approaches demonstrated that protein levels of RXRs are 5 times higher than those of RARs (Fisher et al., 1994), and that in several cell types of normal skin, including keratinocyte, melanocyte, FB and dendritic cells, RXRα exhibit the highest levels of expression (Reichrath et al., 1995). RXRα is also the main isotypes in the hair follicle (Billoni et al., 1997).

In the early 90's, a functional role of retinoic acid receptors in keratinocytes was demonstrated by transfection experiments, in which expression of different retinoic acid receptor isotypes resulted in inhibition of the expression of several keratins (Blumenberg et al., 1992; Eichner et al., 1992; Tomic et al., 1990). Moreover, it was shown that introduction of a truncated RARγ protein, lacking the A/B and the DBD domains, into keratinocytes in culture inhibits their ability to undergo terminal differentiation (Aneskievich and Fuchs, 1992). Cotransfection of human keratinocytes with expression vectors for dominant negative mutant retinoic acid and retinoic X receptors reduced the retinol-induced activation of a retinoic acid receptor-dependent reporter gene (Kurlandsky et al., 1994).

Targeted disruption of the RARα (Lufkin et al., 1993), RARβ (Mendelsohn et al., 1994) or RARγ (Lohnes et al., 1993) genes in the mouse resulted in no apparent defects in skin development or in the maintenance of adult skin homeostasis. However, using RARα and RARγ null keratinocytes in culture, Goyette and co-workers showed that both RARα and RARγ mediate RA-induced changes in keratinocyte gene expression (Goyette et al., 2000). Moreover, they demonstrated that RARγ is the principal receptor contributing to all- trans-retinoic acid (RA)-mediated growth arrest in this system. Furthermore, transgenic mice in which a dominant negative form of RARα is expressed in the basal layer of the epidermis exhibited abnormalities in skin development (Saitou et al., 1995) and defects in the formation of cornified layer, due to altered lipid processing, resulting in abnormal water loss

(Attar et al., 1997; Imakado et al., 1995). Similarly, RXRβ null (Kastner et al., 1996) or RXRγ null mice (Krezel et al., 1996) have no defects in skin development or in the maintenance of adult skin homeostasis. Moreover, RXRα$^{+/-}$/RXRβ–null/RXRγ-null mice are viable and do not exhibit evident skin defects, indicating that one copy of the RXRα alleles is sufficient to exert most of the RXRs functions during development and for the maintenance of skin homeostasis in adult mice (Krezel et al., 1996). In contrast, mice carrying a null mutation of the RXRα gene die in utero at 14.5 dpc (Kastner et al., 1994). Therefore, to investigate the role of RXRα in adult mouse skin, Feng and co-workers generated transgenic mice in which a dominant negative form of RXRα (dnRXRα) is expressed in the suprabasal keratinocytes of the epidermis (Feng et al., 1997). They showed that suprabasal expression of the dnRXRα results in loss of RAR-induced basal keratinocyte proliferation but does not affect normal keratinocytes proliferation and differentiation.

Using a spatio- temporally-controlled targeted somatic mutagenesis system, Chapellier and co-workers demonstrated that selective ablation of RARα, RARγ or RXRα in keratinocytes of the suprabasal (SB) layers of mouse epidermis does not affect epidermal homeostasis (Chapellier et al., 2002). However, they found that mice lacking RARγ in SB keratinocytes and mice lacking RXRα in all epidermal layers, exhibited impaired response to RA-induced basal keratinocyte proliferation, suggesting that RXRα/RARγ heterodimers in SB keratinocytes mediate RA effect on basal keratinocyte proliferation via paracrine signals (Chapellier et al., 2002). This results, together with those obtained by Goyette and co-workers, using mutant keratinocytes in culture (Goyette et al., 2000), and by Feng and co-workers, using dnRXRα transgenic mice, indicate that RXRα/RARγ heterodimers in SB keratinocytes are the mediators of RA effects in epidermal keratinocytes, but play a minor role in normal skin homeostasis.

RXRα is the main retinoic acid receptor isotype expressed in the basal keratinocytes of the epidermis and, as a heterodimeric partner for other nuclear receptors, it may play a key role not only in response of RA, but also in other signaling pathways affecting keratinocyte proliferation and differentiation. To study the role of RXRα in basal keratinocytes, the laboratory of Professor Pierre Chambon and Dr. Daniel Metzger generated transgenic mouse lines in which a constitutively active or a tamoxifen-inducible Cre recombinase are expressed selectively in the basal keratinocyte of the epidermis (Indra et al., 2000; Indra et al., 1999). Using these basal keratinocyte specific Cre transgenic lines they showed that selective ablation of RXRα alleles in epidermal keratinocytes of adult mice (RXRα$^{ep-/-}$ mice) results in progressive hair loss, hair follicle degeneration, epidermal hyperplasia, and skin inflammation (**figure 12**). They also demonstrated that RXRα plays a key role in anagen initiation during hair cycle and in the control of epidermal keratinocyte proliferation and terminal differentiation (Li et al., 2001b; Li et al., 2000). All together, these data demonstrate that keratinocytic-RXRα plays a key role in the maintenance of skin homeostasis.

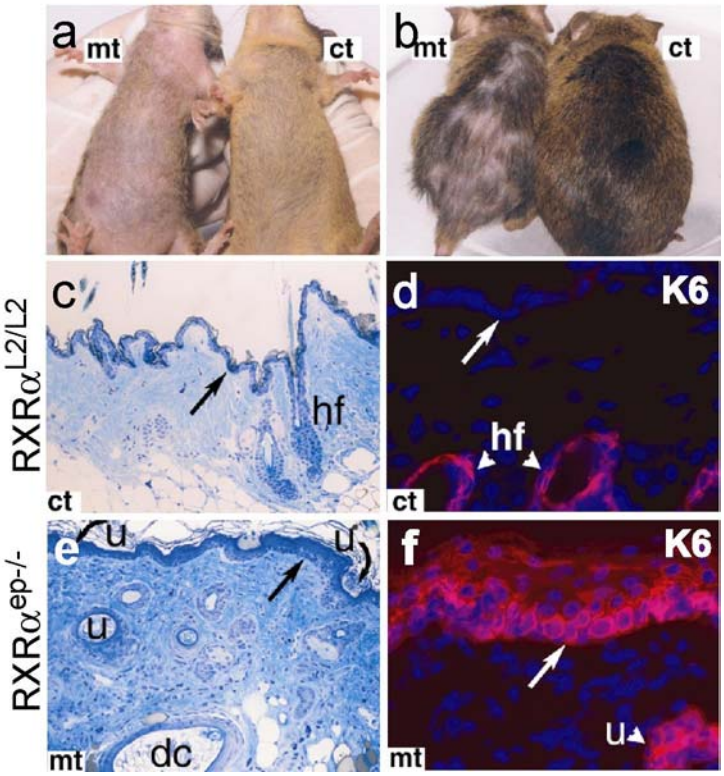

Figure 12. Phenotype of RXRα$^{ep-/-}$ mice. Ventral (a) and dorsal (b) view of RXRα$^{ep-/-}$ and RXRα$^{L2/L2}$ mice. (c,e) Hematoxylin-eosine staining. (d,f) Immunohistochemical analysis of K6 expression. Adapted from Li et al. (2000).

1.6.2 The Vitamin D Receptor

Vitamin D and skin homeostasis.

Vitamin D_3 (VD_3) plays an important role in the control of epithelial homeostasis (Bikle, 1996; Gamady et al., 2003; Kitano et al., 1991; Pavlovitch et al., 1984; Pavlovitch et al., 1981; Pillai et al., 1988b; Smith et al., 1986; Su et al., 1994; van de Kerkhof, 1995). VD_3 is absorbed from the diet (from fish oils, eggs yolk and liver, in the form of cholecalciferol) or produced in the skin by epidermal keratinocytes in response to UV radiation (Bikle, 1995; Bikle et al., 1986; Holick, 1987; Holick et al., 1981; Nemanic et al., 1983; Pillai et al., 1988a; Su et al., 1994). VD_3 deficiency results in alterations in calcium metabolism that give rise to bone malformations, rickets, and decrease in epidermal thickness (Fitzpatrick et al., 2000; Holick, 2003; Hughes et al., 1991; Kolb, 1986; Pavlovitch et al., 1984). In the epidermis, calcium plays an important role in the control of keratinocyte differentiation. It has been shown that VD_3 can affect keratinocyte differentiation by controlling their calcium uptake (Bikle, 1996; Bittiner et al., 1991; Jones and Sharpe, 1994; Pillai et al., 1988b; Ratnam et al., 1999; Rougui et al., 1996; Su et al., 1994). Experimental data demonstrate that VD_3 induces growth arrest of keratinocytes in culture (Kitano et al., 1991; McLane et al., 1990), and that

topical application of VD_3 on psoriatic lesions reduces the number of proliferative cells (Kitano et al., 1991). Thus, VD_3 has pro-differentiation and anti-proliferative effects in epidermal keratinocytes, which is most likely related to the effect of VD_3 on keratinocyte calcium metabolism (Bikle, 1995; Su et al., 1994). Thus, epidermal keratinocytes are not only a source of VD_3, but also a target of its action.

The Vitamin D Receptor in the Skin

VDR is expressed in all layers of the epidermis and in the hair follicle (Milde et al., 1991; Reichrath et al., 1996; Reichrath et al., 1994). The observation that VDR expression in the hair follicle varies during different stages of the hair cycle (Reichrath et al., 1994), suggested that VDR may play an important role in hair cycling. To study the role of VDR in vivo, Yoshizawa and co-workers generated a mice in which a null mutation of the VDR gene was introduced (Yoshizawa et al., 1997). VDR null mice exhibited a progressive alopecia and degeneration of the hair follicles (Li et al., 2000; Yoshizawa et al., 1997), which results from a defect in the initiation of the hair cycle (entrance to anagen) (Sakai and Demay, 2000). The hair cycle defect leading to the development of alopecia in VDR null mice can be prevented by restoration of VDR expression in epidermal keratinocytes (Chen et al., 2001), but not by the normalization of mineral ion homeostasis (Amling et al., 1999), demonstrating that VDR is required in keratinocytes for normal hair cycle. Interestingly, using hair-reconstitution assays in nude mice, Sakai and co-workers demonstrated that hair cycle defects in VDR null mice are the result of abnormal epidermal-mesenchymal interactions due to an impairment in the capacity of keratinocytes to respond to dermal signals (Sakai et al., 2001). The hair cycle defects in VDR null mice are similar to those observed in $RXR\alpha^{ep-/-}$ mice, suggesting that RXRα/VDR heterodimers in keratinocytes play a major role in the control of hair cycle (Li et al., 2001b; Li et al., 2000).

In contrast to its role in hair cycling, VDR seem to play a minor role in the control of keratinocyte proliferation and differentiation. In 2000, Sakai and Demay reported that the epidermis of VDR null mice was indistinguishable from that of wild-type mice, and that VDR null keratinocytes in culture proliferate and differentiate normally (Sakai and Demay, 2000). However, in a more detailed study Xie and co-workers showed that VDR null mice exhibit defects in the expression of keratinocyte differentiation markers such as fillagrin, involucrin, and loricrin, and the presence of abnormal granules in the granular layer (Xie et al., 2002). These results suggest that in addition to its role in the control of hair cycle, VDR is also involved in the regulation of keratinocyte differentiation.

1.6.3 The Peroxisome Proliferator Activated Receptors

Fatty acids are structural components of the cornified layer of the epidermis (Grubauer et al., 1989). It has been shown that synthesis of cholesterol and fatty acid by keratinocyte is required for the formation and maintenance of the epidermal permeability barrier (Feingold

et al., 1990; Mao-Qiang et al., 1993; Yang et al., 1995; Zettersten et al., 1997). For a long time, fatty acids were considered as "simple" structural components of the cornified layer. However experimental data demonstrated that several lipid species can regulate keratinocyte growth and differentiation. Ceramides for example, induce differentiation and inhibit proliferation of keratinocytes in culture (Geilen et al., 1997; Jung et al., 1998; Pillai et al., 1996), whereas topical applications of glucosylceramides stimulate keratinocyte proliferation in the epidermis of hairless mice (Marsh et al., 1995). These data suggested that beside their function as structural components of the epidermis, fatty acids may also play a role as modulators of keratinocytes function.

The Peroxisome Proliferator Activated Receptors (PPARs) were initially described as nuclear receptors that can be activated by synthetic compounds called peroxisome proliferators (Dreyer et al., 1993; Dreyer et al., 1992; Green, 1992; Issemann and Green, 1990). Three PPAR isotypes, PPARα, PPARβ and PPARγ, have been identified (Dreyer et al., 1992; Fajas et al., 1998; Fajas et al., 1999). Interestingly, PPARs can be activated by a variety of fatty acids (Dreyer et al., 1993; Keller et al., 1993; Schmidt et al., 1992). Very little was known about the role of fatty acids on the regulation of keratinocyte proliferation and differentiation before the demonstration that PPARs are expressed in the epidermis. The three PPAR isotypes are expressed in mouse epidermis during fetal development and their expression decreases progressively from the interfollicular epithelium after birth (Michalik et al., 2001). In adult skin, PPARα and PPARβ are the prominent isotypes, and it has been shown that expression of PPARα and PPARβ is increased in adult epidermis after various stimuli inducing keratinocyte proliferation and differentiation (Braissant et al., 1996; Braissant and Wahli, 1998; Michalik et al., 2001).

Experiments using skin explants of rat embryos demonstrated that PPARα ligands stimulate differentiation of fetal epidermal permeability barrier (Hanley et al., 1997). In adult mice, topical application of PPARα ligands stimulate differentiation of epidermal keratinocytes in wild-type animals, but not in PPARα null mice (Hanley et al., 2000a; Komuves et al., 2000). In line with this observations, PPARα activators induce an increase in involucrin mRNA levels in cultured human keratinocytes (Komuves et al., 2000). These data suggest that PPARα is involved in the control or keratinocyte differentiation. However, PPARα null mice did not exhibit skin defect, indicating that PPARα plays a minor role in healthy skin. In agreement with the observation that PPARα expression is reactivated in the skin in response to stress, PPARα null mice exhibited retarded wound healing (Michalik et al., 2001), indicating that PPARα is important for re-epithealization of skin wounds.

A role for PPARβ in the control of keratinocyte differentiation has been shown by experiments using keratinocytes in culture in which activators of PPARβ stimulate differentiation (Matsuura et al., 1999; Schmuth et al., 2004; Tan et al., 2001; Westergaard et al., 2001). Comparison of PPARβ heterozygous mice with wild-type control animals revealed a significant increase in the keratinocyte proliferation rate but no evident

phenotypic changes in the epidermis of adult PPARβ mutant mice (Michalik et al., 2001). However, PPARβ null mice exhibit an increased hyperplastic response to TPA treatment, and a retardation in the healing of skin wounds (Michalik et al., 2001; Peters et al., 2000). Interestingly, PPARβ null keratinocytes in culture exhibit impaired adhesion and migration capacities (Michalik et al., 2001), and impaired response to inflammatory-cytokines induced differentiation (Di-Poi et al., 2002; Michalik et al., 2001; Tan et al., 2001). Thus, PPARβ plays key roles in the response of the skin to stress.

Studies in cell culture systems demonstrated that PPARγ ligands inhibit the growth (Ellis et al., 2000) and enhance calcium-induced differentiation of cultured keratinocytes (Evans et al., 1993). In rat skin explants, PPARγ ligands exhibit no effect on the development of skin barrier function or epidermal morphology (Hanley et al., 1997). In adult mice, topical application of PPARγ activators to mouse skin stimulates epidermal proliferation, differentiation, and apoptosis, which results in no net changes in epidermal morphology (Mao-Qiang et al., in press). However, PPARγ activators inhibit TPA-induced cutaneous inflammation, and improve permeability barrier recovery after mechanical barrier disruption (induced by sequential applications of cellophane tape) (Mao-Qiang et al., in press).

Thus, the three PPAR isotypes seem to play a role in the maintenance of skin homeostasis, in particular in response to injury. Because the absence of a single receptor has only minimal effects in skin homeostasis, there might be functional compensations between the three receptor isotypes.

1.6.4 The Liver X Receptors

Liver X Receptors (LXRs) were first identified as orphan nuclear receptors that interact with the RXRs (Miyata et al., 1996; Willy et al., 1995). LXRs are activated by oxidative derivatives of cholesterol called oxysterols. Recently, it has been demonstrated that both LXR isoforms, LXRα and LXRβ, are expressed in keratinocytes in culture (Hanley et al., 2000b) and in fetal skin (Komuves et al., 2002), but only LXRβ has been detected in adult mouse epidermis (Komuves et al., 2002). Epidermal keratinocytes can synthesize oxysterols de novo (Thiboutot et al., 2003), suggesting that activation of LXRs in keratinocytes may play a role in epidermal maturation and homeostasis. The role of LXRs in epidermal homeostasis is further supported by the observations that oxysterols stimulate keratinocyte differentiation both in culture and adult mice skin (Hanley et al., 2000b; Hanley et al., 2001; Komuves et al., 2002), and that oxysterols and LXR synthetic ligands inhibit both TPA-induced (irritant dermatitis) and oxazolone-induced (allergic dermatitis) inflammation by a mechanism involving both LXRα and LXRβ (Fowler et al., 2003). LXRα null mice do not exhibit any epidermal alterations, but LXRβ null mice and LXRα/LXRβ null mice exhibit a slight decrease in the expression of epidermal differentiation markers, and thinning of the epidermis, but not significant functional cutaneous abnormalities (Fowler et al., 2003; Komuves et al., 2002). These data suggest that LXRs may play important roles in the skin in response to stress.

Thus, several non-steroidal NRs including RXRs, RARs, VDR, PPARs and LXRs are expressed in the skin and have overlapping and independent roles in skin homeostasis. RXRα, the predominant RXR receptor in the skin and a heterodimeric partner for other non-steroidal **NRs**, seem to play a central role as an integrator (regulator) of several signaling pathways. Based on the data available to date, a model can be proposed in which specific heterodimers participate selectively in a given process. RXRα/RARs (mainly RXRα/RARγ) are involved in the control of keratinocyte proliferation and differentiation, in particular in response to excess of RA. RXRα/VDR heterodimers play an important role in the control of hair cycling and probably in the regulation of keratinocyte differentiation. RXRα/PPARs seem to play an important function in response to stress, for example during wound healing and after exposure to UV-radiations. Finally, RXRα/LXRs may participate in the control of keratinocyte differentiation and skin inflammation (**figure 13**).

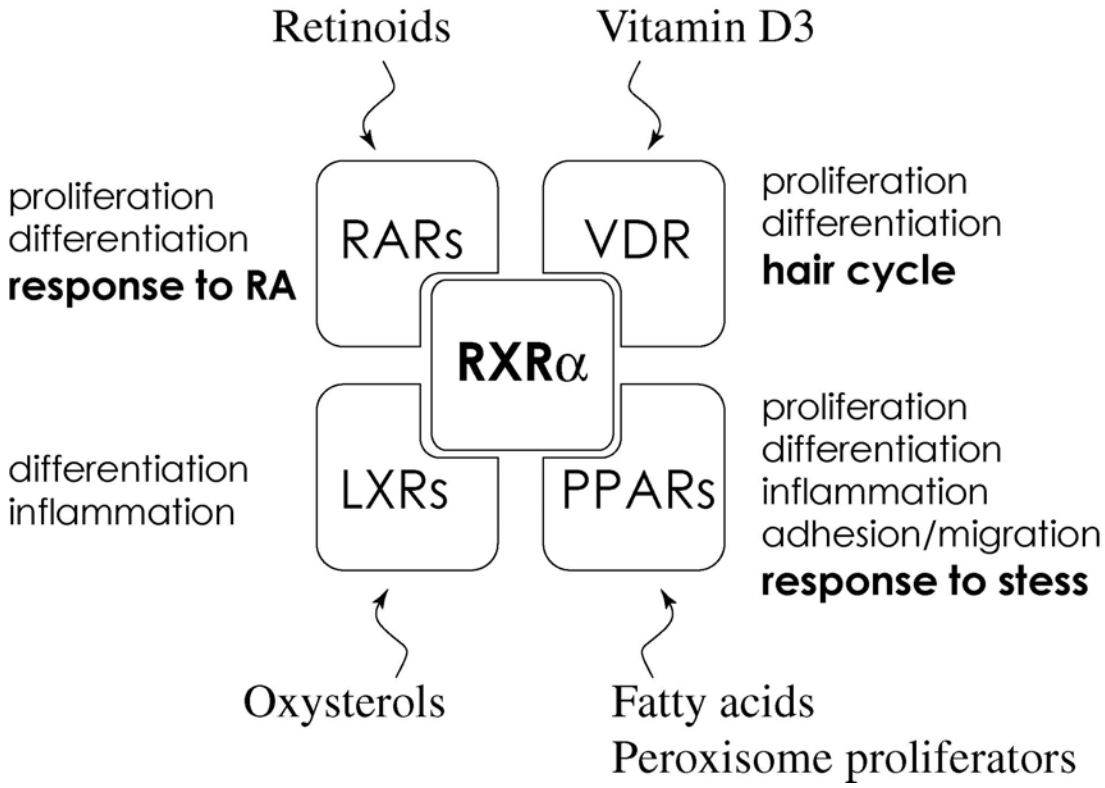

Figure 13. Model for integration of nuclear receptors action in the skin.

1.6.5 Nuclear Receptors and Skin Carcinogenesis

The Retinoic Acid Receptors

In the early 70's, a series of studies by Bollag and co-workers showed that vitamin A has a protective effect against skin tumor formation in mice (Bollag, 1971; Bollag, 1972; Bollag, 1974; Bollag, 1975). During the last three decades a number of experimental and clinical data demonstrated that retinoids can efficiently inhibit skin tumor formation, tumor growth, and malignant progression. Using mouse models in which skin tumors are induced by topical application of chemical carcinogens (Athar et al., 1991; De Luca et al., 1993; Verma et al., 1980; Weeks et al., 1979) or by exposure to UV radiations (Athar et al., 1991), several groups have shown that natural or synthetic retinoids exhibit a protective effect against skin tumor formation. Retinoic acid treated mice develop less and smaller tumors than non treated mice (De Luca et al., 1993; De Luca et al., 1996; Gensler et al., 1987; Verma et al., 1979; Weeks et al., 1979), and the rate of malignant conversion is reduced in retinoid-treated mice (Chen et al., 1994a; Chen et al., 1994b; Chen et al., 1995; De Luca et al., 1993; De Luca et al., 1996).

Early clinical trials showed that retinoids exhibit a protective effect in the treatment of melanoma (Levine and Meyskens, 1980) and non-melanoma skin cancers (Lippman et al., 1988; Lippman et al., 1987a; Lippman et al., 1987b). Larger clinical trials demonstrated that administration of retinol decreases the probability of developing secondary SCC of the skin (Moon et al., 1997), and has a preventive effect in patients with a high risk to develop skin cancer (DiGiovanna, 2001) such as xeroderma pigmentosum patients (Anolik et al., 1998; Giannotti et al., 2003; Saade et al., 1999), organ transplantation recipients (George et al., 2002; McNamara et al., 2002; Smit et al., 2004), and patients with Kaposi sarcoma (Aboulafia et al., 2003; Bernstein et al., 2002; Miles et al., 2002). Moreover, it was demonstrated that retinoids induce regression of some dysplastic nevus, the precursors of melanoma, to benign nevi in humans, suggesting that retinoids may also have benefic effect on the treatment of melanoma (Meyskens et al., 1986).

The precise mechanism by which retinoids exert their anti tumor activity in the skin is not clear. However it has been demonstrated that retinoids inhibit growth of HSCC cell lines (Braakhuis et al., 1997; Cooper et al., 1997; Klaassen et al., 2001; Lotan, 1993), and induce apoptosis of HSCC (Braakhuis et al., 1997; Cooper et al., 1997; Giandomenico et al., 1998; Hail and Lotan, 2001; Islam et al., 2000; Liaudet-Coopman et al., 1997; Lotan, 1996) and melanoma cells lines (Danielsson et al., 1999). Interestingly, is has been shown that retinoids have differential effects on normal and malignant keratinocytes. Whereas in the former it induces growth arrest, in the later it induces apoptosis (Hail and Lotan, 2001).

RXRα and RARγ are the main retinoic acid receptors in the skin. In situ hybridization studies on samples of human normal skin, and AK and SCC lesions, showed a progressive decrease

in the transcript levels of RARα and RARγ, and all three RXRs, which is associated with the progression of squamous skin carcinogenesis (Xu et al., 2001). In agreement with these observations, it has been shown that RARα and RARγ mRNA levels are gradually decreased in epidermal keratinocytes during mouse skin carcinogenesis (Darwiche et al., 1995). Interestingly, overexpression of an oncogenic Ha-ras in keratinocytes resulted in a decrease of RARα and RARγ protein levels and transactivation activity (Darwiche et al., 1996). Moreover, blocking RARγ expression in HNSCC cells by antisense RNA resulted in decreased RA-induced growth arrest (Oridate et al., 1996), and overexpression of RARα or RARγ into neoplastic mouse epidermal cells, with low endogenous levels of these receptors, resulted in an increased RA-induced growth arrest and apoptosis (Hatoum et al., 2001). Recently, is has been shown RARγ null, but not RARα null keratinocytes, carrying an activated v-Ha-ras oncogene, formed squamous cell carcinoma when grafted into nude mice (Chen et al., 2004). Furthermore, the expression of RXRα and RARβ in melanoma cells is lower than in normal melanocytes or nevus cells (Boehm et al., 2004), suggesting that expression of RXRα and RARβ is lost during melanoma formation. All together, the above data strongly suggest that RA receptors play an important role in the development of skin cancers and in the anti-tumor effects of retinoids.

The Vitamin D receptor

Vitamin D has anti-proliferative and pro-differentiating properties on epidermal cells, and is an interesting candidate for the treatment of skin cancer. Studies of chemically-induced skin carcinogenesis in mice demonstrated that VD_3 partially blocks tumor formation when topically applied on the back skin of experimental mice (Chida et al., 1985; Pence et al., 1991; Wood et al., 1983). Vitamin D_3 also has antiproliferative (Enepekides et al., 1999) and pro-apoptotic effects on squamous cell carcinoma cells in culture (Bernardi et al., 2002; McGuire et al., 2001; Satake et al., 2003). Furthermore, VD_3 can induce growth arrest (Evans et al., 1996) and inhibit invasiveness of malignant melanoma cells in vitro and in vivo models (Yudoh et al., 1999). However, clinical use of VD_3 is not yet validated because of its strong hypercalcemic effects. Synthetic analogs of VD_3 with anti-proliferative pro-differentiating effect but with low calcemic activities have been developed (Brown et al., 1990; Hansen et al., 2000; Mathiasen et al., 1993; Posner et al., 2001; Posner et al., 2002; Posner et al., 1998). Several of this compounds exhibit enhanced anti-proliferative and pro-differentiating effects on cancer cells in culture (Akutsu et al., 2001; Hansen and Maenpaa, 1997; Koshizuka et al., 1999; Prudencio et al., 2001). They can induce growth arrest (Posner et al., 2001) and apoptosis (Danielsson et al., 1998; Danielsson et al., 1997) in melanoma cell lines in culture, and it has been demonstrated that they partially inhibit chemical carcinogenesis in vivo (El Abdaimi et al., 1999; Kensler et al., 2000). Interestingly, it has been shown that vitamin D in combination with retinoids exhibit synergistic effects in the induction of apoptosis on melanoma cells (Danielsson et al., 1999), the suppression of SCC growth (Enepekides et al., 1999), and the inhibition of tumor-induced angiogenesis in nude mice (Majewski et al., 1995; Majewski et al., 1993).

The vitamin D receptor is expressed in several cell types in the skin, where it plays a key role in the control of hair cycle and keratinocyte differentiation. A link between the susceptibility to develop skin cancer and the function of the VDR has been established from the observations that VDR expression is higher in BCC lesions compared to normal skin (Reichrath et al., 1999b) and that polymorphisms of the VDR gene, which would be expected to result in impaired function, are associated with susceptibility and prognosis in malignant melanoma (Hutchinson et al., 2000b). Recently, Zinser and co-workers demonstrated that pre-treatment of VDR null mice with medroxyprogesterone acetate (PMA), a synthetic progesterone analog that enhances epithelial proliferation, followed by oral administration of the carcinogen DMBA results in spontaneous development of skin tumors in 85% of treated animals (Zinser et al., 2002).

The Peroxisome Proliferator Activated Receptors

The three PPAR isotypes play important roles in skin homeostasis. They seem to be particularly important under conditions of stress such as wound healing and after exposure to UV radiations. However, their role during skin carcinogenesis has been poorly investigated. It has been shown that PPARα ligands possess receptor mediated, anti-inflammatory activities in both irritant and allergic contact dermatitis animal models (Sheu et al., 2002), and that they can reverse expression of inflammatory cytokines induced by UVB in keratinocytes in culture (Kippenberger et al., 2001). Interestingly, activators of PPARα partially inhibit tumor formation in a two step chemical carcinogenesis protocol (Thuillier et al., 2000). Moreover, PPARγ mediates the non-steroidal anti-inflammatory drugs (NSAID) antineoplastic effect on human oral squamous carcinoma cells (Nikitakis et al., 2002), and PPARγ specific ligands can block melanoma cell growth in culture (Mossner et al., 2002). Recently, Nicol and co-workers demonstrated that, in response to a chemical carcinogenesis protocol, PPARγ+/- mice exhibit an increased number of tumors per mouse when compared to wild-type mice (Nicol et al., 2004). Similarly, Kim and co-workers showed that PPARβ null mice subjected to a chemical skin carcinogenesis protocol exhibited an enhanced onset of tumor formation, tumor size and tumor multiplicity when compared to wild-type mice (Kim et al., 2004). Thus, RXRα and its heterodimeric partners may play important roles in the development of skin cancer, but their precise role is not clear.

2. Results

2.1 Malignant transformation of DMBA/TPA-induced papillomas and melanocytic growths in the skin of mice selectively lacking RXRα in epidermal keratinocytes (Manuscript in preparation)

Indra A.K.[1], Castaneda, E.[1], Antal, M.C.[2], Jiang, M.[1], Messaddeq, N.[1], Gariglio, P.[3], Kato, S.[4], Wahli, W.[5], Desvergne, B.[5], Metzger, D.[1,2] and Chambon, P.[1, 2*].

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire (CNRS, INSERM, ULP, Collège de France); [2]Institut Clinique de la Souris, B.P.10142, 67404, ILLKIRCH, Strasbourg, France; [3]Depto. Genetica y Biol. Mol. Cinvestav-IPN, Mexico; [4]Institute of Molecular & Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan;[5]Unité CIG Science, Centre Intégratif de Génomique (CIG) UNIL, Lausanne

*Corresponding author
IGBMC, BP 163 67404 Illkirch Cedex, France
(Tel: 33 +3 88 65 32/13/15/ ; Fax : 33 +3 88 65 32 03
e-mail: chambon@igbmc.u-strasbg.fr)

Short title : Role of RXRα in skin tumorigenesis

Key words: conditional targeted somatic mutagenesis, RXRα, epidermis, skin cancer, chemical carcinogenesis, DMBA/TPA, carcinoma, melanoma, mouse.

Summary:

Ligands for several nuclear receptors, including those of Retinoid X Receptors (RXRs), Retinoic Acid Receptors (RARs), Peroxisome Proliferator-activated Receptors (PPARs) and Vitamin D3 Receptor (VDR) have a suppressive effect on epidermal tumorigenesis. RXRs, the obligatory heterodimeric partners for RARs, PPARs, VDR and Liver X receptors (LXRs), and in particular RXRα, the major RXR isotype expressed in the epidermis, could be critical in the control of skin tumorigenesis. We show that adult mice, in which RXRα was selectively ablated in keratinocytes, subjected to the two-step skin tumorigenesis protocol, develop an increased number of epidermal tumors, which progress at a much higher frequency to malignant carcinoma, thus establishing a tumor modifier role for this receptor. Moreover, as under similar conditions, RXRα mutant mice also develop a higher number of melanocytic growths, some of which degenerate into melanoma, RXRα-ablated epidermal keratinocytes might control in a paracrine manner melanocyte proliferation and their malignant progression. Finally, our data indicate that PPARγ, PPARα, LXRβ and VDR differentially act as skin tumor modifiers.

Introduction :

The incidence of skin cancers is growing at an alarming rate. Melanoma incidence and mortality rate increases are among the highest of all cancers. While mortality due to non-melanoma skin cancers is lower, squamous cell carcinomas (SCC) are invasive and more than 10% metastasize (Kwa et al., 1992; Stratton et al., 2000).
Retinoids (principally all-trans retinoic acid, the active vitamin A derivative) are involved in the control of growth and differentiation of normal, premalignant and malignant cell types (Altucci and Gronemeyer, 2001). Their effects are mediated through their binding to heterodimers formed between the members of two families of nuclear receptors (NRs), the retinoic acid (RA) (RARα, β and γ) and retinoid X

(RXRα, β and γ) receptors (Chambon, 1996; Mangelsdorf et al., 1995 and Refs therein). Vitamin A deficiency in humans and animal models is associated with epithelial squamous metaplasia prone to malignant conversion (Hong and Itri, 1994; Moon et al., 1994), and aberrant expression and function of RARs have been found in carcinomas, as well as in premalignant lesions (Sun and Lotan, 2002). Natural and synthetic retinoids are used for the treatment of skin disorders including psoriasis and acnea (Livrea, 2000), as well as chemopreventive agents for epidermal cancers (Altucci and Gronemeyer, 2001; Sun and Lotan, 2002). Altered expression of RARs and RXRs found during carcinogenesis may reduce the ability of epidermal cell to respond to RA and contribute to carcinoma development, both in mouse (Darwiche et al., 1995; Darwiche et al., 1996) and human (Sun and Lotan, 2002). In mice, topical application of RA inhibits papilloma formation and dietary RA has been effective in preventing tumor formation and malignant conversion of chemically-induced mouse skin papillomas (De Luca et al., 1996,; Tennenbaum et al., 1998 and refs therein).

The two-step chemical tumorigenesis mouse model has been extensively used to study initiation, promotion and progression (malignant conversion) of skin tumors, as the evolution of human squamous cell carcinoma (SCC) has many similarities with chemically-induced squamous cell tumors in the mouse (Yuspa, 1998, and refs there in). The initiation stage is achieved through a single topical appplication of 7,12-dimethyl-benz[a]anthracene (DMBA) which is known to result in an activating transversion mutation (A^{182}->T) in the second base of codon 61 of the c-Ha-ras gene (Yuspa, 1998, and refs therein). The second stage, promotion, is accomplished by repeated topical treatment with a tumor promoter, such as the phorbol ester 12-O-tetradecanoylphorbol-13 acetate (TPA), which causes a sustained hyperplasia and inflammation, and then a selective clonal expansion into benign papillomas (Yuspa, 1998, and refs therein). The third stage, progression, is the malignant conversion of benign papillomas to invasive SCC. Cutaneous papillomas and SCCs induced by DMBA/TPA treatment, as well as human SCC, have been shown to contain the Ha-ras gene codon 61 (A^{182} -> T) mutation (Nelson et al., 1992, and refs therein) and targeted deletion of the Ha-ras gene drastically decreases the incidence of DMBA/TPA-induced epidermal papillomas (Ise et al., 2000). Interestingly, benign melanocytic dermal tumors (nevi) are induced upon a single topical DMBA

application. However, such nevi in which N-ras but not Ha-ras mutations at codon 61 have been found, do not progress to malignant melanomas (Husain et al., 1991).

RXRα, RXRβ, RARα and RARγ are expressed in human and mouse epidermis, and RXRα and RARγ are the predominant receptors (Chapellier et al., 2002; Fisher and Voorhees, 1996). The RXRα null mutation is lethal in utero between gestation days 13.5 and 16.5 (Kastner et al., 1994; Sucov et al., 1994), which precluded the genetic study of its physiological function in skin homeostasis. This study became possible by conditional inactivation of RXRα using the Cre/loxP technology and transgenic mice selectively expressing the tamoxifen-inducible Cre-ER^{T2} recombinase in the epidermal basal layer (Li et al., 2000; Metzger et al., 2003). RXRα ablation in epidermal keratinocytes of adult mice resulted in alopecia, epidermal interfollicular hyperplasia with keratinocyte hyperproliferation and aberrant terminal differentiation, accompanied by a skin inflammatory reaction (Li et al., 2001b; Li et al., 2000). Interestingly, RARs appear to be dispensible for homeostatic renewal of epidermal keratinocytes in the adult mouse, while the proliferative effect of topically-applied RA is transduced in suprabasal keratinocytes by RXRα/RARγ heterodimers, resulting in the release of a paracrine signal, which in turn stimulates the proliferation of basal keratinocytes (Chapellier et al., 2002). Importantly, RXRs are also known to heterodimerize with other members of the nuclear receptor superfamily (e.g. PPARs, VDR and LXRs, see (Laudet, 2002)) that are expressed in the epidermis (Fowler et al., 2003; Li et al., 2000; Mao-Qiang et al., 2004; Schmuth et al., 2004, and refs. therein; Sheu et al., 2002). Ligands of PPARs and VDR have been shown to inhibit the growth of squamous cell carcinoma cell lines in vitro and multistage skin tumorigenesis in vivo (Kensler et al., 2000; Kopelovich et al., 2002; Nikitakis et al., 2002) and those of LXRs display anti-inflammatory activity against TPA induced cutaneous inflammation (Fowler et al., 2003). Thus, RXR(s) and in particular RXRα, could to be critical in transducing cellular signals controlling skin carcinogenesis.

To investigate the possible role of keratinocytic RXRα in papilloma formation and their malignant conversion, as well as in the formation of melanocytic growths and their progression to melanoma, we have subjected adult mice, in which RXRα was

selectively ablated in keratinocytes, to the chemical two-step tumorigenesis protocol. Upon DMBA/TPA treatment these mutant mice exhibited an increased number of epidermal tumors which were also bigger in size and progressed with a much higher frequency to malignant carcinoma, thus establishing a role of RXRα in tumor suppression. We also show that these DMBA/TPA-treated mutants develop melanocytic growths, which degenerate into melanoma and metastasize to distal lymph nodes, and appear to be induced in a paracrine manner by signals emanating from the RXRα-ablated epidermal keratinocytes. Furthermore, using mice bearing either a VDR-null mutation or a selective ablation of PPARγ in their epidermal keratinocytes, we also investigated the role of these NRs as possible partners of RXRα in tumor suppression.

Results :

Selective ablation of RXRα in keratinocytes increases the formation and growth of epidermal tumors in DMBA/TPA treated mice

RXRα$^{ep-/-}$ mice (for epidermal keratinocyte-selective RXRα-null genotype) selectively lacking RXRα in keratinocytes of interfollicular epidermis and hair follicle outer root sheath, as well as control (CT) RXRα$^{ep-/+}$ and RXRα$^{L2/L2}$ mice carrying either one RXRα L^{-} null allele in their epidermal keratinocytes or two floxed RXRα L2 alleles in their germ line, were generated by tamoxifen (Tam) treatment of K14-Cre-ER^{T2}/RXRα$^{L2/L2}$, K14-Cre-ER^{T2}/RXRα$^{L2/+}$ and RXRα$^{L2/L2}$ littermates, respectively (see Experimental Procedures). As reported, RXRα$^{ep-/-}$ mice exhibited an epidermal keratinocyte hyperproliferation and abnormal differentiation (with keratin 6 expression), a skin inflammation, and developed an extensive alopecia within 10-15 weeks after RXRα ablation, whereas the skin and fur of CT mice were similar to those of WT mice (data not shown, see (Li et al., 2000; Metzger et al., 2003)).

To induce the formation of papilloma-like tumors, CT and RXRα$^{ep-/-}$ female mice were topically treated, two weeks after Tam administration, with a single dose of the tumor initiator DMBA, and then twice a week with the tumor promoter TPA for 25-30 weeks [RXRα$^{ep-/-(a)}$ mice, see Fig. 1A, and Experimental Procedures]. Seven to eight weeks after the start of TPA treatment, epidermal tumors developed in all CT and RXRα$^{ep-/-(a)}$ mice, and their number and size increased with time (Fig.1B, and data not shown). However, RXRα$^{ep-/-(a)}$ mice developed approximately twice as many tumors as CT mice [after 30 weeks, an average of 12 and 28 tumors were present in CT and RXRα$^{ep-/-(a)}$ mice, respectively (Fig. 1B & 1C)]. Moreover, CT tumors were never longer than 12 mm, whereas ~10 % and 3 % of the RXRα$^{ep-/-(a)}$ tumors were 12 to 16 mm and 30 to 40 mm long, respectively (Fig.1D, and data not shown). The number of tumors was 2-fold lower in males. However, RXRα$^{ep-/-(a)}$ males also exhibited twice as many tumors as CT littermates, and their size was also

increased (data not shown). All further analyses were performed on female mice. In all cases, DMBA initiation was required to generate these tumors, as none appeared in CT or RXR$\alpha^{ep-/-}$ mice treated with TPA alone (data not shown).

To investigate whether RXRα was involved in tumor initiation and/or promotion, mice were first treated with DMBA, and then for 7 weeks with TPA before Tam-induced RXRα selective ablation (RXR$\alpha^{ep-/-(b)}$ mice, Fig.1A). Tumors appeared 6 to 7 weeks after the start of TPA treatment in both CT and RXR$\alpha^{ep-/-(b)}$ mice, and by 30 weeks the latter had developed twice as many tumors as CT mice (Fig. 1E and F, and data not shown). Thus, the lack of RXRα in epidermal keratinocytes does not affect tumor initiation by DMBA, while it enhances tumor promotion. Accordingly, the H-ras codon 61 "activating" mutation that was previously identified in DMBA/TPA-induced epidermal tumors (see Introduction) was similarly present in both CT and RXR$\alpha^{ep-/-}$ tumors (data not shown).

Tumors generated in RXR$\alpha^{ep-/-}$ mice have a "high risk" of malignant conversion

Changes in keratin gene expression occurring during DMBA/TPA tumorigenesis have been used to classify the induced papillomas into "high" and "low" risk for malignant progression. Loss of expression of keratins K1 and K10 that are expressed in the differentiating suprabasal layers of normal epidermis and in "low risk" papillomas, and the concomitant appearance of K13 normally not expressed in skin, are used as indicators of early stages of malignant conversion (Nischt et al., 1988, and reference therein; Rundhaug et al., 1997). Immunohistochemistry of tumors (8-16 mm in length) taken 22 weeks after the start of DMBA/TPA treatment were performed using antibodies against keratins K5, K1, K10 and K13 (Nischt et al., 1988, and reference therein; Rundhaug et al., 1997) (Fig. 2A). K5, "normally" selectively expressed in the basal cell layer, was prominent in that layer and persisted throughout most of the upper (suprabasal) layers of highly stratified tumors from CT and RXR$\alpha^{ep-/-(a)}$ mice, but higher K5 levels were usually found in RXR$\alpha^{ep-/-(a)}$ tumors (Fig. 2A, a and b). K1 and K10, strongly expressed in suprabasal cell layers of more than 70 % of CT

tumors (Fig. 2c and e), were expressed in less than 25 % of RXR$\alpha^{ep-/-(a)}$ tumors (Fig. 2d and f). Tumors strongly expressing K13 were 3-4 times more frequent in RXR$\alpha^{ep-/-(a)}$ than in CT mice, and more than 70 % of the tumor area was stained (instead of 15% in CT mice; Fig. 2A, g and h, and data not shown).

Up-regulation of α6 and β4 integrins, normally expressed in basal cells and mainly found in the basement membrane, as well as their presence into several cell layers above the basement membrane, are associated with a high risk of malignant conversion (Tennenbaum et al., 1993). α6 and β4 integrin expression was much stronger in all RXR$\alpha^{ep-/-(a)}$ tumors and in most cases extended into suprabasal layers (Fig 2A, i and j, and data not shown), while it was restricted to the basal layer in >90 % of CT tumors. Finally, in accordance with an increased vascularity of RXR$\alpha^{ep-/-(a)}$ tumors (data not shown), 70 % of RXR$\alpha^{ep-/-(a)}$ tumors exhibited increased immunostaining of CD31 which labels endothelial intercellular junctions (Fig. 2A, k and l). Taken together, the above data indicate that, upon RXRα ablation in epidermal keratinocytes, DMBA/TPA-induced tumors exhibit a higher progression to malignancy.

DMBA/TPA-induced epidermal tumors are known to degenerate at a low rate into squamous cell carcinoma (SCC), but not into basal cell carcinoma (BCC) (Yuspa, 1998, and refs therein) (see Table 1). Nine tumors from each of 6 CT and 6 RXR$\alpha^{ep-/-(a)}$ mice were examined. As previously reported (Yuspa, 1998), most of CT tumors analysed after 25 weeks of DMBA/TPA treatment were benign papillomas characterised by skin folds integrated by a core of connective tissue and lined by an acanthotic, hyperkeratotic, stratified squamous epithelium (Fig.2Ba, and data not shown). 20 % of the papillomas exhibited a basal cell hyperplasia (Fig. 2Bb), 3% displayed in situ carcinoma (data not shown), but foci of early SCC were only observed 30 weeks after tumor initiation in ~14 % of the tumors (Fig. 2Bc, Table I, and data not shown). In contrast, 25 weeks after tumor initiation, ~30% of RXR$\alpha^{ep-/-(a)}$ papillomas exhibited basal cell hyperplasia (Fig. 2Cb), 10 % displayed in situ carcinoma (not shown), and foci of SCC were observed in 40 % of the tumors (Fig. 2Cc). Five weeks later, 46% of these tumors had progressed to differentiated and

undifferentiated SCC with extensive local invasion of cancer cells into the dermis and underlying muscles (Fig. 2Cd-g, Table I, and data not shown). Well differentiated SCC [Fig. 2Cd, note the keratin pearls (white arrow) with keratinisation in the center], moderately differentiated SCC [Fig. 2Ce**;** the cells are markedly irregular in shape and size, and nuclei are hyperchromatic (blue arrowhead) with prominent nucleoli; note also the internal keratinisation (asterisk)], as well as poorly differentiated SCC [Fig. 2Cf; note the presence of pleiomorphic cancer cells with abnormal nuclei and prominent nucleoli], were observed in 30 to 45 % of the tumors (Table I). Highly aggressive anaplastic SCC (~3 %; Fig. 2Cg), and basal cell carcinoma (~6 %; Fig.2C, h and i), previously not reported in the two step chemical tumorigenesis model, were also found in RXR$\alpha^{ep-/-(a)}$, but not in CT mice (Table I).

Melanoma formation in skin of RXR$\alpha^{ep-/-}$ mice upon DMBA/TPA treatment

A single DMBA application to skin of WT mice induces the formation of nevi, which are benign melanocytic growths (MGs) that become visible after 10-12 weeks, and are characterized by subepidermal accumulation of melanocytes (Epstein, 1992 and refs. therein; Husain et al., 1991). Interestingly, a single DMBA treatment of RXR$\alpha^{ep-/-}$ mice resulted in a ~7-fold increase in the number of MGs which were generally larger than those of CT mice (Fig. 1, G and H, and data not shown). Multiple TPA applications after DMBA initiation further increased the number of MGs in CT and mutant mice (Fig. 1H; compare also Fig. 1C and G, and data not shown). After 30 weeks of DMBA/TPA treatment, ~10 nevi were observed per CT mouse (generally with a diameter below 2 mm), whereas >50 MGs were found per RXR$\alpha^{ep-/-(a)}$ mouse (~75% with a diameter over 2 mm) (Fig. 1H). When RXRα was ablated 8 weeks after DMBA treatment (Fig. 1A), the number of MGs was only ~3 fold higher in RXR$\alpha^{ep-/-(b)}$ than in CT mice, and only 40% of them had a diameter larger than 2 mm (Fig. 1H). These results indicate that both MG initiation and promotion might be increased upon ablation of RXRα.

Histological analysis of MGs (>2mm) generated by DMBA or DMBA/TPA treatment showed an accumulation of melanocytes in the dermis of CT mice, whereas a larger number of densely melanin-laden melanocytes was seen in both the dermis and

hypodermis of RXR$\alpha^{ep-/-}$ skin sections, accompanied by a vertical invasion of the underlying musculature in 5–10 % of the cases (compare Fig. 3A, a and b, and data not shown). However, only few melanocytes (1-2 %) were found in the epidermis of both CT and RXR$\alpha^{ep-/-}$ mice, and in most cases the basement membrane appeared intact (Fig 3A, a and b; and data not shown). As previously reported (Ghadially, 1997), electron microscopy revealed that melanocytes from CT nevi contained mainly stage III and IV melanosomes [Fig. 3A, c and e, and data not shown)]. On the other hand, when compared with CT melanocytes, most of the melanocytes present in MGs from RXR$\alpha^{ep-/-}$ mice showed a ~10-fold increase in melanosomes containing melanin granules, and their nuclei were more elongated (Fig. 3, d and f). Moreover, in ~40% of the RXR$\alpha^{ep-/-}$ melanocytes, 40-50% of the melanosomes had the appearance of vesicles exhibiting a variable electron opacity with a distinct substructure, a characteristic of human melanoma cells (Ghadially, 1997) [Fig 3A d, and f, arrows, and data not shown]. In addition, the membrane of a number of melanosomes was disrupted and smaller melanin granules were scattered in the cellular matrix (Fig. 3Af, and data not shown). In rare cases, oval shaped vesicles contained a longitudinally-oriented component made of rods, which is also characteristic of human melanoma cells (Fig. 3Ag, (Ghadially, 1997)). Taken together, these results indicate the formation of melanomas in the skin of RXR$\alpha^{ep-/-}$ mice.

N-Ras mutations in codon 61 have been detected in either mouse or human nevi and melanomas (Husain et al., 1991; Kumar et al., 2004 and refs. therein). After 30 weeks of DMBA/TPA treatment, we PCR-amplified and cloned the first N-Ras exon from 6 and 8 individual MGs (>2 mm in size) collected from CT and RXR$\alpha^{ep-/-(a)}$ mice, respectively. Ten independent transformants from each MGs were sequenced. Glycine to Valine (GGT→GTT) and Glycine to Alanine (GGT→GCT) transversion mutations at codon 12 were found in 2/8 and 1/8 RXR$\alpha^{ep-/-(a)}$ MGs, respectively (data not shown). None of the 6 control MGs showed mutation at codon 12. In addition, 1/8 RXR$\alpha^{ep-/-(a)}$ and 1/6 control MGs had a Glycine to Valine point mutation of N-ras codon 13 (data not shown). In contrast to human melanoma (Demunter et al., 2001), no mutation of N-Ras codon 18 was detected in any of the

mutant or control MGs. No mutation of N-Ras codons 12 and 13 could be detected in 4 control and 4 mutant epidermal tumors. Fifteen MGs and 20 epidermal tumors (4-8 mm in size) from RXR$\alpha^{ep-/-(a)}$ and CT mice (collected 30 weeks after DMBA/ TPA application) were also analyzed for possible mutation of N-Ras codon 61. None of them had a codon 61 A^{182}/T mutation (analysed by PCR and restriction digestion; data not shown), while an A to C substitution was found in the second base of codon 61 [CAA-> CCA, a change from Glutamine (Gln) to Proline (Pro)] in one RXR$\alpha^{ep-/-(a)}$ MG, but not in CT MGs. In contrast to human melanoma and nevi reports (Laud et al., 2003; Pollock et al., 2003 and refs therein), no B-raf mutation could be detected in 15 MGs from either CT or RXR$\alpha^{ep-/-(a)}$ mice (data not shown).

To demonstrate that RXRα was not ablated in MGs, PCR genotyping was performed on DNA isolated from large nevi of CT and RXR$\alpha^{ep-/-}$ mice (Experimental Procedures). RXRα L2 alleles, but no RXRα L^- alleles, were found in RXR$\alpha^{ep-/-}$ melanocytic tumors induced by either DMBA or DMBA/TPA, whereas as expected only L^- alleles were found in their surrounding epidermis (Fig. 3B). These results demonstrate that the formation of nevi and melanomas in DMBA-treated RXR$\alpha^{ep-/-}$ mice is associated with RXRα ablation in keratinocytes, but not in melanocytes.

Melanoma from RXR$\alpha^{ep-/-}$ mice metastasize to distal lymph nodes

Melanoma cells are known to express specific chemokine receptors and to metastasize preferentially in lymph nodes, lung and liver (Murakami et al., 2002; Payne and Cornelius, 2002). Autopsies performed 30 weeks after DMBA treatment revealed that subiliac lymph nodes were enlarged and pigmented in 2 out of 4 RXR$\alpha^{ep-/-}$ mice, but not in CT mice (0/4) (Fig. 4A, a and b, and data not shown). Histological analysis showed few pigmented cells in lymphosinuses and some lymphatic vessels (10-20%) of mutant mice (Fig. 4Ad, and data not shown). In contrast, no pigmented cells were seen in lymphosinuses from CT mice, and very few (5–6 fold less than in mutant mice) were present in lymphatic vessels (Fig. 4Ac, and data not shown).

After 30 weeks of DMBA/TPA treatment, pigmented cells were found in subiliac lymph nodes of 5/6 RXR$\alpha^{ep-/-(a)}$ mice and 1/6 CT mice (Fig. 4A, e and f, and data not shown). Histological analysis showed enlarged lymphosinuses and clusters of pigmented cells in lymph nodes from mutant mice, but not in those from CT mice (compare Fig. 4A, g and h). Only few pigmented cells**,** but no cell clusters were seen in one lymph node and in lymphatic vessels of 1/6 CT mice (data not shown).

Lymph node immunostaining with an antibody against the S-100 antigen, which normally stains malignant melanocytes and interdigitating reticulum cells (a class of macrophage) (Kindblom et al., 1984), revealed that all pigmented cells in lymphosinuses and paracortex of lymph nodes from RXR$\alpha^{ep-/-}$ mice were positively stained (see Fig. 4B and data not shown). However, when an antibody against PEP8 [Tyrosinase related protein-2 (TRP2)] which specifically labels melanocytes (Kroumpouzos et al., 1994) was used, only 10-20% of the pigmented cells were positively stained, identifying them as malignant melanocytes (Fig.5C and data not shown). Thus, it appears that DMBA/TPA-induced nevi can progress to malignancy and metastasis in mice ablated for RXRα in epidermal keratinocytes.

DMBA/TPA treatment of VDR null mutant mice does not enhance epidermal tumorigenesis, but increases the formation of melanocytic growths.

Like RXR$\alpha^{ep-/-}$ mice, VDR-null (VDR$^{-/-}$) mice develop an alopecia, which suggested that RXRα/VDR heterodimers are indispensible during hair cycling (Li et al., 2001b, and refs therein; Li et al., 2000). It has also been reported that vitamin D3, the active VDR ligand, can induce both cell cycle arrest and differentiation in vitro of human cell lines derived from SCC (Niles, 1995) and melanoma (Yudoh et al., 1999), and reduce tumor formation in the two-step chemical tumorigenesis mouse model (Chida et al., 1985; Kensler et al., 2000). 8-9 week-old VDR null mutant and WT control (CT) females were therefore DMBA/TPA-treated to investigate whether VDR could be involved in the tumor-suppressive effect of RXRα.

Seven to eight weeks after the start of TPA treatment, papillomas developed similarly in all control (CT) and mutant mice (Fig.6A and B, and data not shown), although

they were in general smaller in VDR$^{-/-}$ than in CT mice (see Fig. 5A-C). Histological analysis of 40 tumors, 30 weeks after DMBA initiation revealed that, unlike RXR$\alpha^{ep-/-}$ mutants, 95% of the papillomas were benign, while the others only exhibited foci of early SCC (Table 1, and data not shown).

Melanocytic growths also appeared on DMBA and DMBA/TPA-treated skin of VDR$^{-/-}$ mice, and their number was strikingly high to reach ~70 MGs after 30 weeks of TPA application (instead of ~10 MGs in CT mice, Fig 5D). In both cases, histological analysis of 30 MGs from CT and VDR$^{-/-}$ mice showed the presence of dermal melanin-laden melanocytes (Fig 5E, and data not shown), and electron microscopy revealed that they contained mostly stage III and stage IV melanosomes which are characteristics of benign nevi (Fig 5F). However, the number of melanosomes was approximately 2-fold increased in ~10% of the melanocytes present in VDR$^{-/-}$ MGs (Fig 5F, and data not shown).

Selective ablation of PPARγ in keratinocytes enhances DMBA/TPA-induced epidermal tumorigenesis.

To investigate whether the tumor suppressive effect of RXRα could be due to events controlled by RXRα/PPARγ heterodimers, PPAR$\gamma^{ep-/-}$ (for epidermal keratinocyte-selective PPARγ-null genotype) mice, as well as their control (CT) littermates (PPAR$\gamma^{L2/L2}$) which carry two PPARγ L2 floxed alleles in their germ line (Imai et al., 2004), were obtained by tamoxifen administration to K14-CreER^{T2}/ PPAR$\gamma^{L2/L2}$ and PPAR$\gamma^{L2/L2}$ littermates, respectively. Unlike RXR$\alpha^{ep-/-}$ and VDR$^{-/-}$ mice, PPAR$\gamma^{ep-/-}$ mice did not exhibit an alopecia. Furthermore, in contrast to RXR$\alpha^{ep-/-}$ mice, PPAR$\gamma^{ep-/-}$ mice showed only a modest increase in keratinocyte proliferation 6-8 weeks after Tam-induced ablation ($\approx$15 % when compared to CT mice, as determined from IHC staining of the Ki67 proliferation marker; data not shown), and did not exhibit an abnormal interfollicular epidermis differentiation nor a skin inflammatory reaction (Mao-Qiang et al., 2004, and our unpublished data).

Starting 2 weeks after Tam administration (as in Fig.1A), CT and PPARγ$^{ep-/-(a)}$ female mice were subjected to the DMBA/TPA treatment for 30 weeks. All mice developed epidermal tu mors but their number was two-fold increased in PPARγ$^{ep-/-(a)}$ mice and these tumors were generally larger than those of CT mice (Fig 6A-C). To investigate whether the effect of PPARγ on tumorigenesis was exerted on initiation and/or promotion steps, PPARγ was selectively ablated by Tam administration seven weeks after the start of the DMBA/TPA treatment (PPARγ$^{ep-/-(b)}$ mice; as in Fig 1A). Epidermal tumors appeared approximately one week later in both CT and PPARγ$^{e\tilde{}\square}$ mice. However, after 30 weeks their number was again approximately two-fold increased in PPARγ$^{e\tilde{}\square}$ mice (Fig. 6D), and they were generally larger than in CT mice (Fig. 6E), indicating that the ablation of PPARγ in epidermal keratinocytes stimulates the promotion, but not the initiation step of tumorigenesis. Treatment with TPA alone was not tumorigenic (data not shown). Histological analysis of 40 tumors from PPARγ$^{ep-/-(a}$ mice 30 weeks after tumor promotion revealed that 70% of these tumors were benign papillomas, while 25% had progressed to SCC, and 2.5% to BCC (Table I).

Melanocytic growths also appeared on DMBA/TPA-treated skin of CT, PPARγ$^{e\tilde{}a}$ and PPARγ$^{e\tilde{}\square}$ mice (data not shown). However, in contrast to RXRα$^{ep-/-}$ and VDR$^{-/-}$ mice, their number was identical in PPARγ$^{ep-/-}$ and CT mice (Fig 6F), and in both cases histological analysis of MGs (~2mm in diameter) generated after DMBA/TPA treatment showed the presence of dermal melanin-laden melanocytes (data not shown), similar to those observed in benign nevi (see above), with no evidence of progression to malignant melanomas.

Altered expression of genes involved in cell proliferation, tumor suppression, cell-cell communication, cellular adhesion and melanocytosis in skin of RXRα$^{ep-/-}$, VDR$^{-/-}$ and PPARγ$^{ep-/-}$ mice.

Downregulation of the tumor suppressor PTEN has been reported in a variety of human cancers, including breast, prostate, ovarian epithelial cancer and melanoma

(Wishart and Dixon, 2002; Wu et al., 2003 and refs. therein), while mice lacking PTEN in epidermal keratinocytes develop epidermal hyperplasia and skin tumors (Suzuki et al., 2003), and Ink4a/Arf$^{-/-}$ Pten$^{+/-}$ compound mutant mice exhibit melanoma and squamous cell carcinoma (You et al., 2002). When compared to CT mice, and irrespective of DMBA/TPA treatment, PTEN expression was 2-3 fold downregulated in the skin of RXR$\alpha^{ep-/-(a)}$ mutants (MT), 20 weeks after Tam-induced RXRα ablation (Fig. 7A). Interestingly, PTEN expression was already down-regulated 2 and 4 weeks after the ablation of RXRα in epidermal keratinocytes (Fig. 7B, and data not shown), and a similar PTEN down-regulation also occurred in PPAR$\gamma^{ep-/-(a)}$, but not in VDR mutants (Fig. 7B, and data not shown). In contrast, no change in the expression level of other tumor suppressors, such as p53, Rb, p107, p130, p16 and p19 or of other genes involved in nonmelanoma and melnoma skin cancer, like Focal adhesion kinase (Fak),Transforming growth factor α (TGFα), Gli1, Gli2, Metabotropic glutamate receptor 1 (Grm1) and Micropthalmia transcription factor (MITF) was observed between CT and RXR$\alpha^{ep-/-}$ mutant mice skin, 2 and 4 weeks after RXRα ablation (data not shown). Moreover, even though it has been previously reported that the proinflammatory cytokine TNFα is critical for tumor promotion by TPA (Moore et al., 1999; Suganuma et al., 1999) and TNFα null mice are resistant to DMBA/TPA induced skin tumorigenesis (Moore et al., 1999), no significant change in TNFα RNA level was observed in the skin of RXR$\alpha^{ep-/-}$ and PPAR$\gamma^{ep-/-}$ mice 2 to 8 weeks after Tam administration, nor in the skin of VDR-null mice (data not shown).

The expression of the transmembrane receptor E-cadherin, that is involved in cell-cell communication and cellular adhesion is found in both keratinocytes and melanocytes. E-cadherin has been found to be downregulated in invasive carcinoma and melanomas (Frixen et al., 1991; Hsu et al., 1996; Jamal and Schneider, 2002; Li et al., 2001a). Twenty weeks after RXRα ablation, E-cadherin levels were decreased by ~3-fold in skin from RXR$\alpha^{ep-/-(a)}$ mice, irrespective of DMBA/TPA treatments (see Fig. 7A), and a similar ~2-fold decrease in E-cadherin expression was observed 4 weeks, but not 2 weeks after RXRα ablation (Fig. 7C and data not shown). A

similar decrease was also observed in the skin of VDR null mutants (Fig. 7C), whereas expression of E-cadherin was unaltered in PPARγ$^{ep-/-}$ mutants (see Fig. 7C).

It has been shown that targeted expression of α6β4 integrins in the suprabasal cell layers of transgenic mouse epidermis dramatically increased the frequency of papillomas and carcinomas induced by chemical tumorigenesis (Owens et al., 2003). We have seen above that α6 and β4 integrin expression was much stronger in all RXRα$^{ep-/-}$ tumors, and in most cases extended into suprabasal layers (Fig.2A). Most interestingly, 5-8 weeks after Tam injection, α6β4 integrins, which are normally expressed by basal layer cells and located in the basement membrane, were found to be strongly expressed in the upper hyperplastic suprabasal cell layers of RXRα$^{ep-/-}$ epidermis as evidenced by IHC (data not shown). Similarly, α6β4 integrins were also found to be abnormally expressed in the epidermal suprabasal cell layers of ~25% of PPARγ $^{ep-/-}$ mice, but not in the epidermis of VDR null mice (data not shown).

Overexpression of the hepatocyte growth factor (HGF) in basal keratinocytes of transgenic mice leads to epidermal and dermal melanocytosis (Kunisada et al., 2000). However, no significant change in the overall expression level of HGF and its receptor c-Met was observed in the skin of RXRα$^{ep-/-}$, VDR-/- or PPARγ$^{ep-/-}$ mutant mice 4 weeks after DMBA/TPA treatment (data not shown).

The ligand for the c-kit receptor, the stem cell factor SCF that is produced by keratinocytes, plays an important role in melanocyte proliferation, survival and migration (Kunisada et al., 2001; Yoshida et al., 2001). SCF can be produced in two isoforms by alternative splicing yielding either a mRNA encoding only a membrane-bound isoform (SCF220) or a mRNA encoding a longer membrane-bound isoform (SCF248) from which a soluble secreted protein is released by proteolysis (SCF164). Overexpression of SCF in epidermal keratinocytes leads to epidermal and dermal melanocytosis (Kunisada et al., 1998a; Kunisada et al., 1998b). We therefore investigated whether the increase in melanoma-like melanocytic tumors in

DMBA/TPA-treated RXR$\alpha^{ep-/-}$ mutants could be related to altered expression of SCF. Increased expression (~3 fold) of the SCF220 membrane-bound form was observed in the skin of RXR$\alpha^{ep-/-}$mutants (MT), irrespective of DMBA/TPA treatment (Fig. 7A). Note, however, that this treatment also increased the basal level of SCF220 in CT mice. In contrast, there was little, if any change in the expression level of the SCF248 membrane-bound form in both CT and RXR$\alpha^{ep-/-}$ mice. An increase in the expression level of total SCF was also observed in RXR$\alpha^{ep-/-}$ mice 4 weeks after RXRα ablation (Fig.7D), whereas there was no significant change in its expression in VDR$^{-/-}$ and PPAR$\gamma^{ep-/-}$ mutant mice (Fig.7D). We also looked at the expression pattern of the SCF receptor c-kit, as loss of its expression is often associated in human melanoma cells with progression to a metastatic phenotype (Bar-Eli, 2001; Huang et al., 1998). 20-22 weeks after DMBA/TPA application, the level of c-kit was reduced by 2-fold in skin of RXR$\alpha^{ep-/-}$ mice, but not in VDR$^{-/-}$ and PPAR$\gamma^{ep-/-}$ mutants, when compared to CT mice (Fig. 7A).

The POU domain transcription factor Brn-2 mRNA is overexpressed in human melanoma compared to normal melanocytes, and is strongly upregulated by MAP kinase signalling downstream of receptor tyrosine kinases (RTKs) and BRAF (Goodall et al., 2004). Interestingly, Brn-2 mRNA level was significantly increased in 2/6 MGs from RXR$\alpha^{ep-/-}$ mice, but not in MGs from CT mice (data not shown).

Discussion

Keratinocytic RXRα and PPARγ act as tumor modifiers of both promotion and malignant progression of epidermal tumors induced by topical DMBA/TPA treatment.

Using the well characterized DMBA/TPA-induced two-stage chemical skin carcinogenesis system and keratinocyte-selective gene disruption in adult mice, we have investigated here the role possibly played by RXRα and some of its heterodimerization-partners in epidermal tumorigenesis. Our results show clearly that RXRα acts as a tumor modifier of both the TPA-induced promotion of benign papilloma tumors and their subsequent progression to malignancy. We also show that the DMBA-induced initiation step which results in H-ras activation is not affected by RXRα ablation.

Tumor promotion by TPA is known to induce the expression of the cytokine TNFα in epidermal keratinocytes. This induction, which results in keratinocyte hyperproliferation and skin inflammation, is critical for tumor promotion by TPA (Moore et al., 1999; Suganuma et al., 1999). As a skin inflammation is also generated upon RXRα ablation in epidermal keratinocytes (Li et al., 2001b ; Li et al., 2000), and given the known associations between chronic inflammation and cancer (Coussens and Werb, 2002), the question arises whether the promoting effect of RXRα ablation on papilloma formation could reflect a further increase in TNFα production. We could not detect such an increase , thus making it unlikely that TNFα could be responsible for the more efficient tumor promotion resulting from RXRα ablation in keratinocytes. Note, however, that irrespective of RXRα ablation in keratinocytes, the TPA-induced promotion of epidermal tumors was efficiently suppressed by inhibitors of AP-1 activity, such as the glucocorticoid fluocinolone acetonide (FA) (Huang et al., 1997, our unpublished data).

In quest of partners with which RXRα may heterodimerize in skin keratinocytes in order to modify the TPA-induced promotion and malignant progression of epidermal tumors initiated by DMBA treatment, we also subjected mice ablated for NRs known to be expressed in the skin to topical DMBA/TPA-induced skin tumorigenesis. The three PPAR isotypes were selected for this study, because PPARα (Sheu et al., 2002), PPARβ(δ) (Schmuth et al., 2004) and PPARγ (Mao-Qiang et al., 2004) activators are known to inhibit TPA-induced cutaneous inflammation. The selective ablation of PPARβ in basal keratinocytes had no significant effect on DMBA/TPA-induced skin tumorigenesis (our unpublished results). However, as carcinogenicity induced by DMBA/TPA was enhanced in PPARβ null mice (Kim et al., 2004), PPARβ might exert its tumor modifier activity in non-keratinocytes, and /or might control molecular mechanism in keratinocytes of the forming epidermis that prevented tumorigenesis. In contrast, in PPARγ$^{ep-/-}$ mice ablated for PPARγ in epidermal keratinocytes, the rate of formation of epidermal tumors, as well as their malignant progression to carcinomas, were similar to those observed in RXRα$^{ep-/-}$ mice even though, in contrast to RXRα, the selective ablation of PPARγ in keratinocyte does not result in skin inflammation (Mao-Qiang et al., 2004, and our unpublished results). Thus, PPARγ is likely to be a major heterodimerization partner for RXRα in the process which leads to increased tumor promotion and malignant progression in DMBA/TPA-treated mice. Interestingly, germ line null mutations of PPARα or LXRβ, the activations of which is also known to inhibit TPA-induced cutaneous inflammation (Fowler et al., 2003), similarly resulted in 1.5 - 2-fold increased rates of formation of DMBA/TPA induced tumors (our unpublished results). Selective ablation of PPARα and LXRβ in keratinocytes is therefore required to determine whether, besides RXRα/PPARγ heterodimers, RXRα/PPARα and RXRα/LXRβ heterodimers also act as cell-autonomous epidermal tumor modifiers. On the other hand, germline null mutations of either VDR, RARα, RARγ or RARβ had no significant effect on DMBA/TPA-induced epidermal tumor formation (this study, and our unpublished data).

In a search for genes whose altered expression upon RXRα ablation in skin keratinocytes could possibly account for the DMBA/TPA-induced increased in

epidermal tumor formation and progression to malignancy, we looked at the expression of a number of genes whose misexpression is known to affect DMBA/TPA-induced epidermal tumorigenesis. Although the expression of most of these genes was not significantly different in DMBA/TPA-treated skin of control and RXR$\alpha^{ep-/-}$ mutant mice, changes in the expression of two genes was particularly relevant. The PTEN gene, whose expression was 2-3-fold decreased (Fig. 7), is a tumor suppressor gene that modulates several cellular functions, including cell migration, survival and proliferation, through the inhibition of PI3-kinase-mediated signalling cascades (Sulis and Parsons, 2003). Importantly, selective disruption of the PTEN gene in epidermal keratinocytes has shown that PTEN functions as a tumor suppressor for both spontaneous and induced skin tumors in mice (Suzuki et al., 2003). Moreover, it has been reported that PTEN expression is upregulated by PPARγ ligands, and PPAR response elements have been localized in the genomic sequence upstream of the PTEN coding sequence (Patel et al., 2001). Most interestingly, we found that ablation of PPARγ in skin keratinocytes also significantly reduces PTEN expression in the skin (Fig. 7). Thus, the similarly increased tumor formation and progression to malignancy observed for DMBA/TPA-treated mice upon selective ablation of RXRα or PPARγ in keratinocytes, might be caused at least in part by a downregulation of the expression of the tumor suppressor PTEN, due to the lack of RXRα/PPARγ heterodimers.

The suprabasal expression of $\alpha6\beta4$ integrin, that we observed in DMBA/TPA induced epidermal tumors of RXR$\alpha^{ep-/-}$ mice (see Results section) has been correlated with a high risk of malignant conversion to SCC (Tennenbaum et al., 1993). Interestingly, it has been recently shown that targeted expression of $\alpha6\beta4$ in keratinocytes of the epidermal suprabasal layers of transgenic mice dramatically increases the rate of DMBA/TPA-induced promotion and malignant progression of epidermal tumors, and it was suggested that this ectopic $\alpha6\beta4$ integrin expression promotes tumorigenesis by preventing TGFβ from suppressing clonal expansion of initiated basal keratinocytes (Owens et al., 2003). As approximately 30% of PPAR$\gamma^{ep-/-}$ mice also exhibit suprabasal expression of $\alpha6\beta4$ (our unpublished results) and both PPARγ (Westergaard et al., 2001), and RXRα (Chapellier et al., 2002), are present in

suprabasal cells, it appears very likely that the suprabasal upregulation of α6β4 integrin occurring upon RXRα or PPARγ ablation in epidermal keratinocytes is due to the suppression of a downregulation of α6β4 expression normally exerted directly or indirectly by PPARγ/RXRα heterodimers in suprabasal keratinocytes.

Thus, the increased promotion and malignant progression of DMBA/TPA-induced tumors in mice ablated for RXRα or PPARγ appear to involve RXRα/PPARγ-mediated events that, under wild type conditions, directly or indirectly, either upregulate the expression of suppressor genes (e.g. PTEN) or downregulate the expression of growth- and invasion-promoting genes (e.g. α6β4 integrin).

Keratinocytic RXRα acts through distinct paracrine pathways to prevent the formation of DMBA-induced dermal benign melanocytic growths and their malignant progression to melanomas.

In contrast to human skin melanocytes which are mainly located in the interadnexal epidermis, mouse skin melanocytes drastically decrease in interfollicular epidermis from the fourth day after birth. In adult mice, few melanocytes are present in the dermis, whereas the bulk of them are confined to hair follicles (Hardy, 1992; Hirobe, 1984; Yoshida et al., 1996). In agreement with the initial report of Husain et al. (Husain et al., 1991), we observed that a single topical application of DMBA induces the formation of benign melanocytic growths (MGs) that become visible as nevi corresponding to dermal accumulation of melanocytes. Our present data clearly demonstrate that the selective ablation of RXRα in adult epidermal keratinocytes (RXRα$^{ep-/-}$ mutant mice) followed by DMBA application results in a marked increase of these nevi both in number and size. Moreover, in both control and RXRα$^{ep-/-}$ mice, multiple TPA application further increase the number of nevi to reach a state of dermal melanocytosis. Most interestingly, and irrespective of TPA treatment, a significant proportion of MGs from RXRα$^{ep-/-}$ mice exhibited invasive vertical growth and electron microscopic melanocyte abnormalities characteristic of malignant progression to melanomas. In agreement with the previous report of Husain et al. (Husain et al., 1991), this malignant progression was not found in control mice. Importantly, and as expected, the ablation of RXRα could not be detected in isolated

MGs, while it was efficiently ablated in MG-free epidermis and in the epidermis surrounding MGs.

We therefore conclude that the **genetically** DMBA-initiated proliferation of melanocytes and their possible malignant transformation must be controlled by paracrine/juxtacrine signals which emanate from keratinocytes, and are regulated by RXRα. Morevorer, these signals must be different from those which control the formation of epidermal squamous tumors, as PPARγ$^{ep-/-}$ mutants develop epidermal tumors upon DMBA/TPA treatment as efficiently as RXRα$^{ep-/-}$ mutants, whereas they do not exhibit any increase in MGs formation, when compared to control mice. On the other hand, VDR-null mutants which do not show any enhancement of epidermal tumorigenesis upon DMBA/TPA treatment, exhibit a marked increase in benign MGs, which, however, do not progress to malignancy. Thus, the events which, upon DMBA/TPA treatment, underlie the suppressive effect of RXRα on epidermal tumorigenesis might be mediated by RXRα/PPARγ heterodimers (see above), while those which underlie the suppressive effect of RXRα on the formation of benign MGs could be mediated by RXRα/VDR heterodimers. On the other hand, the events underlying the malignant conversion of benign MGs to melanomas might be mediated by heterodimers between RXRα and NR partner(s) different from those mutated in the present study. That different mechanisms are at work in these tumorigenesis processes, is further supported by the absolute requirement of the tumor promoter TPA to generate DMBA-initiated epidermal tumors in RXRα□□ mutants, whereas this promoter is dispensible to generate benign melanocytic growths and melanomas. Along the same lines, it is remarkable that the topical application of the glucocorticoid FA, which antagonizes the activity of AP-1, fully prevents the occurrence of epidermal tumors in DMBA/TPA-treated RXRα$^{ep-/-}$ mice, while it does not affect the generation of MGs and their malignant progression (our unpublished data).

The non-cell autonomous molecular events that, upon topical DMBA initiation, underlie in the epidermis and dermis the keratinocyte-originated RXRα-mediated control of dermal MG generation and malignant transformation, are unknown. In vitro keratinocyte-melanocyte cocultures, as well as overexpression studies in transgenic mice, have suggested that the proliferation and differentiation of mammalian

melanocytes are regulated by factors produced by keratinocytes. More specifically endothelin ET-1, as well as the stem cell factor (SCF) and the hepatocyte growth factor (HGF) could be such factors (Hirobe et al., 2002). In particular, analysis of transgenic mice expressing SCF in epidermal keratinocytes led to the conclusion that SCF acts as a key factor to support the temporally and spatially coordinated survival, proliferation, differentiation and migration of melanocyte lineages (Kunisada et al., 1998b). Moreover, the soluble form of SCF (Refs in Results section) may be required for the migration of c-kit-expressing melanoblasts/melanocytes, while membrane-bound SCF is required for their survival, proliferation and differentiation (Wehrle-Haller and Weston, 1995). In a preliminary analysis, we found that SCF expression was significantly increased in the skin of RXRα□□̃ mutants, which suggests that the ablation of RXRα in epidermal keratinocytes may directly or indirectly lead to an overproduction of SCF, which in DMBA-initiated melanocytes/melanoblasts could lead to their proliferation and enhance the formation of MGs. More sophisticated studies are obviously required to characterize the epidermal and dermal factors whose expression is altered by ablation of RXRα in epidermal keratinocytes, in order to elucidate how the development of DMBA-initiated melanocytic tumors is environmentally regulated by RXRα-controlled events in epidermal keratinocytes.

Acknowledgements

We thank B. Lane, E. Georges, S. Yuspa and V. Hearing for kindly providing antibodies, N. Ghyselinck for RAR-null mice, S. Kato for VDR-null mice, Deltagen for LXRβ null mice and F.J. Gonzalez for PPARα - null mice. We also thank M. Mark, A. Van Es and R. Matyas for helpful discussion, R. Lorentz, M. Duval, B. Chofflet, S. Bronner and C. Gérard for excellent technical help, M. LeMeur, E. Metzger and the animal facility staff for animal care and for useful suggestions, the Histology service staff and IGBMC common facility for assistance, the secretarial staff for typing and the illustration staff for preparing the figures. This work was supported from funds from Centre National de la Recherche Scientifique, the institut National de la Santé et de la Recherche Médicale, the Collège de France, the Hôpital Universitaire de Strasbourg, de Association pour la Recherche Médicale, the Human Frontier Science Program, the Ministère de l'Éducation Nationale de la Recherche et de la Technologie, the Swiss National Science Foundation, the Etat de Vaud, and the European Community and by a UICC Translational Cancer Research Fellowship funded by Novartis (Switzerland).

(Prior to publication, a copy of the manuscript is to be provided to UICC to be forwarded to Novartis for information)

Experimental Procedures:

Mice and Carcinogenic treatment

To selectively ablate RXRα in epidermal keratinocytes of adult mice, mice carrying LoxP-site-containing (floxed) RXRα L2 alleles were bred with hemizygous K14-Cre-ER$^{T2(tg/0)}$ transgenic mice, to produce K14-Cre-ER$^{T2(tg/0)}$/RXRα$^{L2/L2}$ mice (Li et al., 2000; Metzger et al., 2003). 8-12 week old female mice, were injected with 0.1 mg of tamoxifen (100 μl sunflower oil) for 5 days (Metzger et al., 2003) to produce control (CT) (from K14-Cre-ER $^{T2\ 0/0}$ and K14-Cre-ER $^{T2\ tg/0}$ RXRα $^{L2/+}$ mice) and RXRα$^{ep-/-}$ mice (from K14-Cre-ER $^{T2\ tg/0}$ RXRα $^{L2/L2}$ mice). 2 weeks after the first tamoxifen injection, RXRα ablation in the epidermis was verified by Southern hybridisation of DNA isolated from tail biopsies after separation of the epidermis and the dermis (Li et al., 2000). CT and PPARγ$^{ep-/-}$ mice were generated in a similar way by breeding K14-Cre-ER$^{T2(tg/0)}$ transgenic mice with PPARγ□□□□ mice (Imai et al., 2004).

Groups of control (CT) and mutant (RXRα$^{ep-/-}$, PPARγ$^{ep-/-}$ and VDR$^{-/-}$) animals were housed in a temperature and humidity controlled room with 12h cycles of light and dark. 14 days after last Tam injection, mice in each group were shaved to synchronize the hair cycle and treated the day after with a single dose of 50 μg DMBA in 100 μl acetone. One week after DMBA application, 5 μg TPA (in 200 μl acetone) was applied topically twice a week for 30 weeks. The number and size of the papillomas per mouse were weekly recorded.

Determination of the number and size of the tumors

The length of the papillomas and the diameter of the melanocytic growths (nevi) were measured with a Vernier calliper on isofluorane anesthesised mice.

Histological and electron microscopic analysis

Tumors were excised 25 or 30 weeks after DMBA/TPA application, fixed in Bouin's fixative and embedded in paraffin. All skin biopsies were matched for age, sex and body site and all papillomas were matched for age and sex. 5 μm paraffin sections of tumor and skin, 2 μm semithin sections of skin samples and 70 nm ultrathin sections for EM were made as described (Li et al., 2001b). Subiliac lymph nodes were fixed in 4% paraformaldehyde, photo-graphed, embedded in paraffin and 5 μm-thick sections were stained with hematoxylin and eosin.

Immunohistochemistry

Tumors (8–16 mm) were excised 22 weeks after DMBA/TPA application and immediately embedded in OCT and frozen on dry ice. 10 μm-thick frozen sections from CT and RXRα$^{ep-/-}$ tumors were reacted with primary antibodies [mouse monoclonal anti -K10, rabbit polyclonal anti-K5 (gifts from Prof. Brigitte Lane, Cell Structure research group, University of Dundee]), rabbit polyclonal anti-K1 (Babco), rabbit polyclonal anti-K13 (gift from Prof. S. Yuspa, NIH), rabbit polyclonal α6-integrin (gift from E. Georges), and biotin-conjugated rat monoclonal anti-CD31(Pharmingen) and processed as described (Li et al., 2001b). To detect melanocytes 5 μm paraffin sections of subiliac lymph nodes from CT and mutant animals were reacted with primary antibodies [rabbit polyclonal α-PEP8 (TRP2) [a gift from Vincent Hearing, NIH] or rabbit polyclonal S-100 (Dako)], and processed as described (Pavan and Tilghman, 1994; Sotillo et al., 2001).

Genotyping of the RXRα alleles

Dorsal skin biopsies with and without black spots (nevi / melanocytic growths) were taken from control (CT) and mutant (RXRα$^{ep-/-)}$ animals. Samples were incubated overnight in 4mg/ml Dispase (neutral protease, Boehringer Mannheim) at 4°C. The following day the epidermis was peeled off from the dermis under a stereo-zoom binocular (Leica) and the melanocytic growths were micro-dissected out from the remaining dermal tissue. The hair follicles were carefully plucked from the nevi under the binocular, rinsed several times with 1XPBS to remove the adhering cells and transferred into separate eppendorf tubes along with the corresponding epidermis. Genomic DNA from the epidermis and dermis was isolated as described (Li et al., 2000). (Li et al., 2001b)A semi-quantitative PCR with different dilution of the isolated DNA was performed for 26 cycles in Perkin Elmer DNA light cycler to amplify the L2 and L^{-} RXRα allele as described (Li et al., 2000).

Detection of mutations in the H-ras gene.

Mutation of the codon 61 of the H-ras gene (Acc No. AF081118) was detected by PCR amplification of a 280 bp amplicon followed by restriction digest as described (Nelson et al., 1992). DNA was isolated from papillomas (4-8 mm; 25 CT and 25 MT), lesion free skin (10 CT and 10 MT) and nevi (15 CT and 15 MT) biopsied from 6 CT and 6 RXR$\alpha^{ep-/-}$ animals 25 weeks after DMBA/TPA treatment. List of the primers are available on request.The 280 bp amplicons of exon II (encompassing codon 61) from some papillomas (5 CTand 5 MT) were also cloned and sequenced to detect mutation in the codon 61using primers as described (Ise et al., 2000). Mutations in the codon 12 and 13 of the H-Ras gene exon I were analysed from papillomas (10 CT and 10 MT), lesion free skin biopsies (4 CT and 4 MT) and nevi (4 CT and 4 MT) of 6 CT and RXR$\alpha^{ep-/-}$ animals, respectively, by PCR amplification of exon I using specific primers, followed by cloning and sequencing on both strands as described (Ise et al., 2000).

Detection of mutations in the N-ras gene.

A 120 bp amplicon of the second exon of the N-Ras gene encompassing codon 61 was amplified by PCR using specific primers. DNA was isolated from papillomas (20 CT and 20 MT) and nevi (>2mm size; 15 CT and 15 MT) microdissected under binocular from the remaining adhering cells, from 6 CT and 6 RXR$\alpha^{ep-/-}$ animals 25 weeks after DMBA/TPA treatment**.** As mutation at the codon 61 in N-Ras produces either a NlaIII (CAA->CAT; A to T transversion in the third base) or a BfaI (CAA->CTA; A to T transversion in the second base) restriction site, the PCR amplified products were restriction digested by NlaIII and BfaI respectively, and resolved by agarose gel electrophoresis. The gel was blotted into a nylon membrane and hybridized with a ^{32}P labelled internal oligonucleotide. Primers are available on request. Amplified products were also cloned and 10 transformants for each sample were sequenced on both strands as mentioned by Ise et al.(Ise et al., 2000). Detection of the codon 12 and codon 13 mutation in the N-ras gene was done by PCR amplification of exon I from papillomas (4 CT and 4 MT) and nevi (6 CT and 8 MT) of 6 CT and RXR$\alpha^{ep-/-}$ animals, respectively, using primers as described by Ise et al. (Ise et al., 2000), followed by cloning and sequencing on both strands.

Quantitative RT-PCR

RNA was extracted from approximately 100 mg of dorsal body skin or tumor with Trizol^R Reagent (GIBCO BRL, Life technologies), dissolved in RNase-free water at a concentration of 1 μg/μl after quantification by spectrophotometric analyses. cDNA synthesis was performed with Superscript II reverse transcription kit (GIBCO BRL, Life technologies) using 5 μg of total RNA, according to the manufacturer's instructions. 1 μl of cDNA was used in a 30 μl semiquantitative PCR reaction with 1X PCR buffer, 100 ng of each primer, an optimal concentration of $MgCl_2$ (1.5 mM), 0.2 mM dNTP and 2 U of AmpliTaq (Perkin Elmer, Foster City, Calif.). Real time PCR was performed with a LightCycler (Roche Diagnostics) and the SYBR green protocol. For that purpose 5 μl of each cDNA was diluted to 20 μl and 2 μl of the diluted cDNA was used for each amplification. Amplification specificity was verified by melting curve analysis and the data quantified with the LightCycler software. The primers were [S=5'-GTGCTCTCTTCAACATTAGGT-3'; As=5'-TCCAGTATAAG GCTCCAAAAG-3'] for SCF, [S=5'-CGGATCACAAAGATT TGCGAT-3'; As=5'-ATA GGACCAGACATCACTTTC-3'] for c-Kit, [S=5'-AA GGTTTTCTAC AGCA TCACC-3'; As=5'-CACTGTGATCACTATCTCCAT-3'] for E-cadherin, [S=5'-AGATGACAATCATGTTGCAGC-3' ; As=5'-CATCATCTTGTGAAACAG C AG -3'] for PTEN and [S=5'-GTAATGATCAGTCAACGGG GGAC-3'; As=5'-CCAGCAAGCTTGCAACCTTAACCA-3'] for HPRT. The amplified products in exponential phase were electrophoresed on 2% agarose gel, transferred onto nylon membrane and hybridized with the following radiolabelled nucleotides for SCF (248+164) and SCF(220), [5'-AAGGCCCCTGAAGACTCGGGCCTACAAT GG-3'], c-KIT(I)[5'-CTGCCCGTGAAGTGGATGGCACCAGAGAGC-3'], E-cad- herin, (I)[5'-CAGCCTCTGGATAGAGAAGCCATTGCCAAG-3'], PTEN(I), [5'-GT AATGATTTGTGCATATTTATTGCATCGG-3'] and HPRT(I), [5'-GCTTTCCCTG GTTAAGCAGTACAGCCCC-3'].

Statistical analyses

Where relevant, data were compared by Unpaired Student's t-test with corrections for unequal variance using Statview 5 programme (Abacus Concepts, CA). Values are reported as mean ± SEM and the significance level was set at $p \leq 0.05$.

Figure legends and Tables:

Figure 1. DMBA/TPA-induced epidermal tumors and melanocytic growths in RXR$\alpha^{ep-/-}$ mice.

A. Timing of tamoxifen (Tam)-induced RXRα ablation in epidermal keratinocytes, and DMBA/TPA induced tumorigenesis. Tam-treatment (0.1 mg for 5 consecutive days) was started either 18 days before ("a") or 8 weeks after ("b") topical DMBA application. TPA was topically applied twice a week (blue arrows) for up to 30 weeks.

B. Formation of epidermal tumors in DMBA/TPA treated control (CT) and RXR$\alpha^{ep-/-(a)}$ mice, as determined macroscopically and plotted against the number of weeks of TPA treatment. Values are expressed as mean +/- SEM (n=7 mice).

C. Dorsal view of CT (K14-Cre-ER$^{T2(0/0)}$/RXR$\alpha^{L2/L2}$) (left) and RXR$\alpha^{ep-/-(a)}$ (right) mice after 25 weeks of DMBA/TPA treatment.

D. Epidermal tumor length distribution in CT and RXR$\alpha^{ep-/-(a)}$ mice, after 30 weeks of DMBA/TPA treatment. Values are expressed as mean +/- SEM (n=7 mice).

E. Formation of epidermal tumors in DMBA/TPA-treated CT and RXR$\alpha^{ep-/-(b)}$ mice in which RXRα was ablated in keratinocytes during TPA promotion. Values are expressed as mean +/- SEM (n = 7 mice).

F. Epidermal tumor length distribution in CT and RXR$\alpha^{ep-/-(b)}$ mice, after 30 weeks of DMBA/TPA treatment. Values are expressed as mean +/- SEM (n = 7 mice).

G. Dorsal view of CT (K14-Cre-ER$^{T2(0/0)}$/RXR$\alpha^{L2/L2}$) (left) and RXR$\alpha^{ep-/-}$ (right) mice 25 weeks after DMBA treatment. Arrows point to melanocytic growths.

H. Size distribution of melanocytic growths in CT, RXR$\alpha^{ep-/-}$, RXR$\alpha^{ep-/-(a)}$ and RXR$\alpha^{ep-/-(b)}$ mice. The number of MGs, 1 – 2 and 2 – 4 mm in diameter, was determined on 7 CT, 7 RXR$\alpha^{ep-/-}$, 7 RXR$\alpha^{ep-/-(a)}$, and 7 RXR$\alpha^{ep-/-(b)}$ mice, 30 weeks after DMBA and DMBA/TPA treatments, as indicated. Values are expressed as mean +/- SEM.

Figure 2. Immuno-histochemical and histological analyses of skin tumors induced by DMBA/TPA treatment in RXR$\alpha^{ep-/-}$ mice.

A. Representative sections of tumor biopsies from control (CT) (a, c, e, g, i, k) and RXR$\alpha^{ep-/-(a)}$ (b, d, f, h, j, l) mice, taken after 22 weeks of DMBA/TPA treatment, and stained with antibodies against K5 (a, b), K1 (c, d), K10 (e, f), K13 (g, h), α6 integrin (i, j) and CD31 (k, l) are presented. Red corresponds to antibody staining and blue to DNA DAPI staining. Scale bar in Aa: 25 μm.

B. Representative hematoxylin- and eosin-stained 5 μm-thick paraffin sections from CT biopsies taken after 25 (a - b) and 30 (c) weeks of DMBA/TPA treatment. (a), suprabasal cell hyperplasia (acanthosis) within a benign papilloma; (b), basal cell hyperplasia within a benign papilloma; (c), focus of early squamous cell carcinoma.

C. Representative hematoxylin- and eosin-stained 5 μm-thick paraffin sections from RXR$\alpha^{ep-/-}$ tumor biopsies taken after 25 (a-c) and 30 (d-i) weeks of DMBA/TPA treatment. (a), suprabasal cell hyperplasia within a benign papilloma ; (b), basal cell hyperplasia within a benign papilloma ; (c), focus of early squamous cell carcinoma; (d), (e) and (f), well, moderately and poorly differentiated SCC, respectively; (g) anaplastic SCC with spindle-shaped cells and absence (or minimal) keratinisation; (h) and (i), basal cell carcinoma with densely packed basophilic cells with hyperchromatic nuclei resembling those of epidermal basal cells.

Hyperplastic basal (blue brackets) and suprabasal (black brackets) cell layers in “b” (B and C) and (a,b) [B and C] are indicated. Black arrows in (B)a and (C)a point to basal cell layers; white arrow in C(d) points to a keratin pearl; black and blue arrowheads in C(c) and (C)e, point to a mitosis and abnormal nuclei, respectively; asterisk in C(e) indicates internal cell keratinisation. Scale bar (in Ba) = 33 μm for Ba,b and Ca,b; 22 μm for Bc and Cc-i.

Figure 3.(A) Histological and ultrastructural analyses of melanocytic growths (MGs) from CT (control) and RXR$\alpha^{ep-/-(a)}$ mice. (a) and (b) : Hematoxylin and eosin-stained paraffin sections (5 μm thick) of **(a)** CT (K14-Cre-ER$^{T2(0/0)}$/RXR$\alpha^{L2/L2}$) and **(b)** RXR$\alpha^{ep-/-(a)}$ MGs in skin biopsies taken 25 weeks after DMBA/TPA treatment. The arrow in (b) points to melanocytic invasion of the hypodermis. E, epidermis ; D, dermis ; H, hypodermis. Scale bar=33 μm. **(c) to (g)** :

electron microscopic analyses of CT **(c and e)** and RXR$\alpha^{ep-/-(a)}$ **(d, f and g)** MGs isolated 25 weeks after DMBA/TPA treatment. Note in the inset (g), the oval-shaped subcellular structure containing a longitudinally oriented component made of rods. Arrows in (c),(d) and (f) point to control "normal" dermal melanocytes (in c), and abnormally shaped melanosomes typical of malignant melanoma (in d and f), respectively. Arrowheads in “c” and “e” point towards a melanin-laden melanophage and melanosomes of stage III and IV, respectively. N, nucleus. Scale bars : 2.5 μm for c and d ; 1 μm for e and f and 0.25 μm for the g inset.

(B) RXRα is not ablated in melanocytic growths from RXR$\alpha^{ep-/-}$ mice. The presence of RXRα L2 and L^{-} alleles was analysed by PCR in DNA from epidermis (E), dermis (D) isolated from MG free skin biopsies and melanocytic growths (MG) from CT (lanes 1 and 2) (K14-Cre-ER$^{T2(0/0)}$/RXR$\alpha^{L2/L2}$) and RXR$\alpha^{ep-/-}$ (lanes 3-10) mice, 25 weeks after DMBA or DMBA/TPA treatments, as indicated. The position of the RXRα L2 and L^{-} alleles is indicated.

Figure 4. Metastasis of melanocytes in lymph nodes of DMBA and DMBA/TPA-treated RXR$\alpha^{ep-/-}$ mice.

A. Subiliac lymph nodes from CT (a,e) and RXR$\alpha^{ep-/-}$ (b,f) mice isolated 30 weeks after DMBA (a,b) and DMBA/TPA (e,f) treatment, respectively. Hematoxylin- and eosin-stained 5 μm paraffin sections of subiliac lymph nodes from CT (c,g) and RXR$\alpha^{ep-/-}$ (d,h) mice after DMBA (c,d) and DMBA/TPA (g,h) treatment, respectively. Scale bar (in "c") = 33 μm. The arrow in “d” and arrowheads in “h” point to pigmented cells.

B. Representative sections of subiliac lymph nodes from CT (a) and RXR$\alpha^{ep-/-}$ (b) taken 30 weeks after DMBA application and stained with antibodies to S-100. Arrows point to S-100 positive cells. Scale bar (in "a"), 33 μm.

C. Representative sections of subiliac lymph nodes from CT (a) and RXR$\alpha^{ep-/-}$ (b) taken 30 weeks after DMBA application and stained with antibodies to α-PEP8. The arrow indicates α-PEP8 positive malignant melanocytes. Scale bar (in "a"), 33 μm.

Figure 5. DMBA/TPA-induced epidermal tumors and melanocytic growths in $VDR^{-/-}$ mice.

A. Dorsal view of CT (control, $VDR^{+/+}$)(left) and $VDR^{-/-}$ (right) mice 25 weeks after the start of DMBA/TPA treatment. Arrows point to papilloma-like epidermal tumors.

B. Formation of epidermal tumors in DMBA/TPA treated CT and $VDR^{-/-}$ mice, as determined macroscopically and plotted against the number of weeks after the start of TPA treatment. Values are expressed as mean +/- SEM (n=7 mice).

C. Length distribution of epidermal tumors in CT and $VDR^{-/-}$ mice, as measured 30 weeks after the start of the DMBA/TPA treatment. Values are expressed as mean +/- SEM (n=7 mice).

D. Size distribution of melanocytic growths in CT and $VDR^{-/-}$ mice. The number of MGs (1-2 and 2-4 mm in diameter) was determined on 7 CT and 7 $VDR^{-/-}$ mice, 30 weeks after the start of TPA treatment. Values are expressed as mean +/- SEM

E. Hematoxylin- and eosin-stained paraffin sections (5 μm thick) of (a) CT and (b) $VDR^{-/-}$ MGs in skin biopsies taken after 25 weeks of DMBA/TPA treatment. Scale bar=33 μm.

F. Electron microscopic analysis of (a) CT and (b) $VDR^{-/-}$ MGs taken after 25 weeks of DMBA/TPA treatment. N, nucleus. Scale bar: 2.5 μm.

Figure 6. DMBA/TPA-induced epidermal tumors and melanocytic growths in $PPAR\gamma^{ep-/-(a)}$ and $PPAR\gamma^{ep-/-(b)}$ mice.

A. Formation of papilloma like epidermal tumors in DMBA/TPA-treated control (CT) and $PPAR\gamma^{ep-/-(a)}$ mice, as determined macroscopically and plotted against the number of weeks after the start of the TPA treatment. Values are expressed as mean +/- SEM (n=7 mice).

B. Dorsal view of CT [K14-Cre-$ER^{T2(0/0)}$/$PPAR\gamma^{L2/L2}$] (left) and $PPAR\gamma^{ep-/-(a)}$ (right) mice 25 weeks after the start of DMBA/TPA treatment.

C. Length distribution of epidermal tumors in CT and $PPAR\gamma^{ep-/-(a)}$ mice as measured after 25 weeks of DMBA/TPA treatment. Values are expressed as mean

+/- SEM (n=7 mice).

D. Formation of papilloma-like tumors in DMBA/TPA-treated PPARγ $^{ep-/-(b)}$ mice in which PPARγ was ablated in keratinocytes during TPA promotion. Values are expressed as mean +/- SEM (n = 7 mice).

E. Length distribution of epidermal tumors in CT and PPARγ $^{ep-/-(b)}$ mice, after 25 weeks of TPA treatment. Values are expressed as mean +/- SEM (n = 7 mice).

F. Size distribution (diameter) of MGs in CT, PPARγ $^{ep-/-(a)}$ and PPARγ $^{ep-/-(b)}$ mice. The number of MGs (1 – 2 and 2 – 4 mm in diameter) was determined on 7 CT, 7 PPARγ $^{ep-/-(a)}$ and 7 PPARγ $^{ep-/-(b)}$ mice, after 25 weeks of TPA treatment. Values are expressed as mean +/- SEM.

Figure 7. (A) RT-PCR analyses on skin biopsies from CT and RXRα$^{ep-/-(a)}$ (MT) mice. Semi-quantitative RT-PCR was performed for SCF(248+164), SCF(220), c-kit, E-cadherin, PTEN and HPRT on dorsal skin biopsies from control (CT) and RXRα$^{ep-/-(a)}$ mutant mice collected after 20-22 weeks of DMBA/TPA treatment as indicated. HPRT was used as an internal control. SCF(248+164) and SCF(220) represent the longer and the shorter membrane bound form of SCF after deletion of exon 6, respectively.

(B-D) Quantitative real-time RT-PCR determination of relative RNA levels of PTEN (B), E-cadherin (C) and SCF (D) in dorsal skin biopsies from CT, VDR$^{-/-}$ mice, and RXRα$^{ep-/-}$ or PPARγ$^{ep-/-}$ mice 4 weeks after Tamoxifen-treatment. In all three cases no DMBA/TPA treatment was applied. *P< 0.05, **P<0.01 and #, p≥ 0.05.

Table I. Incidence of malignant progression in papillomas generated by a 30-week DMBA/TPA treatment of mice selectively lacking either RXRα or PPARγ in epidermal keratinocytes, or VDR$^{-/-}$ mice.

Epidermal tumors obtained 30 weeks after DMBA/TPA application from 6 control, 6 RXRα$^{ep-/-(a)}$, 6 PPARγ$^{ep-/-(a)}$ and 6 VDR$^{-/-}$ mice were analysed and histologically classified, as indicated.

References :

Altucci, L., and Gronemeyer, H. (2001). The promise of retinoids to fight against cancer. Nat Rev Cancer *1*, 181-193.

Bar-Eli, M. (2001). Gene regulation in melanoma progression by the AP-2 transcription factor. Pigment Cell Res *14*, 78-85.

Chambon, P. (1996). A decade of molecular biology of retinoic acid receptors. Faseb J *10*, 940-954.

Chapellier, B., Mark, M., Messaddeq, N., Calleja, C., Warot, X., Brocard, J., Gerard, C., Li, M., Metzger, D., Ghyselinck, N. B., and Chambon, P. (2002). Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled. Embo J *21*, 3402-3413.

Chida, K., Hashiba, H., Fukushima, M., Suda, T., and Kuroki, T. (1985). Inhibition of tumor promotion in mouse skin by 1 alpha,25-dihydroxyvitamin D3. Cancer Res *45*, 5426-5430.

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. Nature *420*, 860-867.

Darwiche, N., Celli, G., Tennenbaum, T., Glick, A. B., Yuspa, S. H., and De Luca, L. M. (1995). Mouse skin tumor progression results in differential expression of retinoic acid and retinoid X receptors. Cancer Res *55*, 2774-2782.

Darwiche, N., Scita, G., Jones, C., Rutberg, S., Greenwald, E., Tennenbaum, T., Collins, S. J., De Luca, L. M., and Yuspa, S. H. (1996). Loss of retinoic acid receptors in mouse skin and skin tumors is associated with activation of the ras(Ha) oncogene and high risk for premalignant progression. Cancer Res *56*, 4942-4949.

De Luca, L. M., Tarone, R., Huynh, M., Jones, C. S., and Chen, L. C. (1996). Dietary retinoic acid inhibits mouse skin carcinogenesis irrespective of age at initiation. Nutr Cancer *25*, 249-257.

Demunter, A., Ahmadian, M. R., Libbrecht, L., Stas, M., Baens, M., Scheffzek, K., Degreef, H., De Wolf-Peeters, C., and van Den Oord, J. J. (2001). A novel N-ras mutation in malignant melanoma is associated with excellent prognosis. Cancer Res *61*, 4916-4922.

Epstein, J. H. (1992). Experimental models for primary melanoma. Photodermatol Photoimmunol Photomed *9*, 91-98.

Fisher, G. J., and Voorhees, J. J. (1996). Molecular mechanisms of retinoid actions in skin. Faseb J *10*, 1002-1013.

Fowler, A. J., Sheu, M. Y., Schmuth, M., Kao, J., Fluhr, J. W., Rhein, L., Collins, J. L., Willson, T. M., Mangelsdorf, D. J., Elias, P. M., and Feingold, K. R. (2003). Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. J Invest Dermatol *120*, 246-255.

Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Lochner, D., and Birchmeier, W. (1991). E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol *113*, 173-185.

Ghadially, F. N. (1997). Ultrastructural Pathology of the Cell and Matrix, Vol 2, Fourth edition edn, Butterworth-Heinemann).

Goodall, J., Martinozzi, S., Dexter, T. J., Champeval, D., Carreira, S., Larue, L., and Goding, C. R. (2004). Brn-2 expression controls melanoma proliferation and is directly regulated by beta-catenin. Mol Cell Biol *24*, 2915-2922.

Hardy, M. H. (1992). The secret life of the hair follicle. Trends Genet *8*, 55-61.

Hirobe, T. (1984). Histochemical survey of the distribution of the epidermal melanoblasts and melanocytes in the mouse during fetal and postnatal periods. Anat Rec *208*, 589-594.

Hirobe, T., Furuya, R., Akiu, S., Ifuku, O., and Fukuda, M. (2002). Keratinocytes control the proliferation and differentiation of cultured epidermal melanocytes from ultraviolet radiation B-induced pigmented spots in the dorsal skin of hairless mice. Pigment Cell Res *15*, 391-399.

Hong, W. K., and Itri, L. M. (1994). Retinoids and human cancer. In The Retinoids, M. B. Sporn, A. B. Roberts, and D. S. Goodman, eds. (New York, Raven Press), pp. 597-658.

Hsu, M. Y., Wheelock, M. J., Johnson, K. R., and Herlyn, M. (1996). Shifts in cadherin profiles between human normal melanocytes and melanomas. J Investig Dermatol Symp Proc *1*, 188-194.

Huang, C., Ma, W. Y., Dawson, M. I., Rincon, M., Flavell, R. A., and Dong, Z. (1997). Blocking activator protein-1 activity, but not activating retinoic acid response element, is required for the antitumor promotion effect of retinoic acid. Proc Natl Acad Sci U S A *94*, 5826-5830.

Huang, S., Jean, D., Luca, M., Tainsky, M. A., and Bar-Eli, M. (1998). Loss of AP-2 results in downregulation of c-KIT and enhancement of melanoma tumorigenicity and metastasis. Embo J *17*, 4358-4369.

Husain, Z., Pathak, M. A., Flotte, T., and Wick, M. M. (1991). Role of ultraviolet radiation in the induction of melanocytic tumors in hairless mice following 7,12-dimethylbenz(a)anthracene application and ultraviolet irradiation. Cancer Res *51*, 4964-4970.

Imai, T., Takakuwa, R., Marchand, S., Dentz, E., Bornert, J. M., Messaddeq, N., Wendling, O., Mark, M., Desvergne, B., Wahli, W.*, et al.* (2004). Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. Proc Natl Acad Sci U S A *101*, 4543-4547.

Ise, K., Nakamura, K., Nakao, K., Shimizu, S., Harada, H., Ichise, T., Miyoshi, J., Gondo, Y., Ishikawa, T., Aiba, A., and Katsuki, M. (2000). Targeted deletion of the H-ras gene decreases tumor formation in mouse skin carcinogenesis. Oncogene *19*, 2951-2956.

Jamal, S., and Schneider, R. J. (2002). UV-induction of keratinocyte endothelin-1 downregulates E-cadherin in melanocytes and melanoma cells. J Clin Invest *110*, 443-452.

Kastner, P., Grondona, J. M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J. L., Dolle, P., and Chambon, P. (1994). Genetic analysis of RXR alpha developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. Cell *78*, 987-1003.

Kensler, T. W., Dolan, P. M., Gange, S. J., Lee, J. K., Wang, Q., and Posner, G. H. (2000). Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. Carcinogenesis *21*, 1341-1345.

Kim, D. J., Akiyama, T. E., Harman, F. S., Burns, A. M., Shan, W., Ward, J. M., Kennett, M. J., Gonzalez, F. J., and Peters, J. M. (2004). Peroxisome proliferator-activated receptor beta (delta)-dependent regulation of ubiquitin C expression contributes to attenuation of skin carcinogenesis. J Biol Chem *279*, 23719-23727.

Kindblom, L. G., Lodding, P., Rosengren, L., Baudier, J., and Haglid, K. (1984). S-100 protein in melanocytic tumors. An immunohistochemical investigation of benign and malignant melanocytic tumors and metastases of malignant melanoma and a characterization of the antigen in comparison to human brain. Acta Pathol Microbiol Immunol Scand [A] *92*, 219-230.

Kopelovich, L., Fay, J. R., Glazer, R. I., and Crowell, J. A. (2002). Peroxisome proliferator-activated receptor modulators as potential chemopreventive agents. Mol Cancer Ther *1*, 357-363.

Kroumpouzos, G., Urabe, K., Kobayashi, T., Sakai, C., and Hearing, V. J. (1994). Functional analysis of the slaty gene product (TRP2) as dopachrome tautomerase and the effect of a point mutation on its catalytic function. Biochem Biophys Res Commun *202*, 1060-1068.

Kumar, R., Angelini, S., Snellman, E., and Hemminki, K. (2004). BRAF mutations are common somatic events in melanocytic nevi. J Invest Dermatol *122*, 342-348.

Kunisada, T., Lu, S. Z., Yoshida, H., Nishikawa, S., Mizoguchi, M., Hayashi, S., Tyrrell, L., Williams, D. A., Wang, X., and Longley, B. J. (1998a). Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. J Exp Med *187*, 1565-1573.

Kunisada, T., Yamazaki, H., and Hayashi, S. I. (2001). Review: ligands for receptor tyrosine kinases expressed in the skin as environmental factors for melanocyte development. J Investig Dermatol Symp Proc *6*, 6-9.

Kunisada, T., Yamazaki, H., Hirobe, T., Kamei, S., Omoteno, M., Tagaya, H., Hemmi, H., Koshimizu, U., Nakamura, T., and Hayashi, S. I. (2000). Keratinocyte expression of transgenic hepatocyte growth factor affects melanocyte development, leading to dermal melanocytosis. Mech Dev *94*, 67-78.

Kunisada, T., Yoshida, H., Yamazaki, H., Miyamoto, A., Hemmi, H., Nishimura, E., Shultz, L. D., Nishikawa, S., and Hayashi, S. (1998b). Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. Development *125*, 2915-2923.

Kwa, R. E., Campana, K., and Moy, R. L. (1992). Biology of cutaneous squamous cell carcinoma. J Am Acad Dermatol *26*, 1-26.

Laud, K., Kannengiesser, C., Avril, M. F., Chompret, A., Stoppa-Lyonnet, D., Desjardins, L., Eychene, A., Demenais, F., Lenoir, G. M., and Bressac-de Paillerets, B. (2003). BRAF as a melanoma susceptibility candidate gene? Cancer Res *63*, 3061-3065.

Laudet, V. a. G., H (2002). The Nuclear Receptor FactsBook, First edn (London, UK, Academic Press).

Li, G., Schaider, H., Satyamoorthy, K., Hanakawa, Y., Hashimoto, K., and Herlyn, M. (2001a). Downregulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development. Oncogene *20*, 8125-8135.

Li, M., Chiba, H., Warot, X., Messaddeq, N., Gerard, C., Chambon, P., and Metzger, D. (2001b). RXR-alpha ablation in skin keratinocytes results in alopecia and epidermal alterations. Development *128*, 675-688.

Li, M., Indra, A. K., Warot, X., Brocard, J., Messaddeq, N., Kato, S., Metzger, D., and Chambon, P. (2000). Skin abnormalities generated by temporally controlled RXRalpha mutations in mouse epidermis. Nature *407*, 633-636.

Livrea, M. A. (2000). Vitamin A and Retinoids: An update of Biological Aspects and Clinical Applications (Basel, Switzerland, Birkhauser Verlag).

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and et al. (1995). The nuclear receptor superfamily: the second decade. Cell *83*, 835-839.

Mao-Qiang, M., Fowler, A. J., Schmuth, M., Lau, P., Chang, S., Brown, B. E., Moser, A. H., Michalik, L., Desvergne, B., Wahli, W., *et al.* (2004). Peroxisome proliferator-activated receptor (PPAR)-gamma activation stimulates keratinocyte differentiation. J Invest Dermatol (In Press).

Metzger, D., Indra, A. K., Li, M., Chapellier, B., Calleja, C., Ghyselinck, N. B., and Chambon, P. (2003). Targeted conditional somatic mutagenesis in the mouse: temporally-controlled knock out of retinoid receptors in epidermal keratinocytes. Methods Enzymol *364*, 379-408.

Moon, R. C., Mehta, R. G., and Rao, K. J. V. N. (1994). Retinoids and cancer in experimental animals. In The Retinoids, M. B. Sporn, A. B. Roberts, and D. S. Goodman, eds. (New York, Raven Press), pp. 573-595.

Moore, R. J., Owens, D. M., Stamp, G., Arnott, C., Burke, F., East, N., Holdsworth, H., Turner, L., Rollins, B., Pasparakis, M., *et al.* (1999). Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med *5*, 828-831.

Murakami, T., Maki, W., Cardones, A. R., Fang, H., Tun Kyi, A., Nestle, F. O., and Hwang, S. T. (2002). Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. Cancer Res *62*, 7328-7334.

Nelson, M. A., Futscher, B. W., Kinsella, T., Wymer, J., and Bowden, G. T. (1992). Detection of mutant Ha-ras genes in chemically initiated mouse skin epidermis before the development of benign tumors. Proc Natl Acad Sci U S A *89*, 6398-6402.

Nikitakis, N. G., Hebert, C., Lopes, M. A., Reynolds, M. A., and Sauk, J. J. (2002). PPARgamma-mediated antineoplastic effect of NSAID sulindac on human oral squamous carcinoma cells. Int J Cancer *98*, 817-823.

Niles, R. M. (1995). Use of vitamins A and D in chemoprevention and therapy of cancer: control of nuclear receptor expression and function. Vitamins, cancer and receptors. Adv Exp Med Biol *375*, 1-15.

Nischt, R., Roop, D. R., Mehrel, T., Yuspa, S. H., Rentrop, M., Winter, H., and Schweizer, J. (1988). Aberrant expression during two-stage mouse skin carcinogenesis of a type I 47-kDa keratin, K13, normally associated with terminal differentiation of internal stratified epithelia. Mol Carcinog *1*, 96-108.

Owens, D. M., Romero, M. R., Gardner, C., and Watt, F. M. (2003). Suprabasal alpha6beta4 integrin expression in epidermis results in enhanced tumourigenesis and disruption of TGFbeta signalling. J Cell Sci *116*, 3783-3791.

Patel, L., Pass, I., Coxon, P., Downes, C. P., Smith, S. A., and Macphee, C. H. (2001). Tumor suppressor and anti-inflammatory actions of PPARgamma agonists are mediated via upregulation of PTEN. Curr Biol *11*, 764-768.

Pavan, W. J., and Tilghman, S. M. (1994). Piebald lethal (sl) acts early to disrupt the development of neural crest-derived melanocytes. Proc Natl Acad Sci U S A *91*, 7159-7163.

Payne, A. S., and Cornelius, L. A. (2002). The role of chemokines in melanoma tumor growth and metastasis. J Invest Dermatol *118*, 915-922.

Pollock, P. M., Harper, U. L., Hansen, K. S., Yudt, L. M., Stark, M., Robbins, C. M., Moses, T. Y., Hostetter, G., Wagner, U., Kakareka, J., *et al.* (2003). High frequency of BRAF mutations in nevi. Nat Genet *33*, 19-20.

Rundhaug, J. E., Gimenez-Conti, I., Stern, M. C., Budunova, I. V., Kiguchi, K., Bol, D. K., Coghlan, L. G., Conti, C. J., DiGiovanni, J., Fischer, S. M., *et al.* (1997). Changes in protein expression during multistage mouse skin carcinogenesis. Mol Carcinog *20*, 125-136.

Schmuth, M., Haqq, C. M., Cairns, W. J., Holder, J. C., Dorsam, S., Chang, S., Lau, P., Fowler, A. J., Chuang, G., Moser, A. H., *et al.* (2004). Peroxisome proliferator-activated receptor (PPAR)-beta/delta stimulates differentiation and lipid accumulation in keratinocytes. J Invest Dermatol *122*, 971-983.

Sheu, M. Y., Fowler, A. J., Kao, J., Schmuth, M., Schoonjans, K., Auwerx, J., Fluhr, J. W., Man, M. Q., Elias, P. M., and Feingold, K. R. (2002). Topical peroxisome proliferator activated receptor-alpha activators reduce inflammation in irritant and allergic contact dermatitis models. J Invest Dermatol *118*, 94-101.

Sotillo, R., Garcia, J. F., Ortega, S., Martin, J., Dubus, P., Barbacid, M., and Malumbres, M. (2001). Invasive melanoma in Cdk4-targeted mice. Proc Natl Acad Sci U S A *98*, 13312-13317.

Stratton, S. P., Dorr, R. T., and Alberts, D. S. (2000). The state-of-the-art in chemoprevention of skin cancer. Eur J Cancer *36*, 1292-1297.

Sucov, H. M., Dyson, E., Gumeringer, C. L., Price, J., Chien, K. R., and Evans, R. M. (1994). RXR alpha mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. Genes Dev *8*, 1007-1018.

Suganuma, M., Okabe, S., Marino, M. W., Sakai, A., Sueoka, E., and Fujiki, H. (1999). Essential role of tumor necrosis factor alpha (TNF-alpha) in tumor promotion as revealed by TNF-alpha-deficient mice. Cancer Res *59*, 4516-4518.

Sulis, M. L., and Parsons, R. (2003). PTEN: from pathology to biology. Trends Cell Biol *13*, 478-483.

Sun, S. Y., and Lotan, R. (2002). Retinoids and their receptors in cancer development and chemoprevention. Crit Rev Oncol Hematol *41*, 41-55.

Suzuki, A., Itami, S., Ohishi, M., Hamada, K., Inoue, T., Komazawa, N., Senoo, H., Sasaki, T., Takeda, J., Manabe, M., *et al.* (2003). Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. Cancer Res *63*, 674-681.

Tennenbaum, T., Lowry, D., Darwiche, N., Morgan, D. L., Gartsbein, M., Hansen, L., De Luca, L. M., Hennings, H., and Yuspa, S. H. (1998). Topical retinoic acid reduces skin papilloma formation but resistant papillomas are at high risk for malignant conversion. Cancer Res *58*, 1435-1443.

Tennenbaum, T., Weiner, A. K., Belanger, A. J., Glick, A. B., Hennings, H., and Yuspa, S. H. (1993). The suprabasal expression of alpha 6 beta 4 integrin is associated with a high risk for malignant progression in mouse skin carcinogenesis. Cancer Res *53*, 4803-4810.

Wehrle-Haller, B., and Weston, J. A. (1995). Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. Development *121*, 731-742.

Westergaard, M., Henningsen, J., Svendsen, M. L., Johansen, C., Jensen, U. B., Schroder, H. D., Kratchmarova, I., Berge, R. K., Iversen, L., Bolund, L., *et al.* (2001). Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. J Invest Dermatol *116*, 702-712.

Wishart, M. J., and Dixon, J. E. (2002). PTEN and myotubularin phosphatases: from 3-phosphoinositide dephosphorylation to disease. Phosphatase and tensin homolog deleted on chromosome ten. Trends Cell Biol *12*, 579-585.

Wu, H., Goel, V., and Haluska, F. G. (2003). PTEN signaling pathways in melanoma. Oncogene *22*, 3113-3122.

Yoshida, H., Kunisada, T., Grimm, T., Nishimura, E. K., Nishioka, E., and Nishikawa, S. I. (2001). Review: melanocyte migration and survival controlled by SCF/c-kit expression. J Investig Dermatol Symp Proc *6*, 1-5.

Yoshida, H., Kunisada, T., Kusakabe, M., Nishikawa, S., and Nishikawa, S. I. (1996). Distinct stages of melanocyte differentiation revealed by anlaysis of nonuniform pigmentation patterns. Development *122*, 1207-1214.

You, M. J., Castrillon, D. H., Bastian, B. C., O'Hagan, R. C., Bosenberg, M. W., Parsons, R., Chin, L., and DePinho, R. A. (2002). Genetic analysis of Pten and Ink4a/Arf interactions in the suppression of tumorigenesis in mice. Proc Natl Acad Sci U S A *99*, 1455-1460.

Yudoh, K., Matsuno, H., and Kimura, T. (1999). 1alpha,25-dihydroxyvitamin D3 inhibits in vitro invasiveness through the extracellular matrix and in vivo pulmonary metastasis of B16 mouse melanoma. J Lab Clin Med *133*, 120-128.

Yuspa, S. H. (1998). The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. J Dermatol Sci *17*, 1-7.

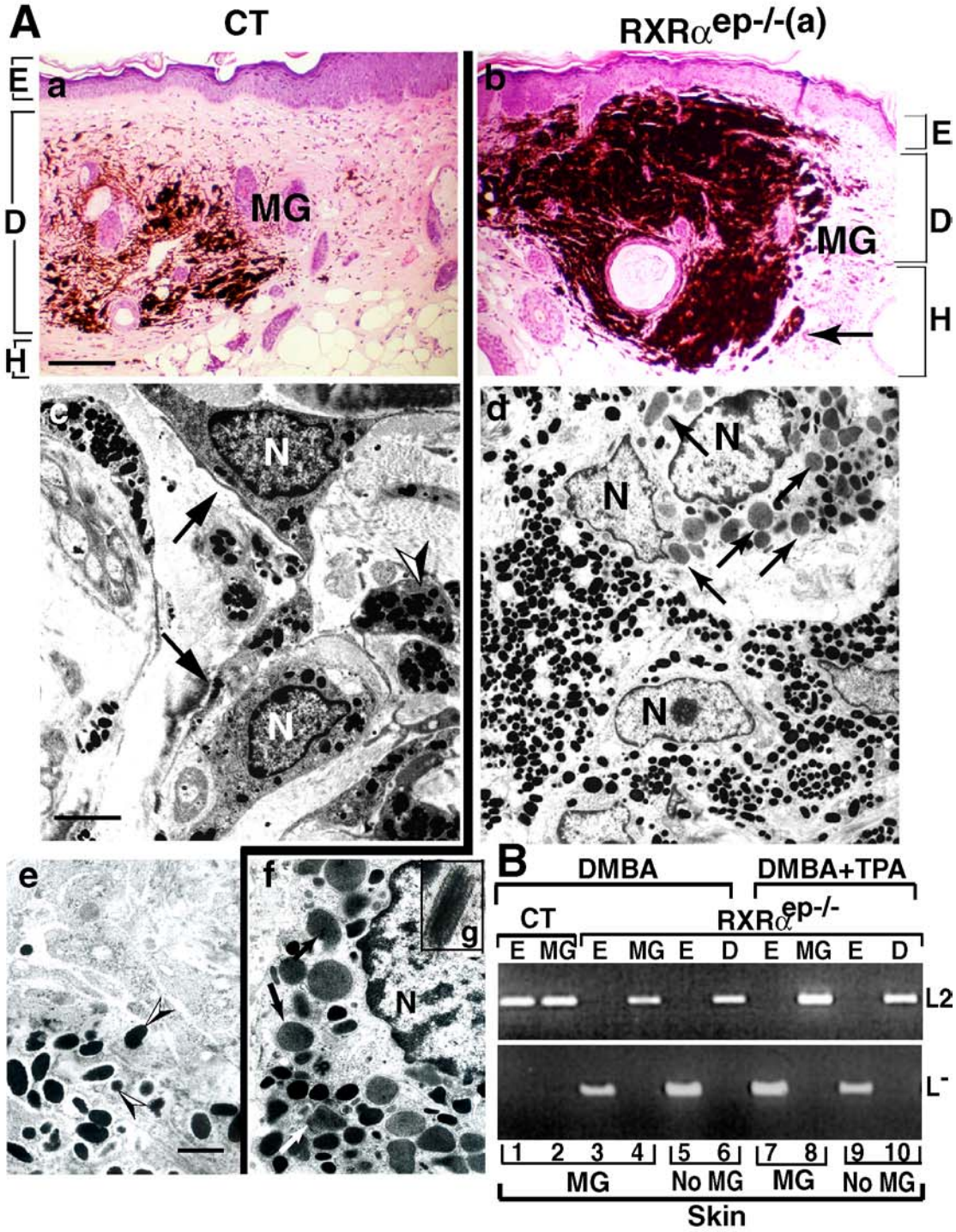
A
CT
RXRαep-/-(a)
E
D
H
a
MG
b
MG
c
N
N
d
N
N
N
e
f
g
N
B
DMBA
DMBA+TPA
CT
RXRαep-/-
E MG E MG E D E MG E D
L2
L-
1 2 3 4 5 6 7 8 9 10
MG
No MG
MG
No MG
Skin

Fig.3

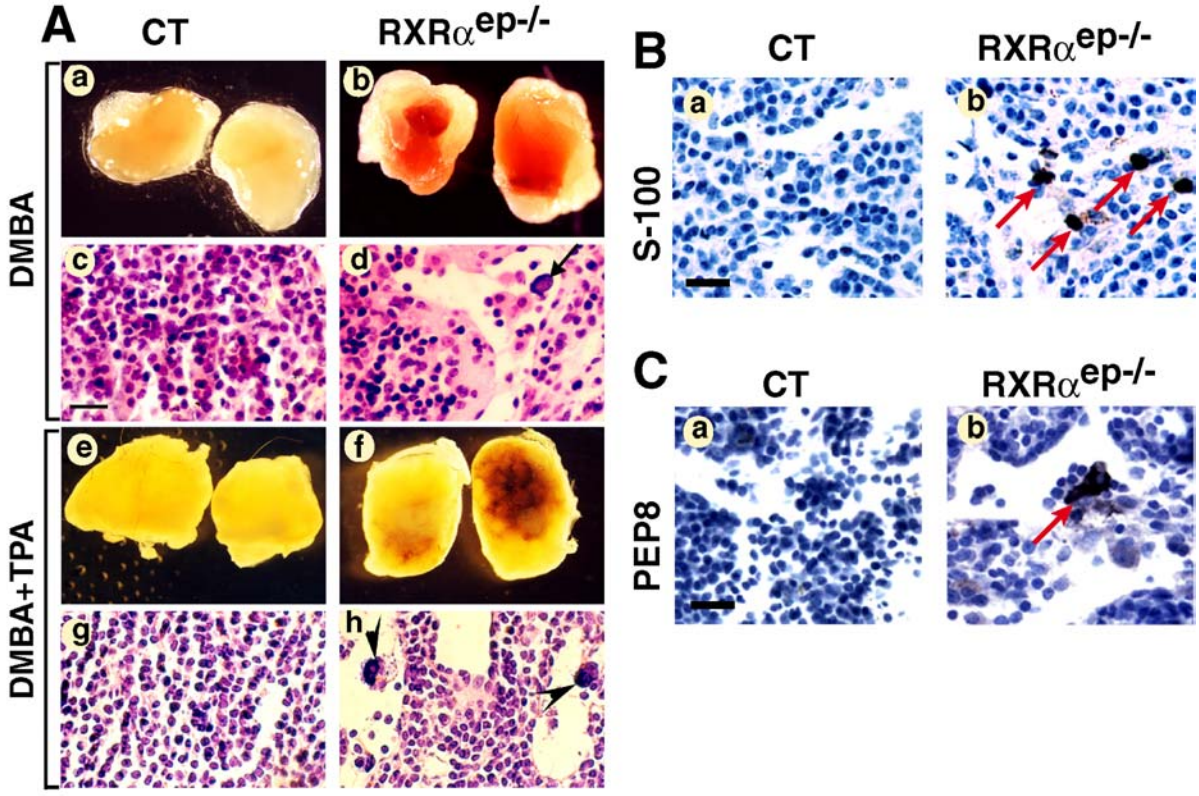
A
CT
RXRαep-/-
DMBA
DMBA+TPA
a
b
c
d
e
f
g
h
B
CT
RXRαep-/-
S-100
a
b
C
CT
RXRαep-/-
PEP8
a
b

Fig.4

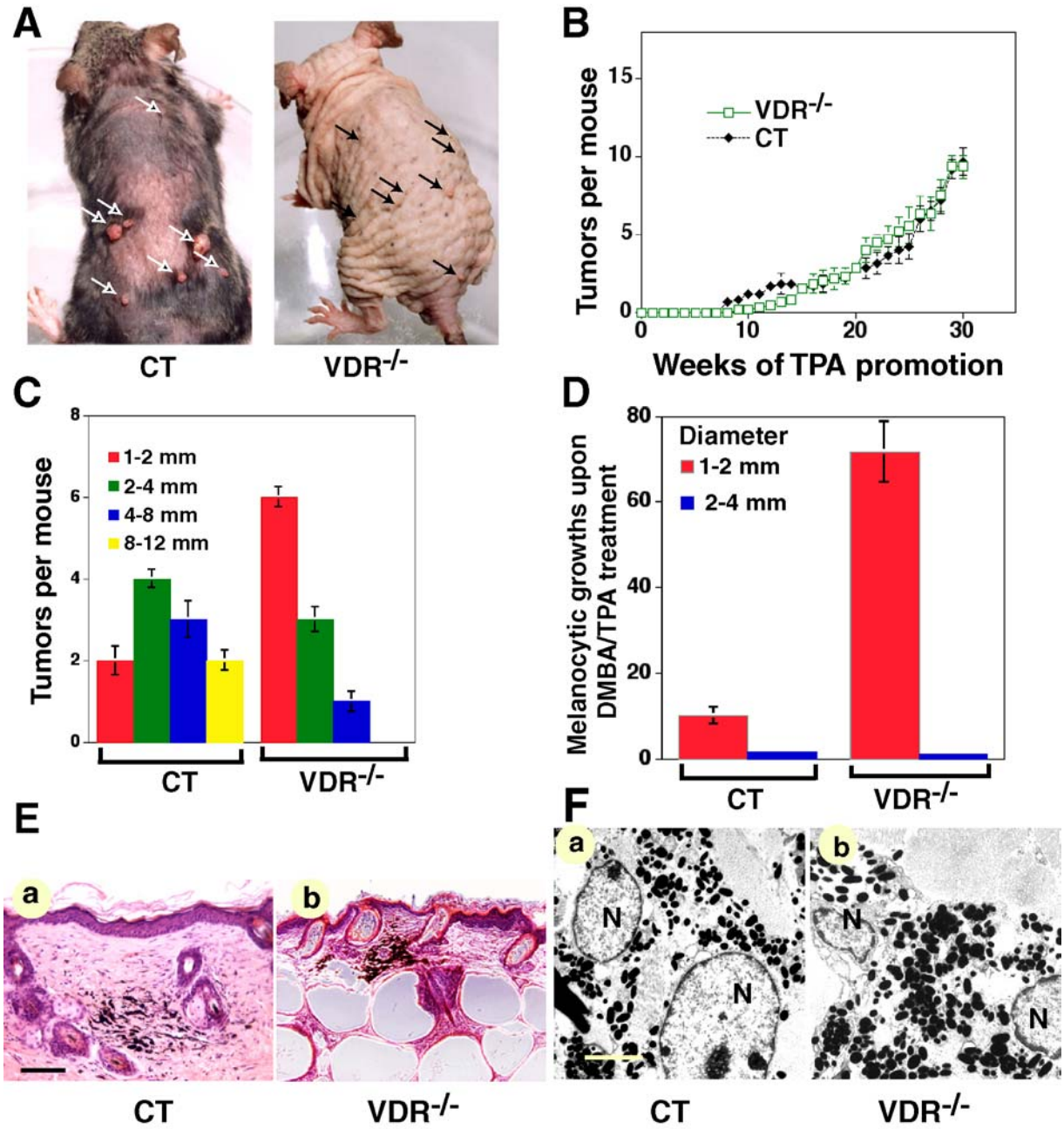
A
CT
VDR-/-
B
Tumors per mouse
VDR-/-
CT
Weeks of TPA promotion
C
Tumors per mouse
1-2 mm
2-4 mm
4-8 mm
8-12 mm
CT
VDR-/-
D
Melanocytic growths upon DMBA/TPA treatment
Diameter
1-2 mm
2-4 mm
CT
VDR-/-
E
a
b
CT
VDR-/-
F
a
b
N
CT
VDR-/-

Fig.5

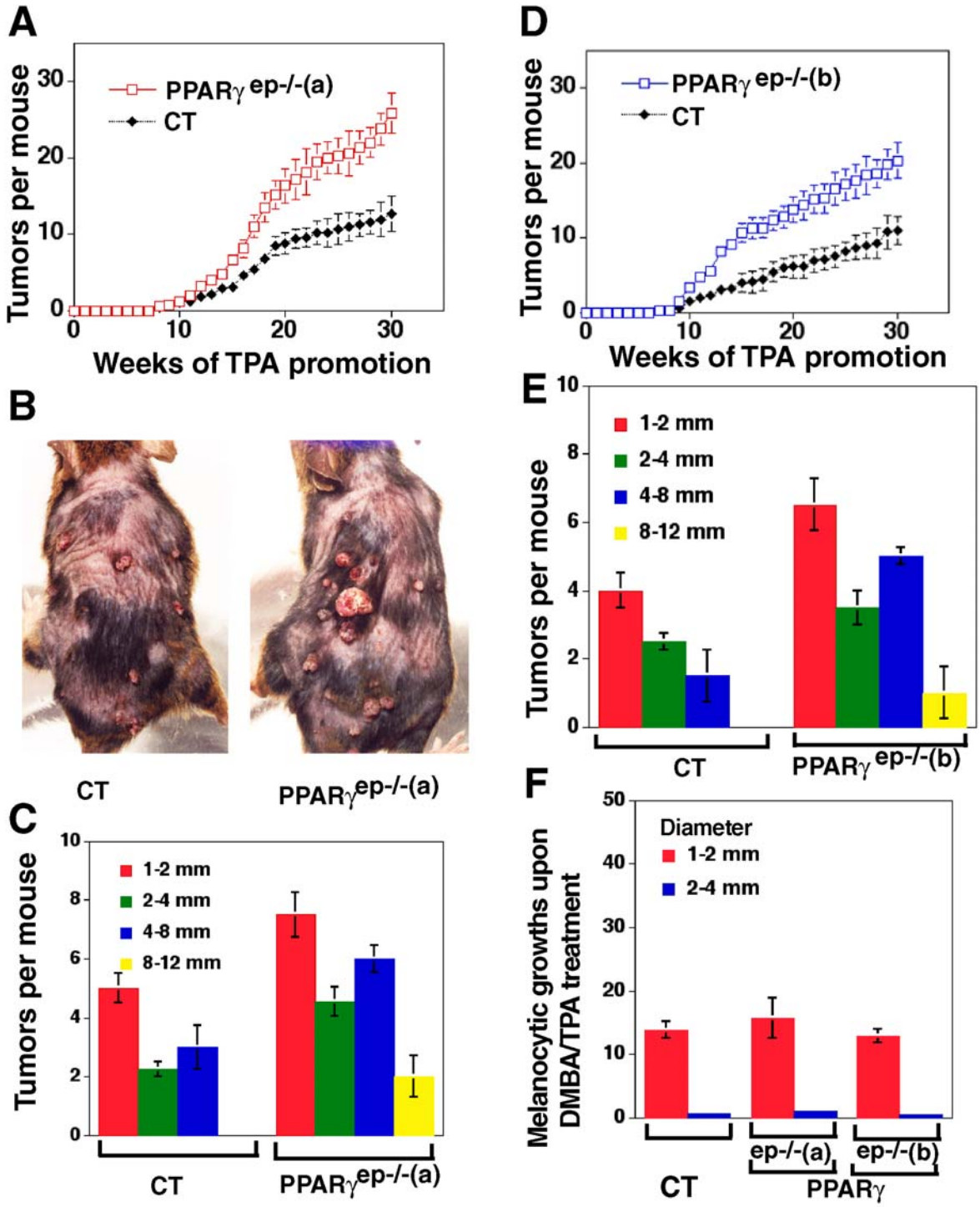
A
PPARγ ep-/-(a)
CT
Tumors per mouse
Weeks of TPA promotion
B
CT
PPARγep-/-(a)
C
1-2 mm
2-4 mm
4-8 mm
8-12 mm
Tumors per mouse
CT
PPARγep-/-(a)
D
PPARγ ep-/-(b)
CT
Tumors per mouse
Weeks of TPA promotion
E
1-2 mm
2-4 mm
4-8 mm
8-12 mm
Tumors per mouse
CT
PPARγ ep-/-(b)
F
Diameter
1-2 mm
2-4 mm
Melanocytic growths upon DMBA/TPA treatment
CT
ep-/-(a)
ep-/-(b)
PPARγ

Fig.6

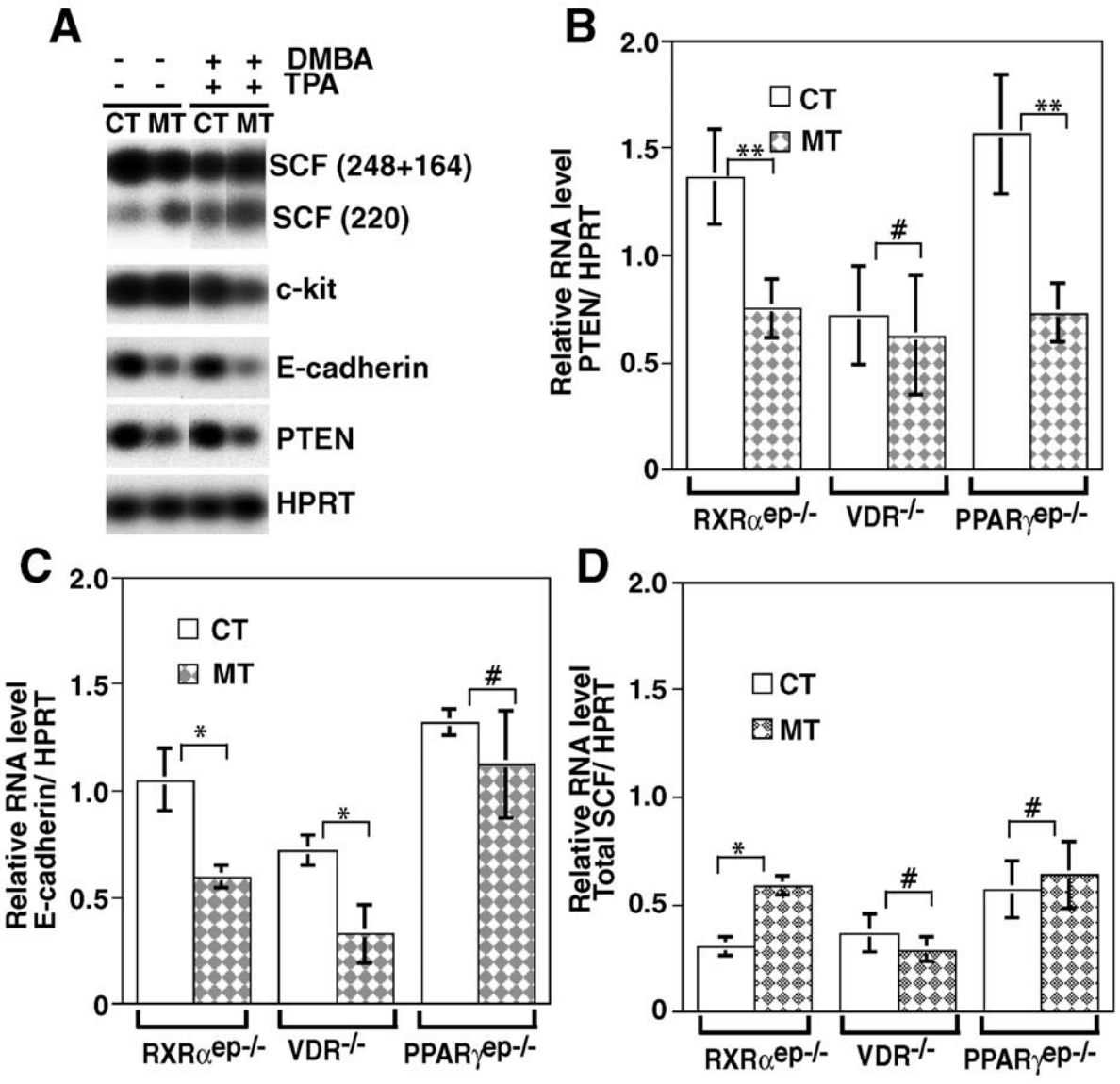
A
- - + + DMBA
- - + + TPA
CT MT CT MT
SCF (248+164)
SCF (220)
c-kit
E-cadherin
PTEN
HPRT
B
Relative RNA level PTEN/ HPRT
CT
MT
RXRαep-/-
VDR-/-
PPARγep-/-
C
Relative RNA level E-cadherin/ HPRT
D
Relative RNA level Total SCF/ HPRT

Fig. 7

Table I

Incidence of malignant progression in papillomas generated by a 30-week DMBA/TPA treatment of mice selectively lacking either RXRα or PPARγ in epidermal keratinocytes, or VDR-/- null mice

Mice	Papillomas			Papillomas with squamous cell carcinomas (SCC)				Papillomas with basal cell carcinomas (BCC)
	Benign	With in situ carcinoma	With FC	Well differentiated	Moderately differentiated	Poorly differentiated	Anaplastic carcinomas	
Control	29/35 (83%)	1/35 (3%)	5/35 (14%)	0/35	0/35	0/35	0/35	0/35
RXRα$^{ep-/-}$	11/35 (32%)	1/35 (3%)	4/35 (11%)	11/35 (31%)	3/35 (9%)	2/35 (9%)	1/35 (3%)	2/35 (6%)
PPARγ$^{ep-/-}$	25/40 (70%)	0/40	2/40 (5%)	7/40 (17.5%)	0/40	2/40 (5%)	1/40 (2.5%)	0/40
VDR$^{-/-}$	38/40 (35%)	0/40	2/40 (5%)	0/40	0/40	0/40	0/40	0/40

FC, foci of early squamous cell carcinoma

2.2 Role of PPARα in skin carcinogenesis.

2.2.1 Introduction.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily (Keller et al., 1993; Wahli et al., 1999),. Three PPAR isotypes, PPARα, PPARδ/β and PPARγ have been identified (Fajas et al., 1998; Fajas et al., 1999; Issemann et al., 1993). PPARs are activated by the peroxisome proliferators, fibrates, fatty acids and eicosanoids (Forman et al., 1997; Kliewer et al., 1997). They modulate the transcription of a variety of target genes involved in lipid metabolism (Wahli et al., 1999), and play a key role in the control of proliferation and apoptosis in various cell types (Asou et al., 1999; Cattley, 2003; Hanley et al., 1998).

All PPAR isotypes are expressed in mouse epidermis during fetal development. Their expression decreases progressively from the interfollicular epithelium after birth (Michalik et al., 2001). In adult epidermis PPARα and PPARβ are the prominent isotypes, and it has been shown that expression of PPARα and PPARβ is increased in adult epidermis after various stimuli (Braissant et al., 1996; Braissant and Wahli, 1998; Michalik et al., 2001).

Several experimental approaches demonstrated that PPARs play key roles in skin development and in the maintenance of skin homeostasis. PPARβ activators stimulate differentiation of keratinocytes in culture (Tan et al., 2001) and in vivo (Schmuth et al., 2004). Moreover, it has been shown that PPARβ plays key roles in skin wound healing, keratinocyte response to inflammation and keratinocyte apoptosis (Di-Poi et al., 2002; Michalik et al., 2001; Tan et al., 2001). Recently, Kim and co-workers demonstrated that PPARβ null mice exhibited an increased susceptibility to DMBA/TPA-induced skin carcinogenesis (Kim et al., 2004). PPARγ activators also stimulate differentiation of epidermal keratinocytes in vivo, and improve permeability barrier recovery after mechanical disruption (Mao-Qiang, et al. in press). Moreover, it was shown that PPARγ mediate the non-steroidal anti-inflammatory drugs (NSAID) antineoplastic effect on human oral squamous carcinoma cells (Nikitakis et al., 2002), and that PPARγ specific ligands can block melanoma cell growth in culture (Mossner et al., 2002). As we show in section 2.1, $PPAR\gamma^{ep-/-}$ mice, in which PPARγ was selectively ablated in epidermal keratinocytes, exhibited an increased susceptibility to

chemically- induced skin carcinogenesis, demonstrating a key role of PPARγ during chemically-induced skin carcinogenesis.

PPARα ligands stimulate the differentiation of fetal epidermal permeability barrier (Hanley et al., 1997), and possess receptor mediated, anti-inflammatory activities in both irritant and allergic contact dermatitis animal models (Sheu et al., 2002). Moreover, PPARα activators can reverse the expression of inflammatory cytokines induced by UVB in keratinocytes in culture (Kippenberger et al., 2001). Interestingly, it has been shown that PPARα activators partially inhibit tumor formation in a two-step chemical carcinogenesis protocol (Thuillier et al., 2000), suggesting that PPARα, may also play an important role during skin carcinogenesis. To investigate the possible role of PPARα in skin carcinogenesis, we subjected PPARα null mice to a chemically-induced skin carcinogenesis protocol.

2.2.2 Material and methods.

Mice and carcinogenic treatment. PPARα null mice were previously described (Lee and Gonzalez, 1996). PPARα null mutants and control wild-type (CT) animals were housed in a temperature and humidity controlled room with 12h cycles of light and dark. 10-12 week-old PPARα (n=10) and WT (n=10) mice were shaved to synchronize the hair cycle and treated the day after with a single dose of 50 μg DMBA in 100 μl acetone. One week after the DMBA application, 5 μg TPA (in 200 μl acetone) was applied topically twice a week. The number of papillomas per mouse was weekly recorded.

2.2.3 Results

PPARα null mice exhibit an increased susceptibility to chemically-induced skin tumors.

To determine whether PPARα is involved in skin carcinogenesis, we subjected PPARα null mice to a two-step chemical carcinogenesis protocol. PPARα null and CT mice were treated with a single dose of DMBA, followed by twice a week applications of TPA. Both DMBA/TPA-treated PPARα null and CT mice developed papilloma-like tumors after 10 and 12 weeks of TPA promotion, respectively (**figure 14A**). After 15 weeks of TPA promotion, all PPARα null mice developed papillomas, whereas only 80% of CT mice exhibited papillomas. Moreover, PPARα null mice developed two to three times more tumors than CT mice (**figure 14B**).

After 27 weeks of TPA promotion, an average of 8 and 20 tumors per mouse were observed on CT and PPARα null mice, respectively (*p<0.001*) (**figure 14 B and C**). Thus, upon DMBA/TPA treatment, PPARα null mice exhibit a lower latency and increased tumor multiplicity than CT mice. Notice that PPARα null and CT mice treated with either TPA or DMBA alone did not develop any tumor (data not shown).

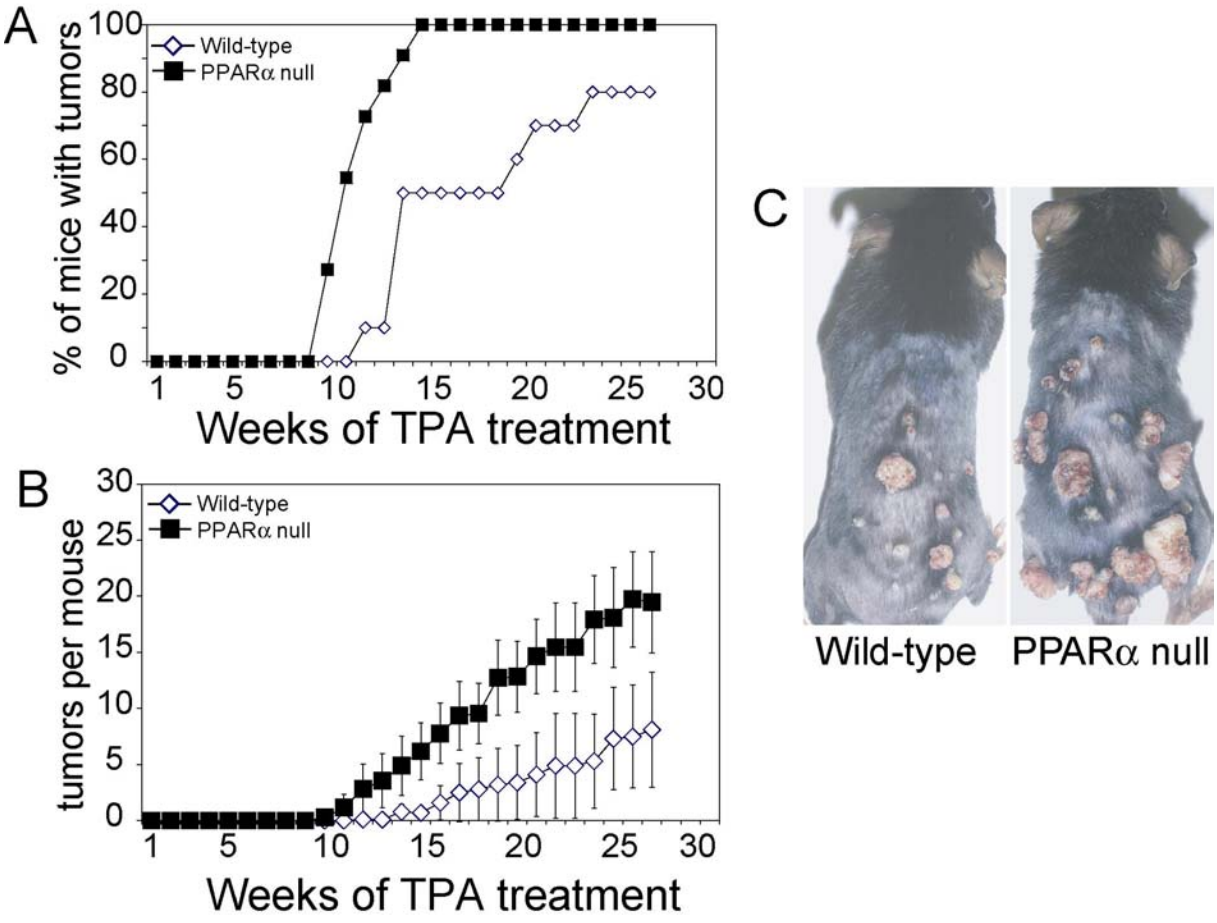

Figure 14. 10-12 week-old PPARα null (n=11) and wild-type (n=8) mice were subjected to a two-step chemical carcinogenesis protocol. (**A**) Incidence of mice with tumors. (**B**) average number of tumors per mouse +/- SEM. (**C**) Dorsal view of DMBA/TPA treated wild-type and PPARα null mice, after 25 weeks of TPA treatment.

2.2.4 Perspectives.

To investigate whether PPARα null mice exhibit an enhanced response to TPA treatment, we will compare the morphological and biochemcal alterations induced by topical application of TPA to back skin of PPARα and WT mice, including epidermal thickness, keratinocyte hyperproliferation and suprabasal expression of integrins α6β4. We have taken skin biopsies from unchallenged PPARα null and WT mice, or PPARα null and WT mice treated either with acetone or with two topical applications of TPA that we will use for histological and IHC analysis.

In our previous study, both RXRα$^{ep-/-}$ and PPARγ$^{ep-/-}$ mice exhibit increased suceptibility to skin tumor formation and high frequency malignant conversion, in response to DMBA/TPA treatment. To determine if PPARα plays a role in the malignant conversion of DMBA/TPA-induced skin tumors, DMBA/TPA treated PPARα and CT mice will be sacrificed after 30 weeks of TPA promotion, tumor biosies will be taken and analyzed by histology.

Moreover, we found that the increased susceptibility to papilloma and carcinoma formation in RXRα$^{ep-/-}$ and PPARγ$^{ep-/-}$ mice was correlated with a downregulation of the tumor suppressor Pten, in the skin of these animals. Moreover, RXRα$^{ep-/-}$ mice also exhibited increased expression of SCF. To determine if the expression of Pten and SCF is also deregulated in the skin of PPARα mice, we have taken skin biopsies from PPARα null and CT mice and extracted the total RNA. We will measure the transcript levels of Pten and SCF in these samples by real-time quantitative RT-PCR. Because PPARα is also expressed in other cells types than keratinocytes, the generation of keratinocyte-specific PPARα mutant mice will help to better understand its role during skin carcinogenesis.

2.3 Interaction between RXRα and the Ha-ras oncogene during mouse skin carcinogenesis

2.3.1 Introduction.

Retinoids, the active derivatives of vitamin A, are involved in the control of growth and differentiation of normal, pre-malignant and malignant cell types (Altucci and Gronemeyer, 2001). Vitamin A deficiency in humans and animal models is associated with epithelial squamous metaplasia (Hong and Itri, 1994; Moon et al., 1994). On the other hand, natural and synthetic retinoids can efficiently block chemically-induced skin tumor formation (Tennenbaum et al., 1998) and prevent malignant conversion in mice (Chen et al., 1994a; Chen et al., 1995; De Luca et al., 1996), and are used as chemo preventive agents for epithelial cancers in humans (Altucci and Gronemeyer, 2001; Lippman and Lotan, 2000; Niles, 2000). Retinoids action is mediated by members of two families of nuclear receptors (NRs), the retinoic acid receptors (RARα, β and γ) and the retinoic X receptors (RXRα, β and γ) (Chambon, 1996; Mangelsdorf and Evans, 1995). Aberrant expression and function of RARs and RXRs that may contribute to carcinoma development, have been found during skin carcinogenesis, both in mouse (Darwiche et al., 1995; Darwiche et al., 1996) and human (Xu et al., 2001). Previous studies from our laboratory demonstrated that selective ablation of RXRα in epidermal keratinocytes during skin morphogenesis ($RXR\alpha^{ep-/-(c)}$ mice) (Li et al., 2001b), or induced in keratinocytes of adult mice ($RXR\alpha^{ep-/-}$ mice) (Li et al., 2000), results in extensive alopecia, epidermal hyperplasia, hair follicle degeneration, and skin inflammation (Li et al., 2001b; Li et al., 2000), demonstrating a key role for RXRα in skin homeostasis. Moreover, as shown in section 2.1, $RXR\alpha^{ep-/-}$ mice are more susceptible to chemically-induced skin tumors than wild-type mice. Indeed, $RXR\alpha^{ep-/-}$ mice treated with DMBA/TPA exhibit an increased epithelial tumor number, growth rate and incidence of malignant conversion when compared to control mice. Furthermore, $RXR\alpha^{ep-/-}$ mice developed malignant melanoma in response to a single topical application of DMBA. Thus, keratinocytic RXRα has a suppressive effect on DMBA/TPA-induced skin tumorigenesis. Moreover, when RXRα was ablated during the promotion stage of the two-step carcinogenesis protocol, several weeks after the DMBA initiation, resulted in similar increase in tumor formation and malignant conversion, indicating that keratinocytic RXRα plays a critical role during the promotion stage of chemical carcinogenesis.

As discussed in section 1.3.1, topical application of DMBA into mouse skin induces activation mutations in the H-ras oncogene in keratinocytes, (Pazzaglia et al., 2001; Quintanilla et al., 1986a). Mutation and activation of Ha-ras oncogene are critical events for the formation of skin tumors. Indeed, Ha-ras null mice develop significantly less tumors than wild-type mice in response to a DMBA/TPA protocol (Ise et al., 2000), and the tumors induced in Ha-ras null mice exhibit mutations in the K-ras gene, suggesting that activation of the K-ras gene can replace the Ha-ras activation at the initiation step (Ise et al., 2000). To further investigate the role of RXRα in the development of skin cancer, an in particular in the Ha-ras mediated carcinogenesis, we used the Tg.AC mouse model (described in section 1.3.2) as an alternative model to the two-stage chemical skin carcinogenesis protocol. Tg.AC transgenic mice carry an activated v-Ha-ras oncogene under the control of a partial ζ-globin promoter (Leder et al., 1990), and are considered as "genetically pre-initiated mice". Tg.AC mice develop skin tumors in response to specific carcinogens and tumor promoters in a very rapid and highly selective fashion (Battalora et al., 2001; Leder et al., 1990; Leder et al., 2002; Tennant et al., 2001). The Tg.AC mouse has been used to identify carcinogens (Tennant et al., 1995; Tennant et al., 1996) and for the study of genes involved in skin carcinogenesis (Humble et al., 1998; Lan et al., 2000; Leder et al., 2002). To study the role of RXRα during the promotion stage of Ha-ras-induced skin tumors, we generated Tg.AC/RXR$\alpha^{ep-/-(c)}$ and Tg.AC/RXR$\alpha^{ep-/-}$ mice, carrying the Ha-ras oncogene and in which the selective ablation of the RXRα alleles either occurred in epidermal keratinocytes during fetal epidermal morphogenesis or was induced in adult mice, repectively.

2.3.2 Materials and methods.

Mice. K14-Cre$^{tg/o}$/RXR$\alpha^{L2/L2}$ (RXR$\alpha^{ep-/-(c)}$) and K14-Cre-ER$^{T2(tg/o)}$/RXR$\alpha^{L2/L2}$ (RXR$\alpha^{ep-/-}$) mice, in which RXRα alleles are selectively ablated in epidermal keratinocytes during embryonic development or in adult mice, respectively, were described (Li et al., 2001b; Li et al., 2000). Tg.AC transgenic mice, carrying an activated Ha-ras oncogene under the control of a partial ζ-globin promoter (Leder et al., 1990), were purchased from Taconic. To selectively ablate RXRα alleles in epidermal keratinocytes of Tg.AC mice during skin morphogenesis, K14-Cre$^{tg/o}$/RXR$\alpha^{L2/L2}$ mice, carrying both RXRα floxed alleles and hemizygous for the K14-Cre transgene, were crossed with Tg.AC mice hemizygous for the ζ-globin Ha-ras transgene. The resulting Tg.AC/K14-Cre$^{tg/o}$/RXR$\alpha^{L2/+}$ and Tg.AC/RXR$\alpha^{L2/+}$ mice were intercrossed to generate Tg.AC/K14-Cre$^{tg/o}$/RXR$\alpha^{L2/L2}$ (Tg.AC/RXR$\alpha^{ep-/-(c)}$) and Tg.AC/RXR$\alpha^{L2/L2}$ control mice. To

selectively ablate RXRα alleles in epidermal keratinocytes of adult Tg.AC mice, K14-Cre-ER$^{T2(tg/o)}$/RXRα$^{L2/L2}$ mice were bred with hemizygous Tg.AC mice to generate Tg.AC/K14-Cre-ER$^{T2(tg/o)}$/RXRα$^{L2/+}$ and Tg.AC/RXRα$^{L2/+}$ mice. Breeding of Tg.AC/K14-Cre-ER$^{T2(tg/o)}$/RXRα$^{L2/+}$ with Tg.AC/RXRα$^{L2/+}$ mice resulted in Tg.AC/K14-Cre-ER^{T2}/RXRα$^{L2/L2}$, carrying the Ha-ras and the K14-Cre-ER^{T2} transgenes and both RXRα alleles floxed, and Tg.AC/RXRα$^{L2/L2}$ mice. 6-7 week-old Tg.AC/K14-Cre-ER$^{T2(tg/o)}$/RXRα$^{L2/L2}$ females were injected intraperitonealy with 0.1 mg of tamoxifen for 5 consecutive days (Indra et al., 1999) to generate Tg.AC/RXRα$^{ep-/-}$ mice. Tg.AC/RXRα$^{L2/L2}$ littermates were injected with the same dose of tamoxifen and used as controls (CT) in all experiments. Mice were genotyped for the presence of K14-Cre and K14-Cre-ER^{T2} transgenes and for the presense of wt, L2 or L- RXRα alleles by PCR as described (Li et al., 2000). The presence of the ζ-globin Ha-ras transgene was detected by PCR using primers directed to the SV40 polyadenylation sequence from the transgene (Fwd: 5'-AATTCTGAAGGAAAGTCC-3', reverse: 5'-TGGACAAACTACCTACAG–3'). The Tg.AC responder genotype was confirmed by Southern blotting (Kantz et al., 1999; Thompson et al., 2001). DNA was digested with BamHI and probed with a radiolabeled 660 bp DNA segment specific for the ζ-globin promoter of the transgene. The ζ-globin probe was obtained by PCR amplification from genomic DNA of Tg.AC transgenic mice (ζ-globin primers: fwd 5'-GACAGGGATGGCTCAGACAA–3'; reverse: 5'-CTGCTCGACAAGCTTTGATCT–3').

TPA treatment. Mice were housed in a temperature and humidity controlled room with 12h cycles of light and dark. Back skin of 8-9 week-old Tg.AC/RXRα$^{ep-/}$ and Tg.AC/RXRα$^{L2/L2}$ mice was shaved using electric clippers. One week after shaving, 5 μg of TPA (in 200 μl of acetone) were applied topically twice a week to the back skin of the animals. TPA promotion was continued for 13 weeks.

DMBA treatment. Back skin of 6-8 week-old RXRα$^{ep-/-(c)}$ and RXRα$^{L2/L2}$ mice was shaved and one day after shaving, a single dose of 50 μg of DMBA in 100 μl acetone was topically applied.

Histology and immunohistochemistry. Mice were anesthetized, skin and tumor biopsies were surgically removed, fixed in 4% paraformaldehide over night at 4°C and embedded in paraffin. 7 μm paraffin sections were stained with Hematoxylin and eosine for histological analysis. For IHC analysis, paraffin sections were deparaffinized, permeabilized with PBS Triton 0.1% for 2 min, blocked with 5% normal goat serum for 1h, incubated with a rabbit

polyclonal anti-keratin 13 antibody (gift from Prof. S. Yuspa, NIH) o/n at 4°C and with a goat anti-rabbit fluorescent secondary antibody for 1h and mounted with DAPI.

Real-Time RT-PCR. After surgical removal, skin biopsies were immediately frozen in liquid nitrogen**.** RNA was extracted from skin biopsies with Trizol^{R} Reagent (Gibco BRL, Life technologies) following the manufacturer's instructions. 3-5 μg of RNA were used for reverse transcription reaction using Superscript II reverse transcriptase (Gibco BRL, Life technologies) and 0.2 mM oligo dT in a final volume of 20 μl, following the manufacturer's instructions. cDNA preparations were diluted 1:10 or 1:20 in ultra pure (milliQ) water, and 2 μl of diluted cDNA were used for quantitative real-time PCR, using the Quantitect PCR amplification kit (Qiagen) in a final reaction volume of 10 μl, in a LightCycler instrument (Roche Diagnostics). To generate calibration curves, sequential dilutions of 3 μg of cDNA from CT mice skin were prepared. cDNA was diluted 10, 100, 1000 and 10000 times. A calibration curve with the 4 dilutions and the undiluted cDNA was generated for each transcript. The relative levels of expression for each gene were normalized to HPRT. The results represent the fold difference in the amount of transcript compared to the CT levels. Data shown represent the average of 3 independent quantifications. The primers used for RT-PCR were HPRT (s=5'-GTAATGATCAGTCAACGGGGGAC-3'; as=5'-CCAGCAAGCTTGCAACCTTAACCA-3'), odc (s=5'-GACGATAAGGATGCGTTCTATGTT-3'; as=5'-CTGGCAGCAT ACTTGATTTGAGA-3'), c-jun (s=5'-GAGCGGATCAAGGCAGAGAGGAAG; as=5'-TGTGCCACCTGTTCCCTGAGC-3'), cox-2 (s=5'-TCAAAACCGTGGGGAATGTAT-3'; as=5'-AGGATGTAGTGCACTGTGTTT-3'), c-myc (s=5'-AAAACGACAAGAGGCG GACAC-3'; as=5'-GCTTGTGCTCGTCTGCTTGAA-3'), IL1-RI (s=5'-CATCAAGGCAGTGGGAACTT-3'; as=5'-ACTTTTGCAACACT TGGCCT-3'), IL1-RII (s=5'-GCATCATTGGGGTCAAGACT-3'; as=5'-GATCCTCCC TTGTGACTGGA-3') and icIL-1ra (s=5'-CTGGGTACTTACAAGGACC-3'; as=5'-CGT GACTATAAGGGGCT-3').

2.3.3 Results.

Selective ablation of RXRα in epidermal keratinocytes of "genetically pre-initiated" adult mice increases the susceptibility to TPA-induced skin tumorigenesis.

To investigate whether selective ablation of RXRα in epidermal keratinocytes of Tg.AC mice has an effect on the formation of TPA-induced tumors, we generated Tg.AC/RXR$\alpha^{ep-/-}$ mice, bearing the Ha-ras oncogene and in which RXRα was selectively ablated in epidermal keratinocytes at adult stage through tamoxifen (Tam) administration to 6-7 week-old Tg.AC/K14-Cre-ER^{T2}/RXR$\alpha^{L2/L2}$ mice. As previously seen in RXR$\alpha^{ep-/-}$ mice (Li et al., 2000), Tg.AC/RXR$\alpha^{ep-/-}$ mice developed progressive alopecia, hair follicle degeneration and epidermal hyperplasia starting 7-8 weeks after Tam administration (data not shown). To induce skin tumors on Tg.AC/RXR$\alpha^{L2/L2}$ (CT) and Tg.AC/RXR$\alpha^{ep-/-}$ (MT) mice, TPA was topically applied twice a week to back skin of 9-10 week-old CT and MT mice and tumor appearance, number and size were recorded weekly. After 4 weeks of TPA treatment, papilloma-like tumors appeared in 12% of CT and MT mice, and after 8 weeks of promotion all mice developed tumors (**fig 15A**). However, MT mice developed two to three times more tumors than their CT littermates (**fig 15B**), to the extend that after 13 weeks of TPA treatment, CT and MT mice exhibited an average of 20 and 50 papilloma-like tumors, respectively. Because of the large number of tumors developed in CT and MT mice in response to TPA applications, the treatment was stopped after 13 weeks. Moreover, when TPA promotion was started at the age of 23 weeks**,** CT mice exhibited a 2-3 fold increase in tumor number and growth rate, when compared to CT mice in which the TPA treatment was started at the age of 10 weeks (data not shown), in agreement with previous reports showing that tumor incidence and multiplicity in Tg.AC mice in response to TPA-promotion are higher in older animals (Battalora et al., 2001). However, under these conditions, MT mice still developed two to three times more tumors than CT mice (data not shown). Furthermore, irrespective of the age at which the TPA treatment was started, 62% (10 out of 16) of MT mice developed more than 50 papillomas, whereas only 7% (1 out of 15) of CT mice developed more than 50 tumors (data not shown).

Figure 15. Selective ablation of RXRα in epidermal keratinocytes of adult Tg.AC mice increase the susceptibility to TPA-induced skin tumorigenesis. 9-10 week-old Tg.AC/RXRα$^{ep-/-}$ and Tg.AC/RXRα$^{L2/L2}$ mice were treated twice a week with topical applications of TPA. **(A)** Percentage of mice with tumors. **(B)** Average number of tumor per mouse +/- SEM. **(C)** Tumor size (length) distribution after 13 weeks of TPA treatment. The total number of tumors per category is shown. (**D**) IHC analysis of K13 expression. High-risk papillomas from MT (a) and CT (b) mice, and low-risk papilloma from CT mice (c). Scale bar 100 μm for a and b, 25 μm in c. (**E**) Hematoxylin-eosine staining of TPA-induced tumors from Tg.AC/RXRα$^{L2/L2}$ and Tg.AC/RXRα$^{ep-/-}$ mice, after 13 weeks of TPA treatment. (a) squamous papilloma from CT mice; (b) squamous papilloma with hyperplasia and (c) focal carcinoma from MT mice. Arrowhead in (b) points to an area of atypical hyperplasia. Arrow in (c) points to epidermal cells invading into the dermis. Scale bar 50 μm for a and b, 25 μm in c.

The length of more than 99% of the tumors from both CT and MT mice in which TPA was started at the age of 9-10 weeks, measured one week after the end of TPA treatment, varied between 0.5 and 6 mm. Aproximately 1% of the tumors had a size between 6 and 8 mm in both cases. No significant difference in tumor size distribution was observed between CT and MT mice (**Fig 15C**, and data not shown). However, when the tumor size was measured 10 weeks after the end of TPA treatment, 3 MT mice exhibited tumors larger than 15 mm in length, whereas in CT mice the tumors were never larger than 8 mm (data not shown). Thus, tumors of MT mice grew at a much higher rate than those of CT mice after the TPA treatment had been stopped.

To investigate whether ablation of RXRα in epidermal keratinocytes of Tg.AC mice has an effect on the malignant conversion of TPA-induced skin tumors, tumor biopsies from CT and MT mice, in which TPA was started at the age of 9-10 weeks, were removed 2 weeks after the end of TPA treatment and analyzed by IHC. Keratin 13 (K13) was shown to be expressed in papillomas with a "high risk" for malignant conversion, but not in low risk papillomas or normal skin (Glick et al., 1993; Tennenbaum et al., 1996). 60% of the tumors from MT mice exhibited large areas of K13 expression (**figure 15Da and data not shown**), whereas only few tumors (less than 1%) from CT mice exhibited such large areas of K13 staining (**figure 15Db and data not shown**). In more than 90% of tumors from CT mice K13 expression was either not detected, or in only a few cells (**figure 15Dc**). Histological analysis revealed that most of the tumors from CT and MT mice were benign squamous papillomas (**figure 15E a and b**), with few tumors exhibiting atypical basal cell hyperplasia (**figure 15Eb**). 8% of the tumors from MT mice were identified as focal carcinoma (**FC**) (**figure 15Ec**), whereas no such tumors were observed in CT mice. No evidence of squamous cell carcinoma (SCC) or basal cell carcinoma (BCC) was found in the tumors from either CT or MT mice (**Table 6**). Note that topical application of TPA to CT or MT mice did not results in formation of melanocytic growths (MGs). This is in contrast to what occurs when RXR$\alpha^{ep-/-}$ mice are subjected to a DMBA/TPA two-step chemical tumorigenesis protocol. Indeed, initiation with a single application of DMBA, followed by twice a week applications of TPA resulted in the formation of epithelial tumors and MGs in RXR$\alpha^{ep-/-}$ mice (see section 2.1).

Thus, ablation of RXRα in genetically pre-initiated adul mice results in an increased incidence of TPA-induced epidermal tumors, which exhibit features of high-risk papillomas.

Selective ablation of RXRα in keratinocytes of Tg.AC mice during skin morphogenesis results in spontaneous papilloma formation and a high rate of progression to malignancy.

To investigate the effect of RXRα ablation during skin morphogenesis in the tumor susceptibility of Tg.AC mice, we generated Tg.AC/RXRα$^{ep-/-(c)}$ mice, carrying the Ha-ras oncogene and in which the RXRα alleles are selectively ablated in epidermal keratinocytes early during skin formation, from Tg.AC/K14-Cre/RXRα$^{L2/L2}$ mice. As reported for RXRα$^{ep-/-(c)}$ mice (Li et al., 2001b), Tg.AC/RXRα$^{ep-/-(c)}$ mice exhibited a progressive alopecia, which started in ventral skin, near the hind legs, and with time spreads over the whole body. Hair loss begined at 4-5 weeks of age, and at the age of 16 weeks, all Tg.AC/RXRα$^{ep-/-(c)}$ mice exhibited a severe hair loss (60% loss in dorsal and 80% in ventral skin). Surprisingly, papilloma-like tumors appeared spontaneously on the ventral skin of 12-13 week-old Tg.AC/RXRα$^{ep-/-(c)}$ mice (**figure 16A d**). By 16 weeks of age, all Tg.AC/RXRα$^{ep-/-(c)}$ mice had developed skin tumors (**figure 16B**), both on the ventral and dorsal skin (**figure 16A e,f**). In contrast, no tumors appeared in Tg.AC/RXRα$^{L2/L2}$ (**figure 16A a and b**) or RXRα$^{ep-/-}$ mice (**Figure 16A c and table 6**). The number of tumors in Tg.AC/RXRα$^{ep-/-(c)}$ mice increased with age, and an average of 16 tumors per mouse was observed in 26-28 week-old mice (**figure 16C**). Some of the tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice exhibited a high growth rate, reaching more than 15 mm in length in 16-25 week-old mice (data not shown).

To investigate whether spontaneous skin tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice progress to malignancy, tumor biopsies were taken from 16-25 week-old mice. The IHC analysis of **K13** expression revealed that all tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice exhibited large areas with high levels of K13 expression (**figure 16D a and b**), indicating that these tumors are at a high risk of conversion into malignancy. Histological analysis showed that most of the tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice were benign squamous papillomas (**figure 16Ea**). However, 11.5%, 7.7% and 3.8% of 20-25 week-old Tg.AC/RXRα$^{ep-/-(c)}$ mice developed ISC (**figure 16Eb**), FC (**figure 16Ec**) and SCC (data not shown), respectively (**table 6**).

Figure 16. Selective ablation of RXRα in epidermal keratinocytes of Tg.AC mice during skin morphogenesis results in formation of spontaneous skin tumors in adult mice. (**A**) ventral (a,c,d) an dorsal (b,e,f) view of 38 week-old Tg.AC/RXRα$^{L2/L2}$ (a,b), 20 week-old RXRα$^{ep-/-(c)}$ **(c)** and 16 week-old (**d**) and 20 week-old (**e,f**) Tg.AC/RXRα$^{ep-/-(c)}$ females; **(B)** Percentage of mice with spontaneous tumors. **(C)** The average number of tumors per mouse +/- SEM. (**D**) IHC analysis of K13 expression in tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice. Scale bar 50 μm. (**E**) Hematoxylin-eosine staining of tumors from Tg.AC/RXRα$^{ep-/-(c)}$ mice. (**a**) benign papilloma; (**b**) in situ carcinoma; (**c**) focal carcinoma. Arrows point to the basement membrane. Arrowheads in (c) point to cells invading into the dermis through the basement membrane. Scale bar 20 μm for a and b; 10 μm in c.

These data demonstrate that selective ablation of RXRα in epidermal keratinocytes of genetically pre-initiated mice during skin morphogenesis is sufficient to induce the formation of high-risk papillomas at adult stage, that with time progress to malignancy in the absence of any tumor promoter.

It has been shown that the formation of skin tumors in Tg.AC mice is correlated with the transcriptional activation of the Ha-ras transgene in epidermal keratinocytes (Hansen et al., 1995; Hansen and Tennant, 1994a). While Ha-ras transgene expression is not detected in the skin of unchallenged Tg.AC mice, expression of the transgene is induced after topical application of TPA, and is detected in all tumors induced in Tg.AC mice (Battalora et al., 2001; Cannon et al., 1997; Hansen and Tennant, 1994a). Moreover, the induction of the Ha-ras transgene expression and the sensitivity to tumor promotion in Tg.AC mice increase with age (Battalora et al., 2001). To investigate whether RXRα ablation in keratinocytes of Tg.AC mice during skin formation results in the transcriptional activation of the Ha-ras transgene, we measured the levels of the Ha-ras transgene transcript in the skin of Tg.AC/RXRα$^{ep-/-(c)}$ mice 2, 4, 8 and 12 weeks after birth, by quantitative RT-PCR. No significant increase in the expression levels of the Ha-ras transgene was observed in the skin of Tg.AC/RXRα$^{ep-/-(c)}$ mice, when compared to Tg.AC/RXRα$^{L2/L2}$ mice (data not shown). This indicated that ablation of RXRα in keratinocytes of Tg.AC mice during epidermal morphogenesis does not alter the expression of the Ha-ras transgene post-natally.

Selective ablation of RXRα in keratinocytes during skin morphogenesis predisposes mice to develop papillomas and melanocytic growths in response to DMBA initiation.

To determine whether ablation of RXRα in epidermal keratinocytes during skin morphogenesis also predisposes mice to develop skin tumors in response to DMBA initiation, which is known to result in an activating mutation in codon 61 of the c-Ha-ras gene (Balmain et al., 1988; Quintanilla et al., 1986b), 6-8 week-old RXRα$^{ep-/-(c)}$ and RXRα$^{L2/L2}$ mice were treated with a single topical application of DMBA to the back skin. 15 weeks after DMBA initiation, RXRα$^{ep-/-(c)}$ mice developed skin papillomas in the area were DMBA was applied. 25 weeks after initiation, 6 out of 13 RXRα$^{ep-/-(c)}$ mice developed 2-3 papillomas (**figure 17A b and c**, and data not shown), whereas RXRα$^{L2/L2}$ mice developed none (**figure 17Aa**). Histological analysis revealed that the tumors from RXRα$^{ep-/-(c)}$ mice, taken 25 weeks after DMBA treatment, were benign squamous papillomas (**figure 17Ba-c**), some of which exhibited large areas of atypical hyperplasia (**figure 17B b and c**), and the presence of mitotic bodies in basal and suprabasal layers (**figure 17Bd**). Moreover, IHC analysis demonstrated that papillomas from RXRα$^{ep-/-(c)}$ mice exhibit large areas of K13 expression (**figure 17C**), indicating that these are high-risk papillomas. Thus, selective ablation of RXRα in mouse epidermal keratinocytes during epidermal morphogenesis predisposes mice to the formation of Ha-ras-mediated epithelial tumors.

In agreement with our previous results (section 2.1), showing that RXRα$^{ep-/-}$ mice develop a large number of melanocytic growths (MGs), some of which progressed to melanoma, in response to a single DMBA application, DMBA-treated RXRα$^{ep-/-(c)}$ mice exhibited 13 times more MGs than CT mice, 25 weeks after DMBA initiation (**figure 18A and B**). Histological analysis showed that the MGs from DMBA-treated RXRα$^{ep-/-(c)}$ mice were accumulation of densely-laden melanocytes in both dermis and hypodermis (**figure 18Cb**), resembling the melanoma lesions developed in DMBA/TPA treated RXRα$^{ep-/-}$ mice (see section 2.1), whereas the MGs from CT mice exhibited only few melanocytes growing in the dermis (**figure 18Ca**).

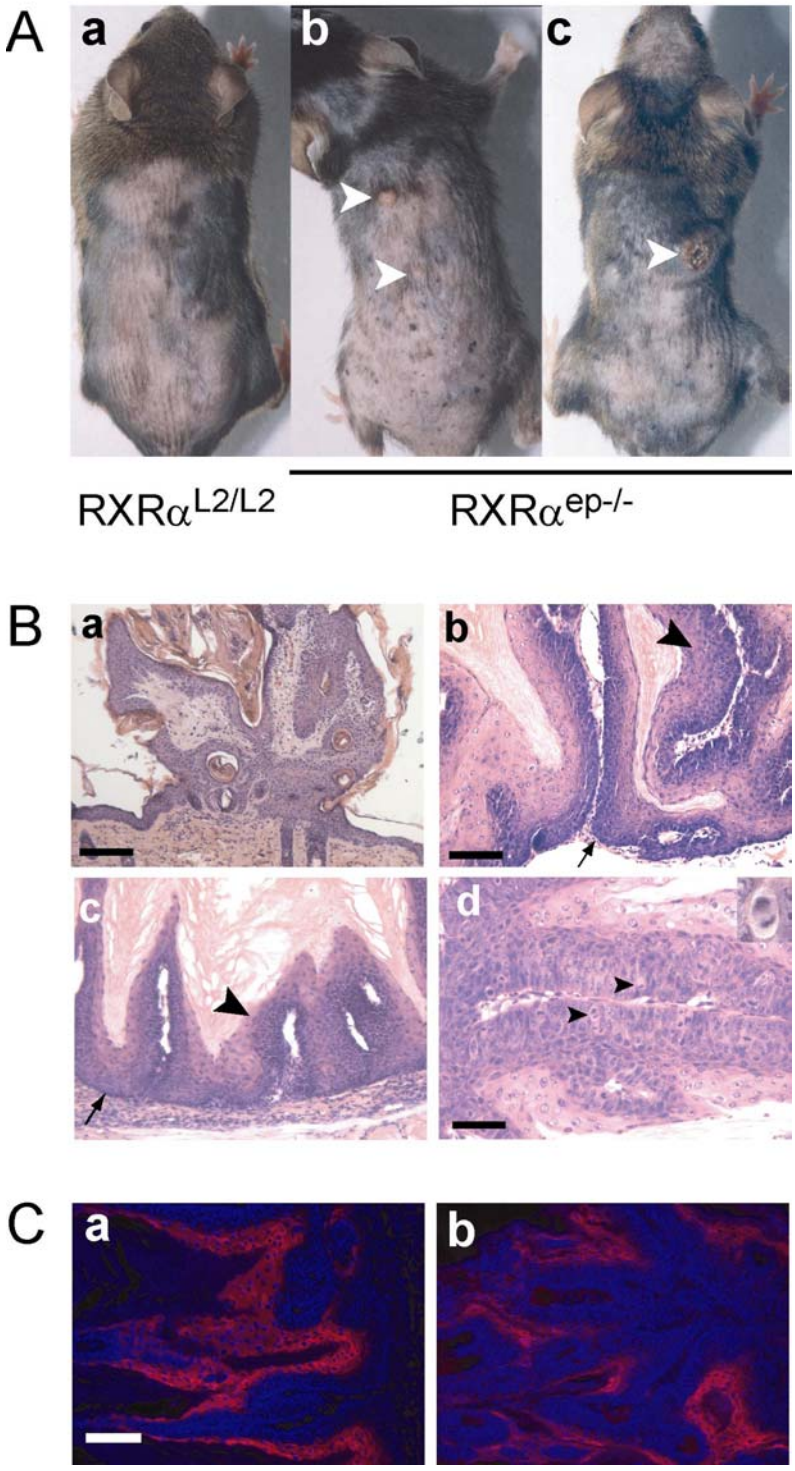

Figure 17. Formation of skin papillomas in RXRα$^{ep-/-(c)}$ mice in response to a single DMBA application. (A) Dorsal view of RXRα$^{L2/L2}$ (a) and RXRα$^{ep-/-(c)}$ (b,c) mice, 25 weeks after the application of DMBA. White arrowheads point to papillomas. (B) Hematoxylin-eosine staining of tumors from DMBA-treated RXRα$^{ep-/-(c)}$ mice, 25 weeks after DMBA initiation. (a) benign papilloma, (b,c) benign papillomas with atypical hyperplasia (arrowheads). (d) higher magnification of a papilloma. Arrows point to basement membrane. Arrowheads in (d) point to mitotic figures. The insert is a higher magnification of a mitotic figure. Scale bar 50 μm in a; 25 μm in b and c; 10 μm in d. (C) IHC detection of keratin 13 expression in papillomas from RXRα$^{ep-/-(c)}$ mice. Scale bar 50 μm.

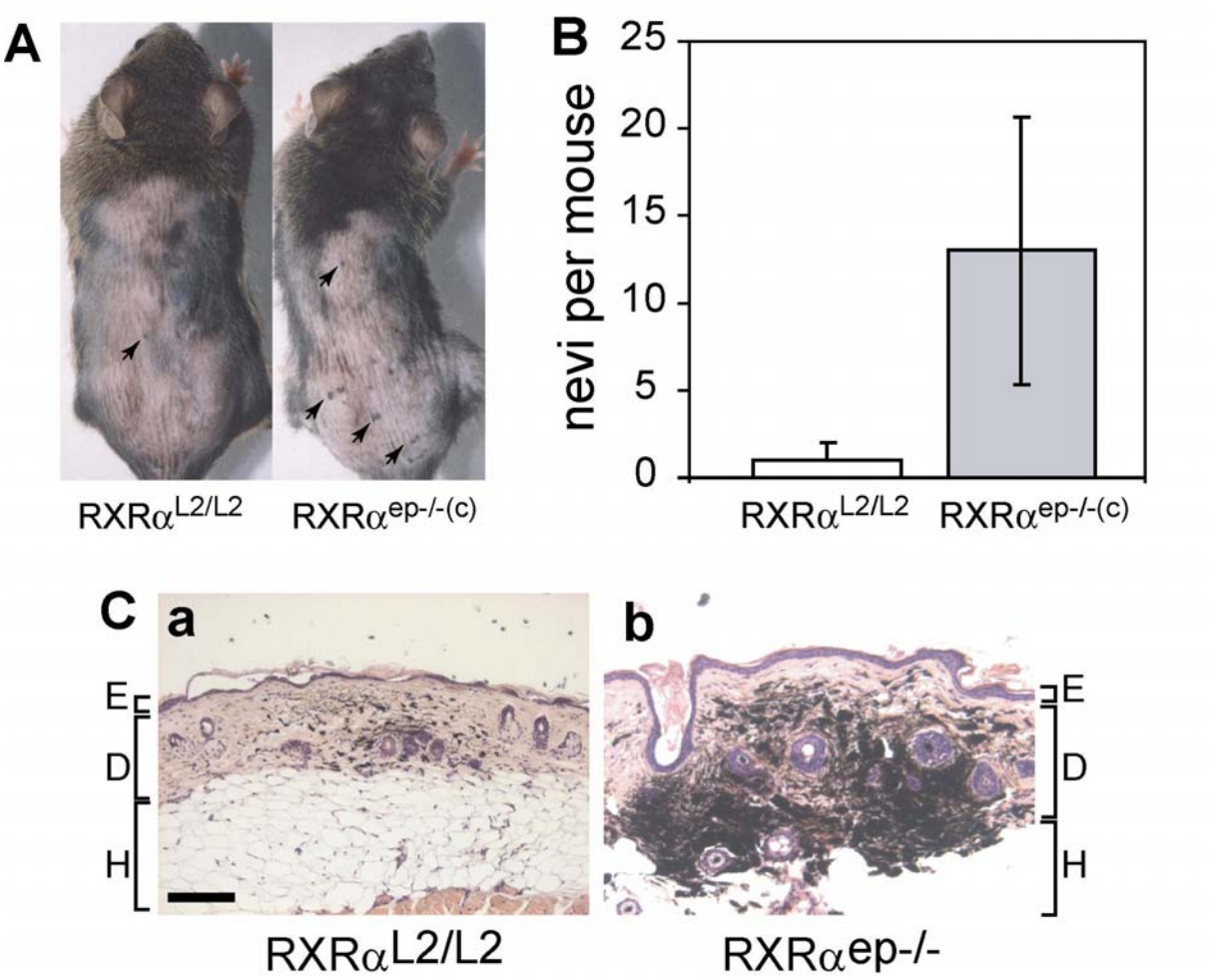

Figure 18. Formation of melanocytic growths (MGs) in RXRα$^{ep-/-(c)}$ mice in response to a single DMBA application. (A) Dorsal view of RXRα$^{L2/L2}$ and RXRα$^{ep-/-(c)}$ mice, 25 weeks after the application of DMBA. Arrows point to MGs. (B) Average number of nevi per mouse, 25 weeks after DMBA initiation +/- SEM. (C) Hematoxylin-eosine staining of MGs from RXRα$^{L2/L2}$ (a) and RXRα$^{ep-/-(c)}$ (b) mice, 25 weeks after the application of DMBA. Notice the MGs from DMBA-treated RXRα$^{ep-/-(c)}$ mice growing deep into the dermis and hypodermis. E, epidermis; D, dermis; H, hypodermis. Scale bar 50 μm

Mice genotype	Treatment	Papilloma incidence	Papilloma per mouse	MG's incidence	Mouse with malignancy			
					ISC	FC	SCC	BCC
Tg.AC/RXR$\alpha^{L2/L2}$	-	0% (0/25)	0	0% (0/25)	0% (0/25)	0% (0/25)	0% (0/25)	0% (0/25)
Tg.AC/RXR$\alpha^{ep-/-}$	-	0% (0/18)	0	0% (0/18)	0% (0/18)	0% (0/18)	0% (0/18)	0% (0/18)
Tg.AC/RXR$\alpha^{L2/L2}$	TPA[a]	100% (15/15)[b]	24+/-11*[b]	0% (0/15)[b]	0% (0/15)[c]	0% (0/15)[c]	0% (0/15)[c]	0% (0/15)[c]
Tg.AC/RXR$\alpha^{ep-/-}$	TPA[a]	100% (16/16)[b]	56+/-27*[b]	0% (0/16)[b]	13% (2/16)[c]	0% (0/16)[c]	0% (0/16)[c]	0% (0/16)[c]
Tg.AC/RXR$\alpha^{ep-/-(c)}$	-	100% (26/26)[d]	16 +/- 7[d]	0% (0/26)[d]	**11.5**% (3/26)[d]	**7.7**% (2/26)[d]	**3.8**% (1/26)[d]	0% (0/26)[d]
RXR$\alpha^{L2/L2}$	DMBA[e]	0% (0/10)[f]	0[f]	30% (3/10)[f]	0% (0/10)[f]	0% (0/10)[f]	0% (0/10)[f]	0% (0/10)[f]
RXR$\alpha^{ep-/-(c)}$	DMBA[e]	46% (6/13)[f]	2+/-1[f]	85% (11/13)[f]	0% (0/13)[f]	0% (0/13)[f]	0% (0/13)[f]	0% (0/13)[f]

MG's= melanocytic growths; ISC= in situ carcinoma; FC= focal carcinoma; SCC= squamous cell carcinoma; BCC= basal cell carcinoma
a = TPA was topically applied to 9-10 week-old mice, twice a week during 13 weeks; b= after 13 weeks of TPA promotion; c= tumor biopsies taken 2-4 weeks after the end of TPA treatment; d= 20-25 week-old mice; e= 6-8 week-old mice were treated with a single dose of DMBA; f= 25 weeks after DMBA treatment; * p=0.005

Skin alterations in Tg.AC mice ablated for RXRα in epidermal keratinocytes during epidermal morphogenesis.

To determine whether skin alterations observed in Tg.AC/RXRα$^{ep-/-(c)}$ mice are related to those induced by topical application of TPA, skin biopsies from unchallenged Tg.AC/RXRα$^{L2/L2}$ (CT) and Tg.AC/RXRα$^{ep-/-}$ mice, CT and Tg.AC/RXRα$^{ep-/-}$ mice treated with two topical applications of TPA, and unchallenged Tg.AC/RXRα$^{ep-/-(c)}$ mice were removed and prepared for histological analysis. Topical application of TPA to skin of CT mice resulted in a 3-4 fold increase in epidermal thickness, when compared to the skin of untreated CT mice. Untreated 20-22 week-old Tg.AC/RXRα$^{ep-/-}$ mice exhibited already a 2-fold increase in epidermal thickness when compared to untreated CT mice, and in response to TPA treatment, the epidermal thickness was further by 7-8 fold, when compared to untreated CT mice. Interestingly, the epidermis of untreated Tg.AC/RXRα$^{ep-/-(c)}$ mice at the age of 8 weeks, was 3-4 fold thicker than that of Tg.AC/RXRα$^{L2/L2}$ mice (**figure 19A and B**). Preliminary results indicate that Tg.AC/RXRα$^{ep-/-(c)}$ mice exhibit epidermal keratinocyte hyperproliferation, similar to the keratinocyte hyperproliferation induced by topical application of TPA to the back skin of Tg.AC/RXRα$^{L2/L2}$ mice (not shown).

It has been shown that topical application of TPA to back skin of wild-type mice, results in significant increase in the transcript levels of genes such as ornithine decarboxylase (odc), c-jun , cyclooxygenase-2 (cox-2), c-myc, the IL-1 receptor type I (IL1-RI), and the intracellular IL-1 receptor antagonist (icIL-1ra) (Chun et al., 2003; Kakar and Roy, 1994; Limtrakul Pn et al., 2001). To examine whether ablation of RXRα in epidermal keratinocytes during epidermal morphogenesis results in gene expression changes similar to those induced by TPA application, we compared the transcript levels of these genes in the skin of Tg.AC/RXRα$^{ep-/-(c)}$ mice and TPA-treated Tg.AC/RXRα$^{L2/L2}$ mice. As expected, topical application of TPA to back skin of Tg.AC/RXRα$^{L2/L2}$ mice resulted in significant increase in the mRNA levels of odc, c-jun, cox-2, c-myc, icIL-1ra and IL1-RI, when compared to untreated Tg.AC/RXRα$^{L2/L2}$ mice (**figure 19C**). In contrast, no significant increase in the transcript levels of these genes was observed in the skin of Tg.AC/RXRα$^{ep-/-(c)}$ mice (**figure 19C**). Thus, changes on gene expression induced by selective ablation of RXRα in keratinocytes during skin development, leading to the formation of skin papillomas, appear to be different from those induced by topical application of TPA to adult skin.

Figure 19. Phenotypic changes in the skin of Tg.AC/RXRα$^{ep-/-(c)}$ mice. (A) Hematoxylin-eosine stained skin sections from 10 week-old Tg.AC/RXRα$^{L2/L2}$ females non treated (a) or treated with two topical applications of TPA (b), and untreated 20 week-old Tg.AC/RXRα$^{ep-/-}$ (c) and 8 week-old Tg.AC/RXRα$^{ep-/-(c)}$ (d) females. Scale bar 20 μm (B) Quantification of epidermal thickness from samples described in (A). Results are the average epidermal thickness in μm +/- SEM. *** $p < 0.001$. (C) Quantitative RT-PCR analysis of mRNA levels in skin samples from 10 week-old Tg.AC/RXRα$^{L2/L2}$ females non treated or treated with two topical applications of TPA, and untreated 8 week-old Tg.AC-RXRα$^{ep-/-(c)}$ females. (A). Data shown are the relative mRNA levels +/- SEM, normalized against HPRT, and represents the average of three independent quantifications on six mice per group. * $p = 0.1$, *** $p < 0.001$.

2.3.4 Discussion.

We have previously shown that ablation of RXRα in epidermal keratinocytes of adult mice results in an increased susceptibility to DMBA/TPA-induced skin tumorigenesis (see section 2.1). Here we show that keratinocytic RXRα exerts a tumor suppressor effect during the promotion stage of Ha-ras-mediated skin papillomas. Indeed, selective ablation of RXRα in epidermal keratinocytes of genetically pre-initiated (Tg.AC) adult mice, carrying an activated Ha-ras oncogene, results in increased skin tumor formation in response to TPA promotion, and a higher incidence of high-risk papillomas.

Interestingly, selective ablation of RXRα in epidermal keratinocytes of Tg.AC mice during skin morphogenesis (Tg.AC/RXRα$^{ep-/-(c)}$ mice), is sufficient on its own, to induce the formation of high-risk papillomas, which with time progress to malignancy. Furthermore, ablation of RXRα in keratinocytes during epidermal morphogenesis (RXRα$^{ep-/-(c)}$ mice), is sufficient for the promotion of skin tumors in response to a single applicaiton of DMBA, which also exhibit features of high-risk papillomas. Interestingly, in contrast to the 100% incidence of skin tumors observed in 16 week-old Tg.AC/RXRα$^{ep-/-(c)}$ mice, only 60% of RXRα$^{ep-/-(c)}$ mice developed skin tumors 25 weeks after DMBA initiation. Moreover, Tg.AC/RXRα$^{ep-/-(c)}$ mice developed an average of 16 tumors, whereas RXRα$^{ep-/-(c)}$ mice developed only 2-3 tumors. These differences might be explained by the fact that in Tg.AC/RXRα$^{ep-/-(c)}$ mice all the epidermal cells carry the activated Ha-ras oncogene, whereas in RXRα$^{ep-/-}$ mice, DMBA application may induce the activation mutations of the Ha-ras oncogene only in few epidermal cells.

Thus, the cellular alterations resulting from the genetic ablation of RXRα in keratinocytes during epidermal morphogenesis are probably sufficient to promote the clonal expansion of Ha-ras initiated keratinocytes. In contrast, ablation of RXRα in keratinocytes of adult mice most likely potentiates the tumor promotion effect of TPA, but is not sufficient on its own to promote the clonal expansion of initiated keratinocytes. It has been suggested that epidermal keratinocyte stem cells are the target of carcinogens and the precursors of skin tumors in response to tumor promoters (Morris et al., 2000). Ablation of RXRα early during epidermal morphogenesis may affect keratinocyte commitment, resulting in the generation of keratinocytes with enhanced proliferative potential. In the presence of Ha-ras mutations, RXRα null keratinocytes will clonaly expand and form tumors. Further studies are required to

understand how ablation of RXRα in epidermal keratinocytes, early during development, results in the promotion of Ha-ras mediated skin tumors.

2.3.5 Perspectives.

In section 2.1, we show that epithelial tumors induced by DMBA/TPA-treatment in RXRα$^{ep-/-}$ mice, progressed with high frequency to SCC after 30 weeks of TPA promotion. In this study, application of TPA to Tg.AC/RXRα$^{ep-/-}$ mice resulted in the formation of a large number of papillomas after few weeks of TPA treatment. The promotion was interrupted after 13 weeks, and the mice were sacrificed and tumor biopsies collected 2 to 10 weeks after the end of the treatment. Under this experimental conditions, TPA-induced tumors in Tg.AC/RXRα$^{ep-/-}$ mice did not progress to SCC, most likely because of the shortness of the TPA treatment (only 13 weeks compared to 30 weeks in the DMBA/TPA protocol). To further investigate if RXRα ablation in the epidermis of adult Tg.AC mice has an effect on the incidence of progression to SCC, lower doses of TPA will be used for the promotion of skin tumors in Tg.AC/RXRα$^{ep-/-}$ mice, which may allow us to continue the TPA treatment for longer than 13 weeks and assess the effect of RXRα ablation on the frequency of malignant conversion. On the other hand, we found that the tumors from 20-25 week-old Tg.AC/RXRα$^{ep-/-(c)}$ mice progressed to SCC, but the frequency of progression was low. However, it is likely that in older animals the frequency of conversion will increase. To address this question, we have generated new groups of Tg.AC/RXRα$^{ep-/-(c)}$ mice, from which tumor biopsies will be taken and analyzed by histology, at 30, 35 and 40 weeks of age. Similarly, we have generated new groups of RXRα$^{ep-/-(c)}$ mice that have been treated with DMBA, and from which tumor biopsies will be taken 30 and 35 weeks after DMBA initiation. Finally, a detailed comparison of the cellular and molecular changes induced upon selective ablation of RXRα in epidermal keratinocytes during embryonic development, or at the adult stage is required to understand the mechanisms underlying the formation of spontaneous papillomas and carcinomas in Tg.AC/RXRα$^{ep-/-(c)}$ but not in Tg.AC/RXRα$^{ep-/-}$ mice. To address this question, we are collecting skin samples from Tg.AC/RXRα$^{ep-/-(c)}$ and Tg.AC/RXRα$^{ep-/-}$ mice at different time points after birth and after tamoxifen administration, respectively, that we will analyze by histology, immunohistochemistry, and real-time quantitative RT-PCR.

3. General Discussion

3.1 Keratinocytic RXRα and its heterodimeric partners as suppressors of epithelial tumors.

RXRα is the main retinoic acid receptor in the epidermis (Reichrath et al., 1997), where it plays critical roles in the maintenance of skin homeostasis (Li et al., 2001b; Li et al., 2000). To investigate the possible role of RXRα during skin carcinogenesis, we took advantage of a mouse model previously generated in the laboratory, in which RXRα alleles are selectively ablated in epidermal keratinocytes of adult mice ($RXR\alpha^{ep-/-}$ mice). We demonstrate that $RXR\alpha^{ep-/-}$ mice exhibit a higher susceptibility to develop papillomas and carcinomas than control mice, in response to a DMBA/TPA treatment, indicating that keratinocytic RXRα has a tumor suppressor effect on the formation of DMBA-TPA-induced skin tumors. Because RXRα is a heterodimeric partner for several non-steroidal NRs, including the VDR, PPARs and LXRs, the increased susceptibility to tumor formation and malignant conversion in $RXR\alpha^{ep-/-}$ mice, may result from alterations of more than one signaling pathway in keratinocytes. To identify the heterodimeric partner(s) that may function, together with RXRα in keratinocytes, to suppress the formation of chemically-induced epithelial tumors, we used mouse lines in which null mutations of various NRs have been introduced, either in the germ line or selectively in epidermal keratinocyte at the adult stage.

We found that, in response to DMBA/TPA treatment, $PPAR\gamma^{ep-/-}$ mice exhibit higher susceptibility to the formation of papillomas and carcinomas than control mice. This data indicate that in keratinocytes, RXRα/PPARγ heterodimers act as suppressors of chemically-induced epithelial tumorigenesis. PPARα null mice also exhibited increased incidence of papilloma formation compared to control mice, in response to DMBA/TPA treatment. Because PPARα is also expressed in dermal fibroblasts (Billoni et al., 2000a), the possibility that the lack of PPARα in fibroblasts may contribute to the increased tumor incidence observed in the PPARα null mice, after DMBA/TPA treatment, can not be excluded. However, the observations that PPARα expression is reactivated in mouse epidermal keratinocytes during wound healing or after TPA treatment (Michalik et al., 2001), and that PPARα activators induce apoptosis and decrease proliferation of keratinocytes in vivo (Komuves et al., 2000), supports the idea that keratinocytic RXRα/PPARα heterodimers may also play key roles as suppressors of epithelial tumor formation. We also show that VDR null mice exhibited similar tumor incidence and multiplicity than WT mice upon DMBA/TPA treatment, indicating that VDR does not play crucial roles during epithelial carcinogenesis.

It has been shown that initiated keratinocytes exhibit abnormal response to several differentiation signals (Parkinson, 1985). In contrast to normal keratinocytes, in which TPA induces differentiation, initiated cells respond to TPA treatment by undergoing proliferation, which results in the clonal expansion of the initiated cells and the subsequent formation of squamous papillomas (Karen et al., 1999). Accelerated keratinocyte differentiation in Cox-1 null and Cox-2 null mice for example, resulted in a decreased incidence of skin tumors induced by DMBA/TPA treatment (Tiano et al., 2002). In contrast, resistance to Ca++ induced differentiation in keratinocytes of transgenic mice overexpressing Cyclin D1 in the epidermis, resulted in increased tumor formation in response to DMBA/TPA (Yamamoto et al., 2002). We have previously shown that RXR$\alpha^{ep-/-}$ mice exhibited abnormal keratinocyte terminal differentiation, as indicated by the significant decrease in the expression of filaggrin in the suprabasal layers of the epidermis (Li et al., 2001b; Li et al., 2000). PPARα and PPARγ play important roles in the control of keratinocyte differentiation in vivo (Komuves et al., 2000). Thus, disruption of RXRα/PPARα or RXRα/PPARγ heterodimers in keratinocytes may result in impairment of keratinocyte differentiation, contributing to the increased tumor formation in response to DMBA/TPA treatment.

Stimulation of keratinocyte proliferation by TPA, during the promotion stage of the chemical carcinogenesis protocol, plays a key role in the formation of skin tumors. A single topical application of TPA to mice skin results in a strong increase in keratinocyte proliferation in the basal layer of the epidermis (Karen et al., 1999). Interestingly, it has been shown that selective ablation of the tumor suppressor BRCA-1 in mice epidermis resulted in increased keratinocyte proliferation and increased susceptibility to DMBA/TPA induced tumors (Berton et al., 2003). Similarly, overexpression of DP-1, a subunit of the E2F transcription factor family, in the basal keratinocytes of mice epidermis, results in increased keratinocyte proliferation and enhanced tumor formation in response to DMBA/TPA treatment (Wang et al., 2001). Ablation of RXRα or PPARγ in epidermal keratinocytes results in keratinocyte hyperproliferation, which may also contribute to the increased susceptibility to tumor formation in response to DMBA/TPA treatment.

Interestingly, both RXR$\alpha^{ep-/-}$ and PPAR$\gamma^{ep-/-}$ mice exhibited increase suprabasal expression of $\alpha6\beta4$ integrins in the epidermis and a decrease of Pten expression in the skin. Recently, it has been demonstrated that targeted expression of $\alpha6\beta4$ integrins in the suprabasal layers of transgenic mouse epidermis dramatically increased the frequency of papillomas, carcinomas and metastases induced by chemical carcinogenesis (Owens et al., 2003). Moreover, the selective ablation of Pten alleles in epidermal keratinocytes results in development of epidermal hyperplasia and skin tumors (Suzuki et al., 2003). Thus, it is likely that the disruption of the RXRα/PPARγ heterodimers in keratinocytes results in deregulation of $\alpha6\beta4$ integrins and Pten expression, leading to an increased susceptibility to epithelial tumor formation in response to DMBA/TPA treatment. Recently, a functional PPAR response element has been identified in the Pten promoter (Patel et al., 2001), strongly supporting the idea that RXRα/PPARγ heterodimers may directly regulate Pten expression in keratinocytes.

When RXRα ablation was induced in mice epidermis 9 weeks after DMBA initiation, during the TPA promotion stage, mutant mice also exhibited higher tumor multiplicity, tumor growth rate and incidence of conversion into malignancy than control mice, indicating that RXRα play minor roles during the initiation stage. Furthermore, the selective ablation of RXRα alleles in epidermal keratinocytes of "genetically pre-initiated" (Tg.AC) adult mice (Leder et al., 1990), also resulted in an increased susceptibility to TPA-induced skin tumors. This data indicate that RXRα plays minor roles in the initiation stage, and suggest that the increased susceptibility to tumor formation in RXRα$^{ep-/-}$ mice is due to alterations in the response to TPA promotion.

A striking observation was that selective ablation of RXRα alleles in epidermal keratinocytes of Tg.AC mice (Leder et al., 1990), during epidermal morphogenesis (Tg.AC/RXRα$^{ep-/-(c)}$ mice), resulted in the formation of spontaneous skin papillomas and carcinomas, in the absence of any tumor promotion agent. Similarly, a single topical application of DMBA to the back skin of RXRα$^{ep-/-(c)}$ mice, in which RXRα alleles were selectively ablated in epidermal keratinocytes during epidermal morphogenesis, results in the spontaneous formation of epithelial tumors. This results demonstrate that skin alterations induced by ablation of RXRα in keratinocytes during epidermal morphogenesis, are sufficient to promote the clonal expansion of a population of initiated keratinocytes, carrying Ha-ras mutations, and for the formation and malignant progression of epithelial tumors. One possible explanation to the spontaneous formation of epithelial tumors in Tg.AC/RXRα$^{ep-/-(c)}$ mice and in RXRα$^{ep-/-(c)}$ mice in response to a single DMBA topical application is that ablation of RXRα early during epidermal morphogenesis may affect keratinocyte commitment, resulting in the generation of epidermal cells with enhanced proliferative potential, which in the presence of Ha-ras mutations will clonally expand and form tumors. Recently, it has been shown that mice carrying a null mutation of the phospholipase C delta1 (PLCδ1) develop progressive alopecia, epidermal hyperplasia and dermal cysts (Nakamura et al., 2003), resembling the phenotype of RXRα$^{ep-/-}$ mice. Interestingly, PLCδ1 null mice developed spontaneous skin tumors and the authors suggest that PLCδ1 is required for skin stem cell lineage commitment. Another possibility is that upon disruption of hair follicles in RXRα$^{ep-/-(c)}$ mice, the keratinocyte stem cells from the bulge will migrate upwards to the interfollicular epidermis, where, exposed to a new microenvironmental stimuli, their proliferation rate will be increased, contributing to the development of epithelial tumors. Several lines of evidence indicate that keratinocytes from the hair follicle contribute in a great extent to the development of epithelial tumors in response to DMBA/TPA treatment (Binder et al., 1997). Similarly, Hansen and co-workers showed that TPA-induced papillomas in Tg.AC mice are mainly from follicular origin (Hansen and Tennant, 1994b). Further studies are required to elucidate the possible role of RXRα in the commitment of keratinocytes during epidermal morphogenesis and in adult skin. Interestingly, PPARγ$^{ep-/-(c)}$ mice, in which PPARγ alleles are selectively ablated in keratinocytes during epidermal morphogenesis (**anexe I**), or PPARα null or VDR null mice did not developed epithelial tumors in response to a single topical application of DMBA, suggesting

that disruption of several RXRα/NR heterodimers may be required to promote the formation of epithelial tumors in response to single topical application of DMBA.

3.2 Keratinocytic RXRα and its heterodimeric partners as suppressors of malignant melanoma.

In addition to epithelial tumors, RXRα$^{ep-/-}$ mice developed a large number of melanocytic growths, which progress to malignant melanoma, in response to DMBA/TPA treatment. In contrast to RXRα$^{ep-/-}$ mice, PPARα null or PPARγ$^{ep-/-}$ mice did not develop melanocytic growths or melanoma, upon DMBA/TPA treatment, suggesting that these receptors are not involved in the formation of melanoma. Interestingly, DMBA/TPA-treated VDR null mice developed a large number of MGs, but in contrast to the MGs induced in RXRα$^{ep-/-}$ by DMBA/TPA treatment, the MGs from VDR null mice did not degenerate into melanoma. In mice, melanocytes are located mainly in the hair follicle. The observation that RXRα$^{ep-/-}$ and VDR null mice (Li et al., 2000), but not in PPARα null or PPARγ$^{ep-/-}$ mice, developed a progressive alopecia and hair follicle degeneration, suggests that degeneration of the hair follicle is an important event for the formation of MGs. Upon disruption of the hair follicle melanocytes might be release to the dermal compartment, where their growth and differentiation properties may change, becoming more susceptible to malignant transformation. At the molecular level, a significant decrease in the expression levels of e-cadherin, an adhesion molecule that plays key roles in keratinocyte-melanocyte communication (Jamal and Schneider, 2002), was observed in the skin of both RXRα$^{ep-/-}$ and VDR null mice. It has been shown that down regulation of e-cadherin is associated with melanoma progression (Li et al., 2001a), and that overexpression of e-cadherin in melanoma cells restores the keratinocyte-mediated growth control (Hsu et al., 2000). Thus, degeneration of the hair follicle and loss of keratinocyte-melanocyte adhesion resulting from the disruption of RXRα/VDR heterodimers in keratinocytes may generate a permissive environment for the growth of DMBA-initiated melanocytes. However, the observation that MGs from RXRα$^{ep-/-}$, but not those of VDR mice progress to malignant melanoma, indicate that additional alterations observed in RXRα$^{ep-/-}$ but not in VDR null mice (ie down regulation of Pten), are required for the degeneration of these MG's to melanoma. It has been shown that down regulation of Pten is associated with melanoma development in human (Wu et al., 2003), and that in mice, invalidation of one Pten allele in a Inka/ARF$^{\Delta 2/3}$ background results in the development of malignant melanoma (You et al., 2002). We found that the levels of Pten mRNA were down regulated in the skin of RXRα$^{ep-/-}$ mice, but not in that of VDR null mice. These data suggest that degeneration of the hair follicle and decreased e-cadherin expression in the skin may be sufficient for the formation of benign melanocytic growths, whereas Pten down regulation and/or other alterations might be required for their malignant transformation.

3.3 General overview.

RXRs are heterodimeric partners for several hormone nuclear receptors including RARs, VDR, PPARs and LXRs. In the epidermis, RXRα is the major RXR isotype. As described in section 2.1, selective ablation of RXRα in epidermal keratinocytes results in increased susceptibility to chemical skin carcinogenesis (DMBA/TPA). RXRα$^{ep-/-}$ mice exhibited an increased incidence of epithelial tumor formation and malignant conversion than control mice. In addition, RXRα$^{ep-/-}$ mice also developed melanoma in response to a single DMBA application. This is associated to changes in gene expression including decrease of Pten and e-cadherin, and increase in SCF and SB expression of α6β4 integrins. These alterations have been associated to cancer development in humans and mouse models. The ablation of any single heterodimeric partner for RXRα recapitulates all the alterations observed in RXRα$^{ep-/-}$ mice, indicating that those alterations are the result of the disruption of more than one single signaling pathway. Interestingly, PPARα null and PPARγ$^{ep-/-}$ mice, but not VDR null mice, exhibited similar increased papilloma incidence, than RXRα$^{ep-/-}$ mice, and PPARγ$^{ep-/-}$ mice also exhibited decrease in Pten expression and SB expression of α6β4 integrins. In contrast, VDR null, but not PPARα null or PPARγ$^{ep-/-}$ mice exhibited hair follicle degeneration, decrease of e-cadherin expression and increased MGs (**table 7**). These data indicate that the two carcinogenic events, development of papillomas and melanoma, observed in RXRα$^{ep-/-}$ mutants can be functionally separated. RXRα/PPARα and RXRα/PPARγ heterodimers seem to play major roles as suppressors of epithelial tumors, whereas keratinocytic RXRα/VDR heterodimers seem to play an important role in the control of melanocytes growth. Interestingly, the combined alterations observed in VDR null mice (hair follicle degeneration, down regulation of e-cadherin and formation of MG's) and in PPARγ$^{ep-/-}$ mice (down regulation of Pten, suprabasal expression of α6β4 integrins and increased papilloma formation) recapitulate almost all the alterations observed in RXRα$^{ep-/-}$ mice.

Table 7. Summary of RXRα and its heterodimeric partners functions during skin carcinogenesis.

								DMBA/TPA			DMBA	
Genotype	KHP	INF	HFD	Pten	SCF	e-cad	SB α6β4	Pap	MG's	Mel	Pap	MG's
RXRα$^{ep-/-}$	+++	+++	+++	↓	↑	↓	+++	+++	+++	+++	-	+
RXRα$^{ep-/-(c)}$	+++	+++	+++	↓	↑	↓	+++	ND	ND	ND	+	+
PPARγ$^{ep-/-}$	+	-	-	↓	NSD	NSD	+	+++	NSD	-	-	-
PPARγ$^{ep-/-(c)}$	+	-	-	↓	NSD	NSD	ND	+++	NSD	-	-	-
PPARα null	-	-	-	ND	ND	ND	ND	+++	NSD	-	-	-
VDR null	-	-	+++	NSD	NSD	↓	-	NSD	+++	-	-	-

KHP = keratinocyte hyperproliferation, INF = skin inflammation, HFD = hair follicle degeneration, Pap = papilloma, Mel = melanoma, MG's = melanocytic growths, SCF = stem cell factor, e-cad = e-cadherin, SB α6β4= suprabasal expression of α6β4 integrins, ND = not determined, NSD = no significant difference when compared to control, ↓ = downregulated, ↑ = upregulated

Thus, ablation of both PPARγ (or PPARα) and VDR in keratinocytes may completely recapitulate the phenotype observed in RXRα$^{ep-/-}$ mice (**table 7**). However, the contribution of other heterodimeric partners of RXRα can not be discarded. The generation of mice lacking PPARα or VDR selectively in epidermal keratinocytes, may help to elucidate the precise role of this receptors during skin carcinogenesis.

We have also shown that ablation of RXRα in keratinocytes during epidermal morphogenesis, but not at adult stage, is sufficient for the promotion of epithelial tumors in response to a single DMBA application. Interestingly, PPARγ$^{ep-/-(c)}$ mice, in which PPARγ was selectively ablated in epidermal keratinocytes during epidermal morphogenesis, or PPARα null and VDR null mice, in which null mutations of the PPARα and VDR genes have been introduced in the germ line, respectively, did not develop epithelial tumors in response to a single topical application of DMBA (**table 7**). We can hypothesize that the simultaneous ablation of more than one RXRα heterodimeric partner, PPARα and PPARγ, PPARα and VDR or PPARγ and VDR for example, may also results in spontaneous development of skin tumors in response to a single topical application of DMBA.

4. Conclusion and Perspectives.

Our data demonstrate that keratinocytic RXRα plays a key role during the promotion stage of the two-step chemical skin carcinogenesis, most likely by preventing the clonal expansion of the initiated cells. Keratinocytic RXRα/PPARγ heterodimers, and probably RXRα/PPARα heterodimers, function as suppressors of DMBA/TPA-induced epithelial tumors. Moreover, keratinocytic RXRα plays key roles in the control of melanocyte growth and melanoma formation in response to a single topical application of DMBA. Degeneration of the hair follicle and down regulation of Pten and e-cadherin resulting from the ablation of keratinocytic RXRα predispose for melanoma formation. Our results suggest that keratinocytic RXRα/VDR heterodimers control melanocyte growth, and disruption of these heterodimers in keratinocytes results in the formation of MG's in response to a single DMBA application. However, the disruption of other RXRα/NR heterodimers may be required for the degeneration of these MG's into melanoma. Thus, keratinocytic RXRα acts as a tumor suppressor of chemically-induced melanoma and non-melanoma skin cancer in mice.

To determine whether PPARα and PPARγ may have overlapping and independent functions during chemically-induced skin carcinogenesis, we are establishing double PPARγ$^{ep-/-}$/PPARα null mice that will be subjected to a DMBA/TPA two-step carcinogenesis protocol. Moreover, generation of keratinocyte-specific PPARα mutant mice may help to better understand the role of keratinocytic PPARα during skin caricnogenesis. Similarly, because VDR is also expressed in other cell types, including melanocytes, the generation of a keratinocyte-specific VDR knockout mice will help to clarify the function of keratinocytic RXRα/VDR heterodimers in the development of carcinoma and melanoma.

We have also shown that ablation of RXRα in keratinocytes during epidermal morphogenesis is sufficient to promote their clonal expansion in presence of an activated Ha-ras oncogene and the formation of epithelial tumors. Our data suggest that RXRα may play critical roles in the commitment of epidermal keratinocytes during development and that ablation of RXRα during epidermal morphogenesis may results in the generation of keratinocytes with increased proliferative potential.

So far, our studies using the chemically-induced carcinogenesis and Tg.AC mice are based on the mutation and activation of the Ha-ras oncogene. It will be interesting to extend this studies to other models in which tumor formation is independent of the Ha-ras oncogene, for example, using UV-induced carcinogenesis or Human Papillomavirus (HPV)-induced carcinogenesis. On the other hand, it will be interesting to investigate the possible role of RXRα and its heterodimeric partners in the development of other epithelial cancers such as cervical, colon and prostate cancer. We have already established a collaboration with the

laboratories of Dr. Patricio Gariglio and Dr. Luis Covarrubias in Mexico to study the role of RXRα in HPV-induced cervical cancer.

Our genetic approach has lead to the identification of NRs heterodimers that play specific functions during skin carcinogenesis, and we hope that it will provide the basis for the development of new anti-cancer strategies based in combinatory treatments, in which inhibition or induction of a specific cellular process could be achieved by targeting a given NRs heterodimer.

5. Annexes

5.1 Annexe I

Skin carcinogenesis in PPARγ$^{ep-/-(c)}$ mice.

We have shown that selective ablation of RXRα in epidermal keratinocytes of adult mice, results in an increased susceptibility to chemically-induced skin carcinogenesis (section 2.1) and that selective ablation of RXRα in epidermal keratinocytes during skin morphogenesis, but not at adult stage, is sufficient on its own for the promotion of epithelial tumors, in response to a single topical application of DMBA. We have also shown that selective ablation of PPARγ in keratinocytes of adult mice (PPARγ$^{ep-/-}$), also results in increased susceptibility to chemically-induced skin carcinogenesis. To investigate if ablation of PPARγ in epidermal keratinocytes during skin morphogenesis is suficient for the promotion of skin tumors in response to a single DMBA application, we generated PPARγ$^{ep-/-(c)}$ mice, in which PPARγ alleles are selective ablated in epidermal keratinocytes during skin morphogenesis. Back skin of 6-8 week-old PPARγ$^{ep-/-(c)}$ and PPARγ$^{L2/L2}$ (CT) mice was shaved and one week later a single dose of 50 mg DMBA was topically applied to the back skin. Mice were observed for a period of 40 weeks. No skin tumors were observed in either PPARγ$^{ep-/-(c)}$ or CT mice (figure 20 A and B and data not shown). However, in agreement with the data shown in section 2.1, PPARγ$^{ep-/-(c)}$ mice were more susceptibile to chemically-induced skin carcinogenesis than CT mice. PPARγ$^{ep-/-(c)}$ mice exhibited increased tumor incidence (figure 20A) and multiplicity (figure 20B) than CT mice in response to DMBA/TPA treatment.

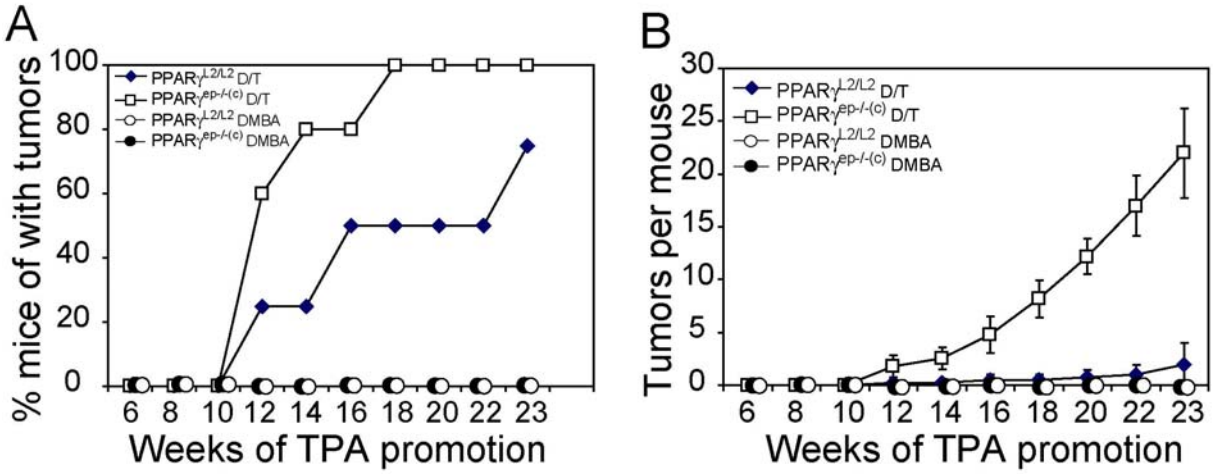

Figure 20. Skin carcinogenesis in PPARγ$^{ep-/-}$ mice. (A) tumor incidence, (B) average tumor per mouse +/- SEM. D/T: BA/TPA treated

6. References

Aboulafia, D.M., Norris, D., Henry, D., Grossman, R.J., Thommes, J., Bundow, D., Yocum, R.C. and Stevens, V. (2003) 9-cis-retinoic acid capsules in the treatment of AIDS-related Kaposi sarcoma: results of a phase 2 multicenter clinical trial. *Arch Dermatol*, **139**, 178-186.

Abremski, K. and Hoess, R. (1984) Bacteriophage P1 site-specific recombination. Purification and properties of the Cre recombinase protein. *J Biol Chem*, **259**, 1509-1514. 1502: Austin S et al. A novel role for site-specifi...[PMID: 7026049]Related Articles, Links.

Adams, J.C. and Watt, F.M. (1990) Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes alpha 5 beta 1 integrin loss from the cell surface. *Cell*, **63**, 425-435.

Adam-Stitah, S., Penna, L., Chambon, P. and Rochette-Egly, C. (1999) Hyperphosphorylation of the retinoid X receptor alpha by activated c-Jun NH2-terminal kinases. *J Biol Chem*, **274**, 18932-18941.

Akiyama, M., Smith, L.T. and Shimizu, H. (2000) Changing patterns of localization of putative stem cells in developing human hair follicles. *J Invest Dermatol*, **114**, 321-327.

Akutsu, N., Lin, R., Bastien, Y., Bestawros, A., Enepekides, D.J., Black, M.J. and White, J.H. (2001) Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. *Mol Endocrinol*, **15**, 1127-1139. 1122: Prudencio J et al. Action of low calcemic 1121alpha...[PMID: 11353784]Related Articles, Links.

Aldaz, C.M., Conti, C.J., Gimenez, I.B., Slaga, T.J. and Klein-Szanto, A.J. (1985) Cutaneous changes during prolonged application of 12-O-tetradecanoylphorbol-13-acetate on mouse skin and residual effects after cessation of treatment. *Cancer Res*, **45**, 2753-2759.

Ali, S., Metzger, D., Bornert, J.M. and Chambon, P. (1993) Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region. *Embo J*, **12**, 1153-1160.

Alonso, L. and Fuchs, E. (2003) Stem cells of the skin epithelium. *Proc Natl Acad Sci U S A*, **100 Suppl 1**, 11830-11835.

Altucci, L. and Gronemeyer, H. (2001) The promise of retinoids to fight against cancer. *Nat Rev Cancer*, **1**, 181-193.

Amling, M., Priemel, M., Holzmann, T., Chapin, K., Rueger, J.M., Baron, R. and Demay, M.B. (1999) Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology*, **140**, 4982-4987.

Andrews, R.C. and Walker, B.R. (1999) Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)*, **96**, 513-523.

Aneskievich, B.J. and Fuchs, E. (1992) Terminal differentiation in keratinocytes involves positive as well as negative regulation by retinoic acid receptors and retinoid X receptors at retinoid response elements. *Mol Cell Biol*, **12**, 4862-4871.

Anolik, J.H., Di Giovanna, J.J. and Gaspari, A.A. (1998) Effect of isotretinoin therapy on natural killer cell activity in patients with xeroderma pigmentosum. *Br J Dermatol*, **138**, 236-241.

Aranda, A. and Pascual, A. (2001) Nuclear hormone receptors and gene expression. *Physiol Rev*, **81**, 1269-1304.

Arbeit, J.M., Munger, K., Howley, P.M. and Hanahan, D. (1994) Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. *J Virol*, **68**, 4358-4368.

Arch, J.R. (2002) Lessons in obesity from transgenic animals. *J Endocrinol Invest*, **25**, 867-875.

Archambault, M., Yaar, M. and Gilchrest, B.A. (1995) Keratinocytes and fibroblasts in a human skin equivalent model enhance melanocyte survival and melanin synthesis after ultraviolet irradiation. *J Invest Dermatol*, **104**, 859-867.

Armstrong, B.K. and Kricker, A. (2001) The epidemiology of UV induced skin cancer. *J Photochem Photobiol B*, **63**, 8-18.

Aslam, F., McCabe, L., Frenkel, B., van Wijnen, A.J., Stein, G.S., Lian, J.B. and Stein, J.L. (1999) AP-1 and vitamin D receptor (VDR) signaling pathways converge at the rat osteocalcin VDR element: requirement for the internal activating protein-1 site for vitamin D-mediated trans-activation. *Endocrinology*, **140**, 63-70.

Asou, H., Verbeek, W., Williamson, E., Elstner, E., Kubota, T., Kamada, N. and Koeffler, H.P. (1999) Growth inhibition of myeloid leukemia cells by troglitazone, a ligand for peroxisome proliferator activated receptor gamma, and retinoids. *Int J Oncol*, **15**, 1027-1031.

Aszterbaum, M., Rothman, A., Johnson, R.L., Fisher, M., Xie, J., Bonifas, J.M., Zhang, X., Scott, M.P. and Epstein, E.H., Jr. (1998) Identification of mutations in the human PATCHED gene in sporadic basal cell carcinomas and in patients with the basal cell nevus syndrome. *J Invest Dermatol*, **110**, 885-888.

Athar, M., Agarwal, R., Wang, Z.Y., Lloyd, J.R., Bickers, D.R. and Mukhtar, H. (1991) All-trans retinoic acid protects against conversion of chemically induced and ultraviolet B radiation-induced skin papillomas to carcinomas. *Carcinogenesis*, **12**, 2325-2329.

Attar, P.S., Wertz, P.W., McArthur, M., Imakado, S., Bickenbach, J.R. and Roop, D.R. (1997) Inhibition of retinoid signaling in transgenic mice alters lipid processing and disrupts epidermal barrier function. *Mol Endocrinol*, **11**, 792-800.

Austin, S., Ziese, M. and Sternberg, N. (1981) A novel role for site-specific recombination in maintenance of bacterial replicons. *Cell*, **25**, 729-736. 723: Orban PC et al. Tissue- and site-specific DNA...[PMID: 1495975]Related Articles, Links.

Bagchi, I.C., Cheon, Y.P., Li, Q. and Bagchi, M.K. (2003) Progesterone receptor-regulated gene networks in implantation. *Front Biosci*, **8**, s852-861.

Balmain, A., Brown, K., Akhurst, R.J. and Fee, F.M. (1988) Molecular analysis of chemical carcinogenesis in the skin. *Br J Cancer Suppl*, **9**, 72-75.

Banks, G.C., Deterding, L.J., Tomer, K.B. and Archer, T.K. (2001) Hormone-mediated dephosphorylation of specific histone H1 isoforms. *J Biol Chem*, **276**, 36467-36473.

Banner, C.D., Gottlicher, M., Widmark, E., Sjovall, J., Rafter, J.J. and Gustafsson, J.A. (1993) A systematic analytical chemistry/cell assay approach to isolate activators of orphan nuclear receptors from biological extracts: characterization of peroxisome proliferator-activated receptor activators in plasma. *J Lipid Res*, **34**, 1583-1591.

Bardot, O., Aldridge, T.C., Latruffe, N. and Green, S. (1993) PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. *Biochem Biophys Res Commun*, **192**, 37-45.

Barrandon, Y. and Green, H. (1987) Three clonal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci U S A*, **84**, 2302-2306.

Anexe

Battalora, M.S., Spalding, J.W., Szczesniak, C.J., Cape, J.E., Morris, R.J., Trempus, C.S., Bortner, C.D., Lee, B.M. and Tennant, R.W. (2001) Age-dependent skin tumorigenesis and transgene expression in the Tg.AC (v-Ha-ras) transgenic mouse. *Carcinogenesis*, **22**, 651-659.

Beato, M., Herrlich, P. and Schutz, G. (1995) Steroid hormone receptors: many actors in search of a plot. *Cell*, **83**, 851-857.

Benkoussa, M., Brand, C., Delmotte, M.H., Formstecher, P. and Lefebvre, P. (2002) Retinoic acid receptors inhibit AP1 activation by regulating extracellular signal-regulated kinase and CBP recruitment to an AP1-responsive promoter. *Mol Cell Biol*, **22**, 4522-4534.

Ben-Zimra, M., Koler, M., Melamed-Book, N., Arensburg, J., Payne, A.H. and Orly, J. (2002) Uterine and placental expression of steroidogenic genes during rodent pregnancy. *Mol Cell Endocrinol*, **187**, 223-231.

Bernardi, R.J., Johnson, C.S., Modzelewski, R.A. and Trump, D.L. (2002) Antiproliferative effects of 1alpha,25-dihydroxyvitamin D(3) and vitamin D analogs on tumor-derived endothelial cells. *Endocrinology*, **143**, 2508-2514.

Bernot, K.M., Coulombe, P.A. and McGowan, K.M. (2002) Keratin 16 expression defines a subset of epithelial cells during skin morphogenesis and the hair cycle. *J Invest Dermatol*, **119**, 1137-1149.

Bernstein, Z.P., Chanan-Khan, A., Miller, K.C., Northfelt, D.W., Lopez-Berestein, G. and Gill, P.S. (2002) A multicenter phase II study of the intravenous administration of liposomal tretinoin in patients with acquired immunodeficiency syndrome-associated Kaposi's sarcoma. *Cancer*, **95**, 2555-2561.

Berry, M., Metzger, D. and Chambon, P. (1990) Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *Embo J*, **9**, 2811-2818.

Berton, T.R., Matsumoto, T., Page, A., Conti, C.J., Deng, C.X., Jorcano, J.L. and Johnson, D.G. (2003) Tumor formation in mice with conditional inactivation of Brca1 in epithelial tissues. *Oncogene*, **22**, 5415-5426.

Bessou-Touya, S., Picardo, M., Maresca, V., Surleve-Bazeille, J.E., Pain, C. and Taieb, A. (1998) Chimeric human epidermal reconstructs to study the role of melanocytes and keratinocytes in pigmentation and photoprotection. *J Invest Dermatol*, **111**, 1103-1108.

Bhattacharjee, R.N., Banks, G.C., Trotter, K.W., Lee, H.L. and Archer, T.K. (2001) Histone H1 phosphorylation by Cdk2 selectively modulates mouse mammary tumor virus transcription through chromatin remodeling. *Mol Cell Biol*, **21**, 5417-5425.

Bickenbach, J.R. and Chism, E. (1998) Selection and extended growth of murine epidermal stem cells in culture. *Exp Cell Res*, **244**, 184-195.

Bickenbach, J.R. and Holbrook, K.A. (1987) Label-retaining cells in human embryonic and fetal epidermis. *J Invest Dermatol*, **88**, 42-46.

Bikle, D.D. (1995) 1,25(OH)2D3-regulated human keratinocyte proliferation and differentiation: basic studies and their clinical application. *J Nutr*, **125**, 1709S-1714S.

Bikle, D.D. (1996) 1,25(OH)2D3-modulated calcium induced keratinocyte differentiation. *J Investig Dermatol Symp Proc*, **1**, 22-27.

Bikle, D.D., Nemanic, M.K., Gee, E. and Elias, P. (1986) 1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation. *J Clin Invest*, **78**, 557-566.

Billoni, N., Buan, B., Gautier, B., Collin, C., Gaillard, O., Mahe, Y.F. and Bernard, B.A. (2000a) Expression of peroxisome proliferator activated receptors (PPARs) in human hair follicles and PPAR alpha involvement in hair growth. *Acta Derm Venereol*, **80**, 329-334.

Billoni, N., Buan, B., Gautier, B., Gaillard, O., Mahe, Y.F. and Bernard, B.A. (2000b) Thyroid hormone receptor beta1 is expressed in the human hair follicle. *Br J Dermatol*, **142**, 645-652.

Billoni, N., Gautier, B., Mahe, Y.F. and Bernard, B.A. (1997) Expression of retinoid nuclear receptor superfamily members in human hair follicles and its implication in hair growth. *Acta Derm Venereol*, **77**, 350-355.

Binder, R.L., Gallagher, P.M., Johnson, G.R., Stockman, S.L., Smith, B.J., Sundberg, J.P. and Conti, C.J. (1997) Evidence that initiated keratinocytes clonally expand into multiple existing hair follicles during papilloma histogenesis in SENCAR mouse skin. *Mol Carcinog*, **20**, 151-158.

Birt, D.F., Yaktine, A. and Duysen, E. (1999) Glucocorticoid mediation of dietary energy restriction inhibition of mouse skin carcinogenesis. *J Nutr*, **129**, 571S-574S.

Bittiner, B., Bleehen, S.S. and MacNeil, S. (1991) 1 alpha,25(OH)2 vitamin D3 increases intracellular calcium in human keratinocytes. *Br J Dermatol*, **124**, 230-235. 237: Kitano Y et al. Suppression of proliferation ...[PMID: 1907555]Related Articles, Links.

Blanco, J.C., Minucci, S., Lu, J., Yang, X.J., Walker, K.K., Chen, H., Evans, R.M., Nakatani, Y. and Ozato, K. (1998) The histone acetylase PCAF is a nuclear receptor coactivator. *Genes Dev*, **12**, 1638-1651.

Blauer, M., Vaalasti, A., Pauli, S.L., Ylikomi, T., Joensuu, T. and Tuohimaa, P. (1991) Location of androgen receptor in human skin. *J Invest Dermatol*, **97**, 264-268.

Blumenberg, M., Connolly, D.M. and Freedberg, I.M. (1992) Regulation of keratin gene expression: the role of the nuclear receptors for retinoic acid, thyroid hormone, and vitamin D3. *J Invest Dermatol*, **98**, 42S-49S.

Bo, W.J. (1956) The relationship between vitamin A deficiency and estrogen in producing uterine metaplasia in the rat. *Anat Rec*, **124**, 619-627.

Bo, W.J. (1957) Relation of vitamin A deficiency and estrogen to induction of keratinizing metaplasia in the uterus of the rat. *Am J Clin Nutr*, **5**, 666-673.

Bodak, N., Queille, S., Avril, M.F., Bouadjar, B., Drougard, C., Sarasin, A. and Daya-Grosjean, L. (1999) High levels of patched gene mutations in basal-cell carcinomas from patients with xeroderma pigmentosum. *Proc Natl Acad Sci U S A*, **96**, 5117-5122.

Boehm, N., Samama, B., Cribier, B. and Rochette-Egly, C. (2004) Retinoic-acid receptor beta expression in melanocytes. *Eur J Dermatol*, **14**, 19-23.

Boelsterli, U.A. (2003) Animal models of human disease in drug safety assessment. *J Toxicol Sci*, **28**, 109-121.

Boldrini, L., Loggini, B., Gisfredi, S., Zucconi, Y., Baldinotti, F., Fogli, A., Simi, P., Cervadoro, G., Barachini, P., Basolo, F., Pingitore, R. and Fontanini, G. (2003) Mutations of Fas (APO-1/CD95) and p53 genes in nonmelanoma skin cancer. *J Cutan Med Surg*, **7**, 112-118.

Bollag, W. (1971) Therapy of chemically induced skin tumors of mice with vitamin A palmitate and vitamin A acid. *Experientia*, **27**, 90-92.

Bollag, W. (1972) Prophylaxis of chemically induced benign and malignant epithelial tumors by vitamin A acid (retinoic acid). *Eur J Cancer*, **8**, 689-693.

Bollag, W. (1974) Therapeutic effects of an aromatic retinoic acid analog on chemically induced skin papillomas and carcinomas of mice. *Eur J Cancer*, **10**, 731-737.

Bollag, W. (1975) Therapy of epithelial tumors with an aromatic retinoic acid analog. *Chemotherapy*, **21**, 236-247.

Bolshakov, S., Walker, C.M., Strom, S.S., Selvan, M.S., Clayman, G.L., El-Naggar, A., Lippman, S.M., Kripke, M.L. and Ananthaswamy, H.N. (2003) p53 mutations in human aggressive and nonaggressive basal and squamous cell carcinomas. *Clin Cancer Res*, **9**, 228-234.

Boonstra, J., Rijken, P., Humbel, B., Cremers, F., Verkleij, A. and van Bergen en Henegouwen, P. (1995) The epidermal growth factor. *Cell Biol Int*, **19**, 413-430.

Booth, B.A., Polak, K.L. and Uitto, J. (1980) Collagen biosynthesis by human skin fibroblasts. I. Optimization of the culture conditions for synthesis of type I and type III procollagens. *Biochim Biophys Acta*, **607**, 145-160.

Bos, J.L. (1989) ras oncogenes in human cancer: a review. *Cancer Res*, **49**, 4682-4689.

Boudjelal, M., Voorhees, J.J. and Fisher, G.J. (2002) Retinoid signaling is attenuated by proteasome-mediated degradation of retinoid receptors in human keratinocyte HaCaT cells. *Exp Cell Res*, **274**, 130-137.

Braakhuis, B.J., Klaassen, I., van der Leede, B.M., Cloos, J., Brakenhoff, R.H., Copper, M.P., Teerlink, T., Hendriks, H.F., van der Saag, P.T. and Snow, G.B. (1997) Retinoid metabolism and all-trans retinoic acid-induced growth inhibition in head and neck squamous cell carcinoma cell lines. *Br J Cancer*, **76**, 189-197.

Bradl, M., Klein-Szanto, A., Porter, S. and Mintz, B. (1991) Malignant melanoma in transgenic mice. *Proc Natl Acad Sci U S A*, **88**, 164-168.

Braissant, O., Foufelle, F., Scotto, C., Dauca, M. and Wahli, W. (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology*, **137**, 354-366.

Braissant, O. and Wahli, W. (1998) Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. *Endocrinology*, **139**, 2748-2754. 2742: Braissant O et al. Differential expression of pe...[PMID: 8536636]Related Articles, Links.

Brakebusch, C., Grose, R., Quondamatteo, F., Ramirez, A., Jorcano, J.L., Pirro, A., Svensson, M., Herken, R., Sasaki, T., Timpl, R., Werner, S. and Fassler, R. (2000) Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. *Embo J*, **19**, 3990-4003.

Brand, N., Petkovich, M., Krust, A., Chambon, P., de The, H., Marchio, A., Tiollais, P. and Dejean, A. (1988) Identification of a second human retinoic acid receptor. *Nature*, **332**, 850-853.

Brandenberger, A.W., Tee, M.K., Lee, J.Y., Chao, V. and Jaffe, R.B. (1997) Tissue distribution of estrogen receptors alpha (ER-alpha) and beta (ER-beta) mRNA in the midgestational human fetus. *J Clin Endocrinol Metab*, **82**, 3509-3512.

Braunstein, S., Kaplan, G., Gottlieb, A.B., Schwartz, M., Walsh, G., Abalos, R.M., Fajardo, T.T., Guido, L.S. and Krueger, J.G. (1994) GM-CSF activates regenerative epidermal growth and stimulates keratinocyte proliferation in human skin in vivo. *J Invest Dermatol*, **103**, 601-604.

Brecher, P.I. and Wotiz, H.H. (1969) Dissociation of estradiol from a uterine nuclear receptor. *Endocrinology*, **84**, 718-726.

Brindley, D.N. (1995) Role of glucocorticoids and fatty acids in the impairment of lipid metabolism observed in the metabolic syndrome. *Int J Obes Relat Metab Disord*, **19 Suppl 1**, S69-75.

Broome Powell, M., Gause, P.R., Hyman, P., Gregus, J., Lluria-Prevatt, M., Nagle, R. and Bowden, G.T. (1999) Induction of melanoma in TPras transgenic mice. *Carcinogenesis*, **20**, 1747-1753.

Brown, A.J., Dusso, A. and Slatopolsky, E. (1999) Vitamin D. *Am J Physiol*, **277**, F157-175.

Brown, A.J., Finch, J.L., Lopez-Hilker, S., Dusso, A., Ritter, C., Pernalete, N. and Slatopolsky, E. (1990) New active analogues of vitamin D with low calcemic activity. *Kidney Int Suppl*, **29**, S22-27.

Brown, R., Gray, R.H. and Bernstein, I.A. (1985) Retinoids alter the direction of differentiation in primary cultures of cutaneous keratinocytes. *Differentiation*, **28**, 268-278.

Brumbaugh, P.F. and Haussler, M.R. (1975) Specific binding of 1alpha,25-dihydroxycholecalciferol to nuclear components of chick intestine. *J Biol Chem*, **250**, 1588-1594.

Bruna, A., Nicolas, M., Munoz, A., Kyriakis, J.M. and Caelles, C. (2003) Glucocorticoid receptor-JNK interaction mediates inhibition of the JNK pathway by glucocorticoids. *Embo J*, **22**, 6035-6044.

Butt, T.R. and Walfish, P.G. (1996) Human nuclear receptor heterodimers: opportunities for detecting targets of transcriptional regulation using yeast. *Gene Expr*, **5**, 255-268.

Byrne, C., Tainsky, M. and Fuchs, E. (1994) Programming gene expression in developing epidermis. *Development*, **120**, 2369-2383.

Caelles, C., Gonzalez-Sancho, J.M. and Munoz, A. (1997) Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev*, **11**, 3351-3364.

Cannon, R.E., Spalding, J.W., Trempus, C.S., Szczesniak, C.J., Virgil, K.M., Humble, M.C. and Tennant, R.W. (1997) Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. *Mol Carcinog*, **20**, 108-114.

Cario-Andre, M., Bessou, S., Gontier, E., Maresca, V., Picardo, M. and Taieb, A. (1999) The reconstructed epidermis with melanocytes: a new tool to study pigmentation and photoprotection. *Cell Mol Biol (Noisy-le-grand)*, **45**, 931-942.

Carr, B.R., Parker, C.R., Jr., Ohashi, M., MacDonald, P.C. and Simpson, E.R. (1983) Regulation of human fetal testicular secretion of testosterone: low-density lipoprotein-cholesterol and cholesterol synthesized de novo as steroid precursor. *Am J Obstet Gynecol*, **146**, 241-247.

Cattley, R.C. (2003) Regulation of cell proliferation and cell death by peroxisome proliferators. *Microsc Res Tech*, **61**, 179-184. 173: Roberts RA et al. PPAR alpha and the regulation...[PMID: 12505304]Related Articles, Links.

Chakravarti, D., LaMorte, V.J., Nelson, M.C., Nakajima, T., Schulman, I.G., Juguilon, H., Montminy, M. and Evans, R.M. (1996) Role of CBP/P300 in nuclear receptor signalling. *Nature*, **383**, 99-103.

Chambon, P. (1994) The retinoid signaling pathway: molecular and genetic analyses. *Semin Cell Biol*, **5**, 115-125.

Chambon, P. (1996) A decade of molecular biology of retinoic acid receptors. *Faseb J*, **10**, 940-954.

Chan, L.S. (1997) Human skin basement membrane in health and in autoimmune diseases. *Front Biosci*, **2**, d343-352.

Chang, C., Kokontis, J., Liao, S.S. and Chang, Y. (1989) Isolation and characterization of human TR3 receptor: a member of steroid receptor superfamily. *J Steroid Biochem*, **34**, 391-395.

Chapellier, B., Mark, M., Messaddeq, N., Calleja, C., Warot, X., Brocard, J., Gerard, C., Li, M., Metzger, D., Ghyselinck, N.B. and Chambon, P. (2002) Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled. *Embo J*, **21**, 3402-3413.

Charles, M.A., Ryffel, G.U., Obinata, M., McCarthy, B.J. and Baxter, J.D. (1975) Nuclear receptors for thyroid hormone: evidence for nonrandom distribution within chromatin. *Proc Natl Acad Sci U S A*, **72**, 1787-1791.

Chen, C.F., Goyette, P. and Lohnes, D. (2004) RARgamma acts as a tumor suppressor in mouse keratinocytes. *Oncogene*.

Chen, C.H., Sakai, Y. and Demay, M.B. (2001) Targeting expression of the human vitamin D receptor to the keratinocytes of vitamin D receptor null mice prevents alopecia. *Endocrinology*, **142**, 5386-5389. 5314: Bikle DD et al. Vitamin D regulated keratinoc...[PMID: 12520529]Related Articles, Links.

Chen, D., Riedl, T., Washbrook, E., Pace, P.E., Coombes, R.C., Egly, J.M. and Ali, S. (2000) Activation of estrogen receptor alpha by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7. *Mol Cell*, **6**, 127-137.

Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y. and Evans, R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*, **90**, 569-580.

Chen, J.D. and Evans, R.M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature*, **377**, 454-457.

Chen, L.C., Kirchhoff, S. and De Luca, L.M. (1994a) Effect of excess dietary retinoic acid on skin papilloma and carcinoma formation induced by a complete carcinogenesis protocol in female Sencar mice. *Cancer Lett*, **78**, 63-67.

Chen, L.C., Sly, L. and De Luca, L.M. (1994b) High dietary retinoic acid prevents malignant conversion of skin papillomas induced by a two-stage carcinogenesis protocol in female SENCAR mice. *Carcinogenesis*, **15**, 2383-2386.

Chen, L.C., Tarone, R., Huynh, M. and De Luca, L.M. (1995) High dietary retinoic acid inhibits tumor promotion and malignant conversion in a two-stage skin carcinogenesis protocol using 7,12-dimethylbenz[a]anthracene as the initiator and mezerein as the tumor promoter in female SENCAR mice. *Cancer Lett*, **95**, 113-118.

Chida, K., Hashiba, H., Fukushima, M., Suda, T. and Kuroki, T. (1985) Inhibition of tumor promotion in mouse skin by 1 alpha,25-dihydroxyvitamin D3. *Cancer Res*, **45**, 5426-5430.

Chin, L., Pomerantz, J., Polsky, D., Jacobson, M., Cohen, C., Cordon-Cardo, C., Horner, J.W., 2nd and DePinho, R.A. (1997) Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. *Genes Dev*, **11**, 2822-2834.

Chu, P.G. and Weiss, L.M. (2002) Keratin expression in human tissues and neoplasms. *Histopathology*, **40**, 403-439.

Chun, K.S., Keum, Y.S., Han, S.S., Song, Y.S., Kim, S.H. and Surh, Y.J. (2003) Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. *Carcinogenesis*, **24**, 1515-1524.

Clark, W.H., Jr. (1991) Human cutaneous malignant melanoma as a model for cancer. *Cancer Metastasis Rev*, **10**, 83-88.

Cockerell, C.J. (2000) Histopathology of incipient intraepidermal squamous cell carcinoma ("actinic keratosis"). *J Am Acad Dermatol*, **42**, 11-17.

Conneely, O.M., Mulac-Jericevic, B., DeMayo, F., Lydon, J.P. and O'Malley, B.W. (2002) Reproductive functions of progesterone receptors. *Recent Prog Horm Res*, **57**, 339-355.

Cooper, M.P., Klaassen, I., Brakenhoff, R.H., Cloos, J., Snow, G.B. and Braakhuis, B.J. (1997) All-trans retinoic acid induced gene expression and growth inhibition in head and neck cancer cell lines. *Oral Oncol*, **33**, 270-274.

Cotsarelis, G., Kaur, P., Dhouailly, D., Hengge, U. and Bickenbach, J. (1999) Epithelial stem cells in the skin: definition, markers, localization and functions. *Exp Dermatol*, **8**, 80-88.

Cotsarelis, G., Sun, T.T. and Lavker, R.M. (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell*, **61**, 1329-1337.

D'Ambrosio, S.M., Slazinski, L., Whetstone, J.W. and Lowney, E. (1981) Excision repair of UV-induced pyrimidine dimers in human skin in vivo. *J Invest Dermatol*, **77**, 311-313.

Danielsson, C., Fehsel, K., Polly, P. and Carlberg, C. (1998) Differential apoptotic response of human melanoma cells to 1 alpha,25-dihydroxyvitamin D3 and its analogues. *Cell Death Differ*, **5**, 946-952.

Danielsson, C., Mathiasen, I.S., James, S.Y., Nayeri, S., Bretting, C., Hansen, C.M., Colston, K.W. and Carlberg, C. (1997) Sensitive induction of apoptosis in breast cancer cells by a novel 1,25- dihydroxyvitamin D3 analogue shows relation to promoter selectivity. *J Cell Biochem*, **66**, 552-562.

Danielsson, C., Torma, H., Vahlquist, A. and Carlberg, C. (1999) Positive and negative interaction of 1,25-dihydroxyvitamin D3 and the retinoid CD437 in the induction of human melanoma cell apoptosis. *Int J Cancer*, **81**, 467-470.

Darwiche, N., Celli, G., Tennenbaum, T., Glick, A.B., Yuspa, S.H. and De Luca, L.M. (1995) Mouse skin tumor progression results in differential expression of retinoic acid and retinoid X receptors. *Cancer Res*, **55**, 2774-2782.

Darwiche, N., Scita, G., Jones, C., Rutberg, S., Greenwald, E., Tennenbaum, T., Collins, S.J., De Luca, L.M. and Yuspa, S.H. (1996) Loss of retinoic acid receptors in mouse skin and skin tumors is associated with activation of the ras(Ha) oncogene and high risk for premalignant progression. *Cancer Res*, **56**, 4942-4949.

de Gruijl, F.R. (1999) Skin cancer and solar UV radiation. *Eur J Cancer*, **35**, 2003-2009.

de Gruijl, F.R., van Kranen, H.J. and Mullenders, L.H. (2001) UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *J Photochem Photobiol B*, **63**, 19-27.

De Luca, L.M., Sly, L., Jones, C.S. and Chen, L.C. (1993) Effects of dietary retinoic acid on skin papilloma and carcinoma formation in female SENCAR mice. *Carcinogenesis*, **14**, 539-542.

De Luca, L.M., Tarone, R., Huynh, M., Jones, C.S. and Chen, L.C. (1996) Dietary retinoic acid inhibits mouse skin carcinogenesis irrespective of age at initiation. *Nutr Cancer*, **25**, 249-257.

De Luca, M., D'Anna, F., Bondanza, S., Franzi, A.T. and Cancedda, R. (1988) Human epithelial cells induce human melanocyte growth in vitro but only skin keratinocytes regulate its proper differentiation in the absence of dermis. *J Cell Biol*, **107**, 1919-1926.

Delerive, P., De Bosscher, K., Besnard, S., Vanden Berghe, W., Peters, J.M., Gonzalez, F.J., Fruchart, J.C., Tedgui, A., Haegeman, G. and Staels, B. (1999) Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem*, **274**, 32048-32054.

DiGiovanna, J.J. (2001) Retinoid chemoprevention in patients at high risk for skin cancer. *Med Pediatr Oncol*, **36**, 564-567.

DiPersio, C.M., van der Neut, R., Georges-Labouesse, E., Kreidberg, J.A., Sonnenberg, A. and Hynes, R.O. (2000) alpha3beta1 and alpha6beta4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development. *J Cell Sci*, **113 (Pt 17)**, 3051-3062.

Di-Poi, N., Tan, N.S., Michalik, L., Wahli, W. and Desvergne, B. (2002) Antiapoptotic role of PPARbeta in keratinocytes via transcriptional control of the Akt1 signaling pathway. *Mol Cell*, **10**, 721-733. 710: Mossner R et al. Agonists of peroxisome prolif...[PMID: 12230498]Related Articles, Links.

DiSepio, D., Sutter, M., Johnson, A.T., Chandraratna, R.A. and Nagpal, S. (1999) Identification of the AP1-antagonism domain of retinoic acid receptors. *Mol Cell Biol Res Commun*, **1**, 7-13.

Domann, F.E., Jr., Levy, J.P., Finch, J.S. and Bowden, G.T. (1994) Constitutive AP-1 DNA binding and transactivating ability of malignant but not benign mouse epidermal cells. *Mol Carcinog*, **9**, 61-66.

Donatien, P., Surleve-Bazeille, J.E., Thody, A.J. and Taieb, A. (1993) Growth and differentiation of normal human melanocytes in a TPA-free, cholera toxin-free, low-serum medium and influence of keratinocytes. *Arch Dermatol Res*, **285**, 385-392.

Dreyer, C., Keller, H., Mahfoudi, A., Laudet, V., Krey, G. and Wahli, W. (1993) Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol Cell*, **77**, 67-76.

Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G. and Wahli, W. (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell*, **68**, 879-887. 872: Issemann I et al. The peroxisome proliferator-a...[PMID: 8240670]Related Articles, Links.

Driscoll, J.E., Seachord, C.L., Lupisella, J.A., Darveau, R.P. and Reczek, P.R. (1996) Ligand-induced conformational changes in the human retinoic acid receptor detected using monoclonal antibodies. *J Biol Chem*, **271**, 22969-22975.

Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R. and Chambon, P. (1994) Activation function 2 (AF-2) of retinoic acid receptor and 9-cis retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *Embo J*, **13**, 5370-5382.

Eckert, R.L., Crish, J.F. and Robinson, N.A. (1997) The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. *Physiol Rev*, **77**, 397-424.

Egner, U., Heinrich, N., Ruff, M., Gangloff, M., Mueller-Fahrnow, A. and Wurtz, J.M. (2001) Different ligands-different receptor conformations: modeling of the hER alpha LBD in complex with agonists and antagonists. *Med Res Rev*, **21**, 523-539.

Eichner, R. (1986) Epidermal effects of retinoids: in vitro studies. *J Am Acad Dermatol*, **15**, 789-797. 783: Magnaldo T et al. Expression of loricrin is neg...[PMID: 1378029]Related Articles, Links.

Eichner, R., Kahn, M., Capetola, R.J., Gendimenico, G.J. and Mezick, J.A. (1992) Effects of topical retinoids on cytoskeletal proteins: implications for retinoid effects on epidermal differentiation. *J Invest Dermatol*, **98**, 154-161.

Einspahr, J.G., Alberts, D.S., Warneke, J.A., Bozzo, P., Basye, J., Grogan, T.M., Nelson, M.A. and Bowden, G.T. (1999) Relationship of p53 mutations to epidermal cell proliferation and apoptosis in human UV-induced skin carcinogenesis. *Neoplasia*, **1**, 468-475.

El Abdaimi, K., Papavasiliou, V., Rabbani, S.A., Rhim, J.S., Goltzman, D. and Kremer, R. (1999) Reversal of hypercalcemia with the vitamin D analogue EB1089 in a human model of squamous cancer. *Cancer Res*, **59**, 3325-3328.

Elder, J.T., Astrom, A., Pettersson, U., Tavakkol, A., Krust, A., Kastner, P., Chambon, P. and Voorhees, J.J. (1992) Retinoic acid receptors and binding proteins in human skin. *J Invest Dermatol*, **98**, 36S-41S.

Elias, P.M., Cullander, C., Mauro, T., Rassner, U., Komuves, L., Brown, B.E. and Menon, G.K. (1998) The secretory granular cell: the outermost granular cell as a specialized secretory cell. *J Investig Dermatol Symp Proc*, **3**, 87-100.

Ellis, C.N., Varani, J., Fisher, G.J., Zeigler, M.E., Pershadsingh, H.A., Benson, S.C., Chi, Y. and Kurtz, T.W. (2000) Troglitazone improves psoriasis and normalizes models of proliferative skin disease: ligands for peroxisome proliferator-activated receptor-gamma inhibit keratinocyte proliferation. *Arch Dermatol*, **136**, 609-616.

Enepekides, D.J., Black, M.J. and White, J.H. (1999) The independent and combined effects of RAR-, RXR-, and VDR-selective ligands on the growth of squamous cell carcinoma in vitro. *J Otolaryngol*, **28**, 83-89.

Evans, C.B., Pillai, S. and Goldyne, M.E. (1993) Endogenous prostaglandin E2 modulates calcium-induced differentiation in human skin keratinocytes. *Prostaglandins Leukot Essent Fatty Acids*, **49**, 777-781.

Evans, S.R., Houghton, A.M., Schumaker, L., Brenner, R.V., Buras, R.R., Davoodi, F., Nauta, R.J. and Shabahang, M. (1996) Vitamin D receptor and growth inhibition by 1,25-dihydroxyvitamin D3 in human malignant melanoma cell lines. *J Surg Res*, **61**, 127-133.

Fajas, L., Fruchart, J.C. and Auwerx, J. (1998) PPARgamma3 mRNA: a distinct PPARgamma mRNA subtype transcribed from an independent promoter. *FEBS Lett*, **438**, 55-60.

Fajas, L., Schoonjans, K., Gelman, L., Kim, J.B., Najib, J., Martin, G., Fruchart, J.C., Briggs, M., Spiegelman, B.M. and Auwerx, J. (1999) Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol Cell Biol*, **19**, 5495-5503. 5494: Wahli W et al. Fatty acids, eicosanoids, and...[PMID: 10086196]Related Articles, Links.

Fang, S., Anderson, K.M. and Liao, S. (1969) Receptor proteins for androgens. On the role of specific proteins in selective retention of 17-beta-hydroxy-5-alpha-androstan-3-one by rat ventral prostate in vivo and in vitro. *J Biol Chem*, **244**, 6584-6595.

Fanjul, A., Dawson, M.I., Hobbs, P.D., Jong, L., Cameron, J.F., Harlev, E., Graupner, G., Lu, X.P. and Pfahl, M. (1994) A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. *Nature*, **372**, 107-111.

Fawell, S.E., Lees, J.A., White, R. and Parker, M.G. (1990) Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. *Cell*, **60**, 953-962.

Feil, P.D. and Bardin, C.W. (1975) Cytoplasmic and nuclear progesterone receptors in the guinea pig uterus. *Endocrinology*, **97**, 1398-1407.

Feil, R., Brocard, J., Mascrez, B., LeMeur, M., Metzger, D. and Chambon, P. (1996) Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A*, **93**, 10887-10890.

Feil, R., Wagner, J., Metzger, D. and Chambon, P. (1997) Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun*, **237**, 752-757.

Feingold, K.R., Man, M.Q., Menon, G.K., Cho, S.S., Brown, B.E. and Elias, P.M. (1990) Cholesterol synthesis is required for cutaneous barrier function in mice. *J Clin Invest*, **86**, 1738-1745.

Feng, X., Peng, Z.H., Di, W., Li, X.Y., Rochette-Egly, C., Chambon, P., Voorhees, J.J. and Xiao, J.H. (1997) Suprabasal expression of a dominant-negative RXR alpha mutant in transgenic mouse epidermis impairs regulation of gene transcription and basal keratinocyte proliferation by RAR-selective retinoids. *Genes Dev*, **11**, 59-71.

Fernandez-Mejia, C., Medina-Martinez, O., Martinez-Perez, L. and Goodman, P.A. (1999) The human insulin gene contains multiple transcriptional elements that respond to glucocorticoids. *Pancreas*, **18**, 336-341.

Ferraris, C., Bernard, B.A. and Dhouailly, D. (1997) Adult epidermal keratinocytes are endowed with pilosebaceous forming abilities. *Int J Dev Biol*, **41**, 491-498.

Finch, J.S., Albino, H.E. and Bowden, G.T. (1996) Quantitation of early clonal expansion of two mutant 61st codon c-Ha- ras alleles in DMBA/TPA treated mouse skin by nested PCR/RFLP. *Carcinogenesis*, **17**, 2551-2557.

Fisher, G.J., Talwar, H.S., Xiao, J.H., Datta, S.C., Reddy, A.P., Gaub, M.P., Rochette-Egly, C., Chambon, P. and Voorhees, J.J. (1994) Immunological identification and functional quantitation of retinoic acid and retinoid X receptor proteins in human skin. *J Biol Chem*, **269**, 20629-20635.

Fisher, G.J. and Voorhees, J.J. (1996) Molecular mechanisms of retinoid actions in skin. *Faseb J*, **10**, 1002-1013.

Fitzpatrick, S., Sheard, N.F., Clark, N.G. and Ritter, M.L. (2000) Vitamin D-deficient rickets: a multifactorial disease. *Nutr Rev*, **58**, 218-222.

Fondell, J.D., Brunel, F., Hisatake, K. and Roeder, R.G. (1996) Unliganded thyroid hormone receptor alpha can target TATA-binding protein for transcriptional repression. *Mol Cell Biol*, **16**, 281-287.

Fondell, J.D., Roy, A.L. and Roeder, R.G. (1993) Unliganded thyroid hormone receptor inhibits formation of a functional preinitiation complex: implications for active repression. *Genes Dev*, **7**, 1400-1410.

Forman, B.M., Chen, J. and Evans, R.M. (1997) Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci U S A*, **94**, 4312-4317.

Forman, B.M., Yang, C.R., Au, M., Casanova, J., Ghysdael, J. and Samuels, H.H. (1989) A domain containing leucine-zipper-like motifs mediate novel in vivo interactions between the thyroid hormone and retinoic acid receptors. *Mol Endocrinol*, **3**, 1610-1626.

Fowler, A.J., Sheu, M.Y., Schmuth, M., Kao, J., Fluhr, J.W., Rhein, L., Collins, J.L., Willson, T.M., Mangelsdorf, D.J., Elias, P.M. and Feingold, K.R. (2003) Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. *J Invest Dermatol*, **120**, 246-255. 242: Komuves LG et al. Oxysterol stimulation of epid...[PMID: 11851872]Related Articles, Links.

Frank, S., Stallmeyer, B., Kampfer, H., Kolb, N. and Pfeilschifter, J. (2000) Leptin enhances wound re-epithelialization and constitutes a direct function of leptin in skin repair. *J Clin Invest*, **106**, 501-509.

Freedberg, I.M., Tomic-Canic, M., Komine, M. and Blumenberg, M. (2001) Keratins and the keratinocyte activation cycle. *J Invest Dermatol*, **116**, 633-640.

Freedman, L.P. and Luisi, B.F. (1993) On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective. *J Cell Biochem*, **51**, 140-150.

Fuchs, E. (1995) Keratins and the skin. *Annu Rev Cell Dev Biol*, **11**, 123-153.

Fuchs, E. (1998) Beauty is skin deep: the fascinating biology of the epidermis and its appendages. *Harvey Lect*, **94**, 47-77.

Gamady, A., Koren, R., Ron, D., Liberman, U.A. and Ravid, A. (2003) Vitamin D enhances mitogenesis mediated by keratinocyte growth factor receptor in keratinocytes. *J Cell Biochem*, **89**, 440-449.

Gandarillas, A., Goldsmith, L.A., Gschmeissner, S., Leigh, I.M. and Watt, F.M. (1999) Evidence that apoptosis and terminal differentiation of epidermal keratinocytes are distinct processes. *Exp Dermatol*, **8**, 71-79.

Geilen, C.C., Wieder, T. and Orfanos, C.E. (1997) Ceramide signalling: regulatory role in cell proliferation, differentiation and apoptosis in human epidermis. *Arch Dermatol Res*, **289**, 559-566.

Gensler, H.L., Watson, R.R., Moriguchi, S. and Bowden, G.T. (1987) Effects of dietary retinyl palmitate or 13-cis-retinoic acid on the promotion of tumors in mouse skin. *Cancer Res*, **47**, 967-970.

George, R., Weightman, W., Russ, G.R., Bannister, K.M. and Mathew, T.H. (2002) Acitretin for chemoprevention of non-melanoma skin cancers in renal transplant recipients. *Australas J Dermatol*, **43**, 269-273.

Georges-Labouesse, E., Messaddeq, N., Yehia, G., Cadalbert, L., Dierich, A. and Le Meur, M. (1996) Absence of integrin alpha 6 leads to epidermolysis bullosa and neonatal death in mice. *Nat Genet*, **13**, 370-373.

Gerster, H. (1997) Vitamin A--functions, dietary requirements and safety in humans. *Int J Vitam Nutr Res*, **67**, 71-90.

Ghazizadeh, S. and Taichman, L.B. (2001) Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. *Embo J*, **20**, 1215-1222.

Ghosh, A.K., Bhattacharyya, S., Lakos, G., Chen, S.J., Mori, Y. and Varga, J. (2004) Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. *Arthritis Rheum*, **50**, 1305-1318.

Giandomenico, V., Vaccari, G., Fiorucci, G., Percario, Z., Vannuchi, S., Matarrese, P., Malorni, W., Romeo, G. and Affabris, G.R. (1998) Apoptosis and growth inhibition of squamous carcinoma cells treated with interferon-alpha, IFN-beta and retinoic acid are associated with induction of the cyclin-dependent kinase inhibitor p21. *Eur Cytokine Netw*, **9**, 619-631.

Giannotti, B., Vanzi, L., Difonzo, E.M. and Pimpinelli, N. (2003) The treatment of basal cell carcinomas in a patient with xeroderma pigmentosum with a combination of imiquimod 5% cream and oral acitretin. *Clin Exp Dermatol*, **28 Suppl 1**, 33-35.

Gibbs, S., Backendorf, C. and Ponec, M. (1996) Regulation of keratinocyte proliferation and differentiation by all-trans-retinoic acid, 9-cis-retinoic acid and 1,25-dihydroxy vitamin D3. *Arch Dermatol Res*, **288**, 729-738.

Giguere, V., Ong, E.S., Segui, P. and Evans, R.M. (1987) Identification of a receptor for the morphogen retinoic acid. *Nature*, **330**, 624-629.

Gijbels, M.J., van der Ham, F., van Bennekum, A.M., Hendriks, H.F. and Roholl, P.J. (1992) Alterations in cytokeratin expression precede histological changes in epithelia of vitamin A-deficient rats. *Cell Tissue Res*, **268**, 197-203.

Giudice, L.C. (1999) Genes associated with embryonic attachment and implantation and the role of progesterone. *J Reprod Med*, **44**, 165-171.

Glass, C.K. (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr Rev*, **15**, 391-407.

Glick, A.B., Kulkarni, A.B., Tennenbaum, T., Hennings, H., Flanders, K.C., O'Reilly, M., Sporn, M.B., Karlsson, S. and Yuspa, S.H. (1993) Loss of expression of transforming growth factor beta in skin and skin tumors is associated with hyperproliferation and a high risk for malignant conversion. *Proc Natl Acad Sci U S A*, **90**, 6076-6080.

Gonzalez, M.V., Jimenez, B., Berciano, M.T., Gonzalez-Sancho, J.M., Caelles, C., Lafarga, M. and Munoz, A. (2000) Glucocorticoids antagonize AP-1 by inhibiting the Activation/phosphorylation of JNK without affecting its subcellular distribution. *J Cell Biol*, **150**, 1199-1208.

Gooren, L.J. and Kruijver, F.P. (2002) Androgens and male behavior. *Mol Cell Endocrinol*, **198**, 31-40.

Govindan, M.V., Devic, M., Green, S., Gronemeyer, H. and Chambon, P. (1985) Cloning of the human glucocorticoid receptor cDNA. *Nucleic Acids Res*, **13**, 8293-8304.

Goyette, P., Feng Chen, C., Wang, W., Seguin, F. and Lohnes, D. (2000) Characterization of retinoic acid receptor-deficient keratinocytes. *J Biol Chem*, **275**, 16497-16505.

Green, S. (1992) Peroxisome proliferators: a model for receptor mediated carcinogenesis. *Cancer Surv*, **14**, 221-232.

Green, S., Walter, P., Greene, G., Krust, A., Goffin, C., Jensen, E., Scrace, G., Waterfield, M. and Chambon, P. (1986) Cloning of the human oestrogen receptor cDNA. *J Steroid Biochem*, **24**, 77-83. 10: Zelent A et al. Cloning of murine alpha and b...[PMID: 2544807]Related Articles, Links.

Greenberg, C.S., Birckbichler, P.J. and Rice, R.H. (1991) Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. *Faseb J*, **5**, 3071-3077.

Gronemeyer, H., Turcotte, B., Quirin-Stricker, C., Bocquel, M.T., Meyer, M.E., Krozowski, Z., Jeltsch, J.M., Lerouge, T., Garnier, J.M. and Chambon, P. (1987) The chicken progesterone receptor: sequence, expression and functional analysis. *Embo J*, **6**, 3985-3994.

Grousson, J. and Peguet-Navarro, J. (1998) Functional relevance of CD40 expression in human epidermis. *Cell Biol Toxicol*, **14**, 345-350.

Grubauer, G., Feingold, K.R., Harris, R.M. and Elias, P.M. (1989) Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res*, **30**, 89-96.

Guiochon-Mantel, A., Loosfelt, H., Lescop, P., Sar, S., Atger, M., Perrot-Applanat, M. and Milgrom, E. (1989) Mechanisms of nuclear localization of the progesterone receptor: evidence for interaction between monomers. *Cell*, **57**, 1147-1154.

Haake, A.R. and Scott, G.A. (1991) Physiologic distribution and differentiation of melanocytes in human fetal and neonatal skin equivalents. *J Invest Dermatol*, **96**, 71-77.

Hail, N., Jr. and Lotan, R. (2001) Synthetic retinoid CD437 promotes rapid apoptosis in malignant human epidermal keratinocytes and G1 arrest in their normal counterparts. *J Cell Physiol*, **186**, 24-34.

Hall, P.F. (1991) Cytochrome P-450 C21scc: one enzyme with two actions: hydroxylase and lyase. *J Steroid Biochem Mol Biol*, **40**, 527-532.

Halliday, G.M., Ho, K.K. and Barnetson, R.S. (1992) Regulation of the skin immune system by retinoids during carcinogenesis. *J Invest Dermatol*, **99**, 83S-86S. 13: De Luca LM et al. Dietary retinoids are essenti...[PMID: 1451104]Related Articles, Links.

Hanley, K., Jiang, Y., Crumrine, D., Bass, N.M., Appel, R., Elias, P.M., Williams, M.L. and Feingold, K.R. (1997) Activators of the nuclear hormone receptors PPARalpha and FXR accelerate the development of the fetal epidermal permeability barrier. *J Clin Invest*, **100**, 705-712.

Hanley, K., Jiang, Y., He, S.S., Friedman, M., Elias, P.M., Bikle, D.D., Williams, M.L. and Feingold, K.R. (1998) Keratinocyte differentiation is stimulated by activators of the nuclear hormone receptor PPARalpha. *J Invest Dermatol*, **110**, 368-375.

Hanley, K., Komuves, L.G., Bass, N.M., He, S.S., Jiang, Y., Crumrine, D., Appel, R., Friedman, M., Bettencourt, J., Min, K., Elias, P.M., Williams, M.L. and Feingold, K.R. (1999) Fetal epidermal differentiation and barrier development In vivo is accelerated by nuclear hormone receptor activators. *J Invest Dermatol*, **113**, 788-795.

Hanley, K., Komuves, L.G., Ng, D.C., Schoonjans, K., He, S.S., Lau, P., Bikle, D.D., Williams, M.L., Elias, P.M., Auwerx, J. and Feingold, K.R. (2000a) Farnesol stimulates differentiation in epidermal keratinocytes via PPARalpha. *J Biol Chem*, **275**, 11484-11491.

Hanley, K., Ng, D.C., He, S.S., Lau, P., Min, K., Elias, P.M., Bikle, D.D., Mangelsdorf, D.J., Williams, M.L. and Feingold, K.R. (2000b) Oxysterols induce differentiation in human keratinocytes and increase Ap-1-dependent involucrin transcription. *J Invest Dermatol*, **114**, 545-553.

Hanley, K., Wood, L., Ng, D.C., He, S.S., Lau, P., Moser, A., Elias, P.M., Bikle, D.D., Williams, M.L. and Feingold, K.R. (2001) Cholesterol sulfate stimulates involucrin transcription in keratinocytes by increasing Fra-1, Fra-2, and Jun D. *J Lipid Res*, **42**, 390-398. 392: Hanley K et al. Farnesol stimulates different...[PMID: 10753967]Related Articles, Links.

Hann, B. and Balmain, A. (2001) Building 'validated' mouse models of human cancer. *Curr Opin Cell Biol*, **13**, 778-784.

Hansen, C.M., Hamberg, K.J., Binderup, E. and Binderup, L. (2000) Seocalcitol (EB 1089): a vitamin D analogue of anti-cancer potential. Background, design, synthesis, pre-clinical and clinical evaluation. *Curr Pharm Des*, **6**, 803-828.

Hansen, C.M. and Maenpaa, P.H. (1997) EB 1089, a novel vitamin D analog with strong antiproliferative and differentiation-inducing effects on target cells. *Biochem Pharmacol*, **54**, 1173-1179.

Hansen, L.A., Spalding, J.W., French, J.E. and Tennant, R.W. (1995) A transgenic mouse model (TG.AC) for skin carcinogenesis: inducible transgene expression as a second critical event. *Prog Clin Biol Res*, **391**, 223-235.

Hansen, L.A. and Tennant, R. (1994a) Focal transgene expression associated with papilloma development in v- Ha-ras-transgenic TG.AC mice. *Mol Carcinog*, **9**, 143-154.

Hansen, L.A. and Tennant, R.W. (1994b) Follicular origin of epidermal papillomas in v-Ha-ras transgenic TG.AC mouse skin. *Proc Natl Acad Sci U S A*, **91**, 7822-7826.

Hansen, L.A., Trempus, C.S., Mahler, J.F. and Tennant, R.W. (1996) Association of tumor development with increased cellular proliferation and transgene overexpression, but not c-Ha-ras mutations, in v-Ha-ras transgenic Tg.AC mice. *Carcinogenesis*, **17**, 1825-1833.

Hara, M., Yaar, M. and Gilchrest, B.A. (1995) Endothelin-1 of keratinocyte origin is a mediator of melanocyte dendricity. *J Invest Dermatol*, **105**, 744-748.

Hard, T., Kellenbach, E., Boelens, R., Maler, B.A., Dahlman, K., Freedman, L.P., Carlstedt-Duke, J., Yamamoto, K.R., Gustafsson, J.A. and Kaptein, R. (1990) Solution structure of the glucocorticoid receptor DNA-binding domain. *Science*, **249**, 157-160.

Hardman, M.J., Sisi, P., Banbury, D.N. and Byrne, C. (1998) Patterned acquisition of skin barrier function during development. *Development*, **125**, 1541-1552.

Hatoum, A., El-Sabban, M.E., Khoury, J., Yuspa, S.H. and Darwiche, N. (2001) Overexpression of retinoic acid receptors alpha and gamma into neoplastic epidermal cells causes retinoic acid-induced growth arrest and apoptosis. *Carcinogenesis*, **22**, 1955-1963.

Hayes, K.C., McCombs, H.L. and Faherty, T.P. (1970) The fine structure of vitamin A deficiency. I. Parotid duct metaplasia. *Lab Invest*, **22**, 81-89.

Hayward, M.G. and Keatinge, W.R. (1981) Roles of subcutaneous fat and thermoregulatory reflexes in determining ability to stabilize body temperature in water. *J Physiol*, **320**, 229-251.

Hennings, H., Glick, A.B., Greenhalgh, D.A., Morgan, D.L., Strickland, J.E., Tennenbaum, T. and Yuspa, S.H. (1993) Critical aspects of initiation, promotion, and progression in multistage epidermal carcinogenesis. *Proc Soc Exp Biol Med*, **202**, 1-8.

Hertle, M.D., Adams, J.C. and Watt, F.M. (1991) Integrin expression during human epidermal development in vivo and in vitro. *Development*, **112**, 193-206.

Herz, J.M., Thomsen, W.J. and Yarbrough, G.G. (1997) Molecular approaches to receptors as targets for drug discovery. *J Recept Signal Transduct Res*, **17**, 671-776.

Herzig, M. and Christofori, G. (2002) Recent advances in cancer research: mouse models of tumorigenesis. *Biochim Biophys Acta*, **1602**, 97-113.

Heyden, A., Lutzow-Holm, C., Clausen, O.P., Thrane, E.V., Brandtzaeg, P., Roop, D.R., Yuspa, S.H. and Huitfeldt, H.S. (1994) Application of cantharidin or 12-O-tetradecanoylphorbol-13-acetate on mouse epidermis induces a cell population shift that causes altered keratin distribution. *Differentiation*, **57**, 187-193.

Hirobe, T. (1994) Keratinocytes are involved in regulating the developmental changes in the proliferative activity of mouse epidermal melanoblasts in serum-free culture. *Dev Biol*, **161**, 59-69.

Hirobe, T. (1995) Structure and function of melanocytes: microscopic morphology and cell biology of mouse melanocytes in the epidermis and hair follicle. *Histol Histopathol*, **10**, 223-237.

Hirose, T., Fujimoto, W., Tamaai, T., Kim, K.H., Matsuura, H. and Jetten, A.M. (1994) TAK1: molecular cloning and characterization of a new member of the nuclear receptor superfamily. *Mol Endocrinol*, **8**, 1667-1680.

Hoeck, W., Rusconi, S. and Groner, B. (1989) Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells. Investigations with a monospecific antiserum against a bacterially expressed receptor fragment. *J Biol Chem*, **264**, 14396-14402.

Holbrook, K.A., Underwood, R.A., Vogel, A.M., Gown, A.M. and Kimball, H. (1989) The appearance, density and distribution of melanocytes in human embryonic and fetal skin revealed by the anti-melanoma monoclonal antibody, HMB-45. *Anat Embryol (Berl)*, **180**, 443-455.

Holick, M.F. (1987) Photosynthesis of vitamin D in the skin: effect of environmental and life-style variables. *Fed Proc*, **46**, 1876-1882.

Holick, M.F. (2003) Vitamin D: A millenium perspective. *J Cell Biochem*, **88**, 296-307.

Holick, M.F., MacLaughlin, J.A. and Doppelt, S.H. (1981) Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. *Science*, **211**, 590-593.

Hong, W.K. and Itri, L.M. (1994) *Retinoids and human cancer*. Raven Press, New York.

Horlein, A.J., Naar, A.M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C.K. and et al. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature*, **377**, 397-404.

Horvai, A.E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T.M., Rose, D.W., Rosenfeld, M.G. and Glass, C.K. (1997) Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc Natl Acad Sci U S A*, **94**, 1074-1079.

Hsieh, J.C., Jurutka, P.W., Galligan, M.A., Terpening, C.M., Haussler, C.A., Samuels, D.S., Shimizu, Y., Shimizu, N. and Haussler, M.R. (1991) Human vitamin D receptor is selectively phosphorylated by protein kinase C on serine 51, a residue crucial to its trans-activation function. *Proc Natl Acad Sci U S A*, **88**, 9315-9319.

Hsu, M.Y., Meier, F. and Herlyn, M. (2002) Melanoma development and progression: a conspiracy between tumor and host. *Differentiation*, **70**, 522-536.

Hsu, M.Y., Meier, F.E., Nesbit, M., Hsu, J.Y., Van Belle, P., Elder, D.E. and Herlyn, M. (2000) E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. *Am J Pathol*, **156**, 1515-1525. 1513: Li G et al. Downregulation of E-cadherin ...[PMID: 11781826]Related Articles, Links.

Hu, X. and Lazar, M.A. (2000) Transcriptional repression by nuclear hormone receptors. *Trends Endocrinol Metab*, **11**, 6-10.

Huang, E.Y., Zhang, J., Miska, E.A., Guenther, M.G., Kouzarides, T. and Lazar, M.A. (2000) Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway. *Genes Dev*, **14**, 45-54.

Huang, Y., Boskovic, G. and Niles, R.M. (2003) Retinoic acid-induced AP-1 transcriptional activity regulates B16 mouse melanoma growth inhibition and differentiation. *J Cell Physiol*, **194**, 162-170.

Hughes, I.A., Lim, H.N., Martin, H., Mongan, N.P., Dovey, L., Ahmed, S.F. and Hawkins, J.R. (2001) Developmental aspects of androgen action. *Mol Cell Endocrinol*, **185**, 33-41.

Hughes, M.R., Malloy, P.J., O'Malley, B.W., Pike, J.W. and Feldman, D. (1991) Genetic defects of the 1,25-dihydroxyvitamin D3 receptor. *J Recept Res*, **11**, 699-716.

Humble, M.C., Szczesniak, C.J., Luetteke, N.C., Spalding, J.W., Cannon, R.E., Hansen, L.A., Lee, D.C. and Tennant, R.W. (1998) TGF alpha is dispensable for skin tumorigenesis in Tg.AC mice. *Toxicol Pathol*, **26**, 562-569.

Husmann, M., Dragneva, Y., Romahn, E. and Jehnichen, P. (2000) Nuclear receptors modulate the interaction of Sp1 and GC-rich DNA via ternary complex formation. *Biochem J*, **352 Pt 3**, 763-772.

Hutchinson, P.E., Osborne, J.E., Lear, J.T., Smith, A.G., Bowers, P.W., Morris, P.N., Jones, P.W., York, C., Strange, R.C. and Fryer, A.A. (2000a) Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. *Clin Cancer Res*, **6**, 498-504. 492: Reichrath J et al. Analysis of 491,425-dihydroxyvit...[PMID: 10433950]Related Articles, Links.

Hutchinson, P.E., Osborne, J.E., Lear, J.T., Smith, A.G., Bowers, P.W., Morris, P.N., Jones, P.W., York, C., Strange, R.C. and Fryer, A.A. (2000b) Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. *Clin Cancer Res*, **6**, 498-504.

Ichikawa, K., Hughes, I.A., Horwitz, A.L. and DeGroot, L.J. (1987) Characterization of nuclear thyroid hormone receptors of cultured skin fibroblasts from patients with resistance to thyroid hormone. *Metabolism*, **36**, 392-399.

Imakado, S., Bickenbach, J.R., Bundman, D.S., Rothnagel, J.A., Attar, P.S., Wang, X.J., Walczak, V.R., Wisniewski, S., Pote, J., Gordon, J.S. and et al. (1995) Targeting expression of a dominant-negative retinoic acid receptor mutant in the epidermis of transgenic mice results in loss of barrier function. *Genes Dev*, **9**, 317-329.

Imokawa, G., Yada, Y. and Miyagishi, M. (1992) Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. *J Biol Chem*, **267**, 24675-24680.

Indra, A.K., Li, M., Brocard, J., Warot, X., Bornert, J.M., Gerard, C., Messaddeq, N., Chambon, P. and Metzger, D. (2000) Targeted somatic mutagenesis in mouse epidermis. *Horm Res*, **54**, 296-300.

Indra, A.K., Warot, X., Brocard, J., Bornert, J.M., Xiao, J.H., Chambon, P. and Metzger, D. (1999) Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen- inducible Cre-ER(T) and Cre-ER(T2) recombinases. *Nucleic Acids Res*, **27**, 4324-4327.

Ise, K., Nakamura, K., Nakao, K., Shimizu, S., Harada, H., Ichise, T., Miyoshi, J., Gondo, Y., Ishikawa, T., Aiba, A. and Katsuki, M. (2000) Targeted deletion of the H-ras gene decreases tumor formation in mouse skin carcinogenesis. *Oncogene*, **19**, 2951-2956.

Islam, T.C., Skarin, T., Sumitran, S. and Toftgard, R. (2000) Retinoids induce apoptosis in cultured keratinocytes. *Br J Dermatol*, **143**, 709-719.

Issemann, I. and Green, S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, **347**, 645-650.

Issemann, I., Prince, R.A., Tugwood, J.D. and Green, S. (1993) The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J Mol Endocrinol*, **11**, 37-47. 33: Fajas L et al. Regulation of peroxisome prol...[PMID: 10409739]Related Articles, Links.

Issing, W.J. and Wustrow, T.P. (1996) Expression of retinoic acid receptors in squamous cell carcinomas and their possible implication for chemoprevention. *Anticancer Res*, **16**, 2373-2377.

Ito, K., Barnes, P.J. and Adcock, I.M. (2000) Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol*, **20**, 6891-6903.

Iwasaki, K., Izawa, M. and Mihara, M. (1996) UV-induced apoptosis in rat skin. *J Dermatol Sci*, **12**, 31-35.

Jahoda, C.A. and Reynolds, A.J. (1996) Dermal-epidermal interactions. Adult follicle-derived cell populations and hair growth. *Dermatol Clin*, **14**, 573-583.

Jaken, S. and Yuspa, S.H. (1988) Early signals for keratinocyte differentiation: role of Ca2+-mediated inositol lipid metabolism in normal and neoplastic epidermal cells. *Carcinogenesis*, **9**, 1033-1038.

Jamal, S. and Schneider, R.J. (2002) UV-induction of keratinocyte endothelin-1 downregulates E-cadherin in melanocytes and melanoma cells. *J Clin Invest*, **110**, 443-452. 442: Hsu MY et al. E-cadherin expression in mela...[PMID: 10793063]Related Articles, Links.

Jee, S.H., Lee, S.Y., Chiu, H.C., Chang, C.C. and Chen, T.J. (1994) Effects of estrogen and estrogen receptor in normal human melanocytes. *Biochem Biophys Res Commun*, **199**, 1407-1412.

Jensen, P.K., Norgard, J.O. and Bolund, L. (1985) Changes in basal cell subpopulations and tissue differentiation in human epidermal cultures treated with epidermal growth factor and cholera toxin. *Virchows Arch B Cell Pathol Incl Mol Pathol*, **49**, 325-340.

Jensen, U.B., Lowell, S. and Watt, F.M. (1999) The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. *Development*, **126**, 2409-2418.

Jenster, G. (1998) Coactivators and corepressors as mediators of nuclear receptor function: an update. *Mol Cell Endocrinol*, **143**, 1-7.

Jiang, G., Lee, U. and Sladek, F.M. (1997) Proposed mechanism for the stabilization of nuclear receptor DNA binding via protein dimerization. *Mol Cell Biol*, **17**, 6546-6554.

Jiang, W., Ananthaswamy, H.N., Muller, H.K. and Kripke, M.L. (1999) p53 protects against skin cancer induction by UV-B radiation. *Oncogene*, **18**, 4247-4253.

Jiveskog, S., Ragnarsson-Olding, B., Platz, A. and Ringborg, U. (1998) N-ras mutations are common in melanomas from sun-exposed skin of humans but rare in mucosal membranes or unexposed skin. *J Invest Dermatol*, **111**, 757-761.

Johnson, B.E. (1978) Formation of thymine containing dimers in skin exposed to ultraviolet radiation. *Bull Cancer*, **65**, 283-297.

Jones, K.T. and Sharpe, G.R. (1994) Intracellular free calcium and growth changes in single human keratinocytes in response to vitamin D and five 20-epi-analogues. *Arch Dermatol Res*, **286**, 123-129.

Jones, P.H., Harper, S. and Watt, F.M. (1995) Stem cell patterning and fate in human epidermis. *Cell*, **80**, 83-93.

Jung, E.M., Griner, R.D., Mann-Blakeney, R. and Bollag, W.B. (1998) A potential role for ceramide in the regulation of mouse epidermal keratinocyte proliferation and differentiation. *J Invest Dermatol*, **110**, 318-323.

Jurutka, P.W., Hsieh, J.C., Nakajima, S., Haussler, C.A., Whitfield, G.K. and Haussler, M.R. (1996) Human vitamin D receptor phosphorylation by casein kinase II at Ser-208 potentiates transcriptional activation. *Proc Natl Acad Sci U S A*, **93**, 3519-3524.

Kakar, S.S. and Roy, D. (1994) Curcumin inhibits TPA induced expression of c-fos, c-jun and c-myc proto-oncogenes messenger RNAs in mouse skin. *Cancer Lett*, **87**, 85-89.

Kang, H.Y., Chung, E., Lee, M., Cho, Y. and Kang, W.H. (2004) Expression and function of peroxisome proliferator-activated receptors in human melanocytes. *Br J Dermatol*, **150**, 462-468.

Kanitakis, J. (2002) Anatomy, histology and immunohistochemistry of normal human skin. *Eur J Dermatol*, **12**, 390-399; quiz 400-391.

Kantz, D.C., Lacks, G.D. and Cannon, R.E. (1999) Chemiluminescence-based method for genotyping Tg.AC responder mice. *Biotechniques*, **27**, 278-280.

Karen, J., Wang, Y., Javaherian, A., Vaccariello, M., Fusenig, N.E. and Garlick, J.A. (1999) 12-O-tetradecanoylphorbol-13-acetate induces clonal expansion of potentially malignant keratinocytes in a tissue model of early neoplastic progression. *Cancer Res*, **59**, 474-481.

Kastner, P., Grondona, J.M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J.L., Dolle, P. and Chambon, P. (1994) Genetic analysis of RXR alpha developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell*, **78**, 987-1003.

Kastner, P., Mark, M., Leid, M., Gansmuller, A., Chin, W., Grondona, J.M., Decimo, D., Krezel, W., Dierich, A. and Chambon, P. (1996) Abnormal spermatogenesis in RXR beta mutant mice. *Genes Dev*, **10**, 80-92.

Katahira, M., Knegtel, R., Schilthius, J., Boelens, R., Eib, D., van der Saag, P. and Kaptein, R. (1992) The structure of the human retinoic acid receptor-beta DNA-binding domain determined by NMR. *Nucleic Acids Symp Ser*, 65-66.

Kato, S., Tora, L., Yamauchi, J., Masushige, S., Bellard, M. and Chambon, P. (1992) A far upstream estrogen response element of the ovalbumin gene contains several half-palindromic 5'-TGACC-3' motifs acting synergistically. *Cell*, **68**, 731-742.

Kaur, P. and Li, A. (2000) Adhesive Properties of Human Basal Epidermal Cells: An Analysis of Keratinocyte Stem Cells, Transit Amplifying Cells, and Postmitotic Differentiating Cells. *J Invest Dermatol*, **114**, 413-420.

Keenan, B.S., Meyer, W.J., Hadjian, A.J. and Migeon, C.J. (1975) Androgen receptor in human skin fibroblasts. Characterization of a specific 17beta-hydroxy-5alpha-androstan-3-one-protein complex in cell sonicates and nuclei. *Steroids*, **25**, 535-552.

Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K. and Wahli, W. (1993) Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci U S A*, **90**, 2160-2164.

Kensler, T.W., Dolan, P.M., Gange, S.J., Lee, J.K., Wang, Q. and Posner, G.H. (2000) Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. *Carcinogenesis*, **21**, 1341-1345.

Kerkela, E., Ala-Aho, R., Jeskanen, L., Rechardt, O., Grenman, R., Shapiro, S.D., Kahari, V.M. and Saarialho-Kere, U. (2000) Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. *J Invest Dermatol*, **114**, 1113-1119.

Kerkela, E., Ala-aho, R., Lohi, J., Grenman, R., V, M.K. and Saarialho-Kere, U. (2001) Differential patterns of stromelysin-2 (MMP-10) and MT1-MMP (MMP-14) expression in epithelial skin cancers. *Br J Cancer*, **84**, 659-669.

Kim, D.J., Akiyama, T.E., Harman, F.S., Burns, A.M., Shan, W., Ward, J.M., Kennett, M.J., Gonzalez, F.J. and Peters, J.M. (2004) PPARbeta {(d)}-dependent regulation of ubiquitin C expression contributes to attenuation of skin carcinogenesis. *J Biol Chem*.

Kim, K.H., Marchuk, D. and Fuchs, E. (1984) Expression of unusually large keratins during terminal differentiation: balance of type I and type II keratins is not disrupted. *J Cell Biol*, **99**, 1872-1877.

Kippenberger, S., Loitsch, S.M., Grundmann-Kollmann, M., Simon, S., Dang, T.A., Hardt-Weinelt, K., Kaufmann, R. and Bernd, A. (2001) Activators of peroxisome proliferator-activated receptors protect human skin from ultraviolet-B-light-induced inflammation. *J Invest Dermatol*, **117**, 1430-1436. 1418: Sheu MY et al. Topical peroxisome proliferat...[PMID: 11851881]Related Articles, Links.

Kitajima, Y., Owaribe, K., Nishizawa, Y. and Yaoita, H. (1992) Control of the distribution of hemidesmosome components in cultured keratinocytes: Ca2+ and phorbol esters. *J Dermatol*, **19**, 770-773.

Kitano, Y., Ikeda, N. and Okano, M. (1991) Suppression of proliferation of human epidermal keratinocytes by 1,25-dihydroxyvitamin D3. Analysis of its effect on psoriatic lesion and of its mechanism using human keratinocytes in culture. *Eur J Clin Invest*, **21**, 53-58. 58: Hashimoto K et al. Growth-inhibitory effects of ...[PMID: 2390499]Related Articles, Links.

Klaassen, I., Brakenhoff, R.H., Smeets, S.J., Snow, G.B. and Braakhuis, B.J. (2001) Metabolism and growth inhibition of four retinoids in head and neck squamous normal and malignant cells. *Br J Cancer*, **85**, 630-635.

Klein, J.D., Price, S.R., Bailey, J.L., Jacobs, J.D. and Sands, J.M. (1997) Glucocorticoids mediate a decrease in AVP-regulated urea transporter in diabetic rat inner medulla. *Am J Physiol*, **273**, F949-953.

Kliewer, S.A., Sundseth, S.S., Jones, S.A., Brown, P.J., Wisely, G.B., Koble, C.S., Devchand, P., Wahli, W., Willson, T.M., Lenhard, J.M. and Lehmann, J.M. (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A*, **94**, 4318-4323. 4312: Forman BM et al. Hypolipidemic drugs, polyunsa...[PMID: 9113986]Related Articles, Links.

Kliewer, S.A., Umesono, K., Mangelsdorf, D.J. and Evans, R.M. (1992) Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. *Nature*, **355**, 446-449.

Koga, T., Shimada, Y., Kuroda, M., Tsujita, Y., Hasegawa, K. and Yamazaki, M. (1990) Tissue-selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Biochim Biophys Acta*, **1045**, 115-120.

Kolb, E. (1986) [Recent knowledge about the significance of 1,25-hydroxyvitamin D and the development of genetically-induced rickets]. *Z Gesamte Inn Med*, **41**, 641-646.

Komuves, L.G., Hanley, K., Lefebvre, A.M., Man, M.Q., Ng, D.C., Bikle, D.D., Williams, M.L., Elias, P.M., Auwerx, J. and Feingold, K.R. (2000) Stimulation of PPARalpha promotes epidermal keratinocyte differentiation in vivo. *J Invest Dermatol*, **115**, 353-360.

Komuves, L.G., Schmuth, M., Fowler, A.J., Elias, P.M., Hanley, K., Man, M.Q., Moser, A.H., Lobaccaro, J.M., Williams, M.L., Mangelsdorf, D.J. and Feingold, K.R. (2002) Oxysterol stimulation of epidermal differentiation is mediated by liver X receptor-beta in murine epidermis. *J Invest Dermatol*, **118**, 25-34. 23: Hanley K et al. Oxysterols induce differentia...[PMID: 10692116]Related Articles, Links.

Kopan, R. and Fuchs, E. (1989) A new look into an old problem: keratins as tools to investigate determination, morphogenesis, and differentiation in skin. *Genes Dev*, **3**, 1-15.

Koshizuka, K., Koike, M., Asou, H., Cho, S.K., Stephen, T., Rude, R.K., Binderup, L., Uskokovic, M. and Koeffler, H.P. (1999) Combined effect of vitamin D3 analogs and paclitaxel on the growth of MCF-7 breast cancer cells in vivo. *Breast Cancer Res Treat*, **53**, 113-120.

Koster, M.I., Huntzinger, K.A. and Roop, D.R. (2002) Epidermal differentiation: transgenic/knockout mouse models reveal genes involved in stem cell fate decisions and commitment to differentiation. *J Investig Dermatol Symp Proc*, **7**, 41-45.

Kraemer, K.H. and Greene, M.H. (1985) Dysplastic nevus syndrome. Familial and sporadic precursors of cutaneous melanoma. *Dermatol Clin*, **3**, 225-237.

Kraus, W.L., Manning, E.T. and Kadonaga, J.T. (1999) Biochemical analysis of distinct activation functions in p300 that enhance transcription initiation with chromatin templates. *Mol Cell Biol*, **19**, 8123-8135.

Kraus, W.L. and Wong, J. (2002) Nuclear receptor-dependent transcription with chromatin. Is it all about enzymes? *Eur J Biochem*, **269**, 2275-2283.

Kreimer-Erlacher, H., Seidl, H., Back, B., Cerroni, L., Kerl, H. and Wolf, P. (2003) High frequency of ultraviolet mutations at the INK4a-ARF locus in squamous cell carcinomas from psoralen-plus-ultraviolet-A-treated psoriasis patients. *J Invest Dermatol*, **120**, 676-682.

Krey, G., Mahfoudi, A. and Wahli, W. (1995) Functional interactions of peroxisome proliferator-activated receptor, retinoid-X receptor, and Sp1 in the transcriptional regulation of the acyl-coenzyme-A oxidase promoter. *Mol Endocrinol*, **9**, 219-231.

Krezel, W., Dupe, V., Mark, M., Dierich, A., Kastner, P. and Chambon, P. (1996) RXR gamma null mice are apparently normal and compound RXR alpha +/-/RXR beta -/-/RXR gamma -/- mutant mice are viable. *Proc Natl Acad Sci U S A*, **93**, 9010-9014.

Krimpenfort, P., Quon, K.C., Mooi, W.J., Loonstra, A. and Berns, A. (2001) Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature*, **413**, 83-86.

Krishnan, V., Wang, X. and Safe, S. (1994) Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 human breast cancer cells. *J Biol Chem*, **269**, 15912-15917.

Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornert, J.M. and Chambon, P. (1986) The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors. *Embo J*, **5**, 891-897. 897: Green S et al. Human oestrogen receptor cDNA...[PMID: 3754034]Related Articles, Links.

Krust, A., Kastner, P., Petkovich, M., Zelent, A. and Chambon, P. (1989a) A third human retinoic acid receptor, hRAR-gamma. *Proc Natl Acad Sci U S A*, **86**, 5310-5314. 5315: Ruberte E et al. Retinoic acid receptors and c...[PMID: 1849812]Related Articles, Links.

Krust, A., Kastner, P., Petkovich, M., Zelent, A. and Chambon, P. (1989b) A third human retinoic acid receptor, hRAR-gamma. *Proc Natl Acad Sci U S A*, **86**, 5310-5314.

Kulesz-Martin, M.F., Blumenson, L.E., Manly, K.F., Siracky, J. and East, C.J. (1991) Tumor progression of murine epidermal cells after treatment in vitro with 12-O-tetradecanoylphorbol-13-acetate or retinoic acid. *Cancer Res*, **51**, 4701-4706.

Kulkarni, R.N., Bruning, J.C., Winnay, J.N., Postic, C., Magnuson, M.A. and Kahn, C.R. (1999) Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell*, **96**, 329-339.

Kulkarni, R.N. and Zisman, A. (2003) Lessons for human diabetes from experimental mouse models. *Curr Diab Rep*, **3**, 168-175.

Kulms, D. and Schwarz, T. (2000) Molecular mechanisms of UV-induced apoptosis. *Photodermatol Photoimmunol Photomed*, **16**, 195-201.

Kumar, R., Angelini, S. and Hemminki, K. (2003) Activating BRAF and N-Ras mutations in sporadic primary melanomas: an inverse association with allelic loss on chromosome 9. *Oncogene*, **22**, 9217-9224.

Kumar, R., Angelini, S., Snellman, E. and Hemminki, K. (2004) BRAF mutations are common somatic events in melanocytic nevi. *J Invest Dermatol*, **122**, 342-348.

Kumar, R., Shoemaker, A.R. and Verma, A.K. (1994) Retinoic acid nuclear receptors and tumor promotion: decreased expression of retinoic acid nuclear receptors by the tumor promoter 12- O-tetradecanoylphorbol-13-acetate. *Carcinogenesis*, **15**, 701-705.

Kumar, R. and Thompson, E.B. (1999) The structure of the nuclear hormone receptors. *Steroids*, **64**, 310-319.

Kunisada, T., Yoshida, H., Yamazaki, H., Miyamoto, A., Hemmi, H., Nishimura, E., Shultz, L.D., Nishikawa, S. and Hayashi, S. (1998) Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. *Development*, **125**, 2915-2923.

Kupper, T.S., Lee, F., Birchall, N., Clark, S. and Dower, S. (1988) Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. A potential autocrine role for interleukin 1 in epidermis. *J Clin Invest*, **82**, 1787-1792.

Kurlandsky, S.B., Xiao, J.H., Duell, E.A., Voorhees, J.J. and Fisher, G.J. (1994) Biological activity of all-trans retinol requires metabolic conversion to all-trans retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. *J Biol Chem*, **269**, 32821-32827.

Kuroki, Y., Shiozawa, S., Kano, J. and Chihara, K. (1995) Competition between c-fos and 1,25(OH)2 vitamin D3 in the transcriptional control of type I collagen synthesis in MC3T3-E1 osteoblastic cells. *J Cell Physiol*, **164**, 459-464.

Kurosumi, K., Kurosumi, U. and Inoue, K. (1979) Morphological and morphometric studies with the electron microscope on the Merkel cells and associated nerve terminals of normal and denervated skin. *Arch Histol Jpn*, **42**, 243-261.

Lacour, J.P. (2002) Carcinogenesis of basal cell carcinomas: genetics and molecular mechanisms. *Br J Dermatol*, **146 Suppl 61**, 17-19.

Lan, L., Trempus, C. and Gilmour, S.K. (2000) Inhibition of ornithine decarboxylase (ODC) decreases tumor vascularization and reverses spontaneous tumors in ODC/Ras transgenic mice. *Cancer Res*, **60**, 5696-5703.

Lancillotti, F., Darwiche, N., Celli, G. and De Luca, L.M. (1992) Retinoid status and the control of keratin expression and adhesion during the histogenesis of squamous metaplasia of tracheal epithelium. *Cancer Res*, **52**, 6144-6152.

Larsen, W.J. (1993) Development of the Integumentary System. *Human Embryology. CHURCHILL LIVINGSTONE INC*, 419-433.

Laudet, V., Hanni, C., Coll, J., Catzeflis, F. and Stehelin, D. (1992) Evolution of the nuclear receptor gene superfamily. *Embo J*, **11**, 1003-1013.

Lavker, R.M., Risse, B., Brown, H., Ginsburg, D., Pearson, J., Baker, M.S. and Jensen, P.J. (1998) Localization of plasminogen activator inhibitor type 2 (PAI-2) in hair and nail: implications for terminal differentiation. *J Invest Dermatol*, **110**, 917-922.

Lavker, R.M. and Sun, T.T. (1982) Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. *Science*, **215**, 1239-1241.

Lavker, R.M. and Sun, T.T. (2000) Epidermal stem cells: properties, markers, and location. *Proc Natl Acad Sci U S A*, **97**, 13473-13475.

Le Douarin, B., vom Baur, E., Zechel, C., Heery, D., Heine, M., Vivat, V., Gronemeyer, H., Losson, R. and Chambon, P. (1996) Ligand-dependent interaction of nuclear receptors with potential transcriptional intermediary factors (mediators). *Philos Trans R Soc Lond B Biol Sci*, **351**, 569-578.

Leder, A., Kuo, A., Cardiff, R.D., Sinn, E. and Leder, P. (1990) v-Ha-ras transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid. *Proc Natl Acad Sci U S A*, **87**, 9178-9182.

Leder, A., Lebel, M., Zhou, F., Fontaine, K., Bishop, A. and Leder, P. (2002) Genetic interaction between the unstable v-Ha-RAS transgene (Tg.AC) and the murine Werner syndrome gene: transgene instability and tumorigenesis. *Oncogene*, **21**, 6657-6668.

Lee, H.Y., Suh, Y.A., Robinson, M.J., Clifford, J.L., Hong, W.K., Woodgett, J.R., Cobb, M.H., Mangelsdorf, D.J. and Kurie, J.M. (2000) Stress pathway activation induces phosphorylation of retinoid X receptor. *J Biol Chem*, **275**, 32193-32199.

Lee, K.C. and Lee Kraus, W. (2001) Nuclear receptors, coactivators and chromatin: new approaches, new insights. *Trends Endocrinol Metab*, **12**, 191-197.

Lee, M.S., Kliewer, S.A., Provencal, J., Wright, P.E. and Evans, R.M. (1993) Structure of the retinoid X receptor alpha DNA binding domain: a helix required for homodimeric DNA binding. *Science*, **260**, 1117-1121.

Lee, S.S. and Gonzalez, F.J. (1996) Targeted disruption of the peroxisome proliferator-activated receptor alpha gene, PPAR alpha. *Ann N Y Acad Sci*, **804**, 524-529.

Lee, Y. and Mahdavi, V. (1993) The D domain of the thyroid hormone receptor alpha 1 specifies positive and negative transcriptional regulation functions. *J Biol Chem*, **268**, 2021-2028.

Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.Y., Staub, A., Garnier, J.M., Mader, S. and et al. (1992) Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell*, **68**, 377-395.

Lemon, B.D. and Freedman, L.P. (1999) Nuclear receptor cofactors as chromatin remodelers. *Curr Opin Genet Dev*, **9**, 499-504.

Leng, X., Tsai, S.Y., O'Malley, B.W. and Tsai, M.J. (1993) Ligand-dependent conformational changes in thyroid hormone and retinoic acid receptors are potentially enhanced by heterodimerization with retinoic X receptor. *J Steroid Biochem Mol Biol*, **46**, 643-661.

Levine, N. and Meyskens, F.L. (1980) Topical vitamin-A-acid therapy for cutaneous metastatic melanoma. *Lancet*, **2**, 224-226.

Levitzki, A. (1988) From epinephrine to cyclic AMP. *Science*, **241**, 800-806.

Li, G., Schaider, H., Satyamoorthy, K., Hanakawa, Y., Hashimoto, K. and Herlyn, M. (2001a) Downregulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development. *Oncogene*, **20**, 8125-8135.

Li, M., Chiba, H., Warot, X., Messaddeq, N., Gerard, C., Chambon, P. and Metzger, D. (2001b) RXR-alpha ablation in skin keratinocytes results in alopecia and epidermal alterations. *Development*, **128**, 675-688.

Li, M., Indra, A.K., Warot, X., Brocard, J., Messaddeq, N., Kato, S., Metzger, D. and Chambon, P. (2000) Skin abnormalities generated by temporally controlled RXRalpha mutations in mouse epidermis. *Nature*, **407**, 633-636.

Li, Q., Imhof, A., Collingwood, T.N., Urnov, F.D. and Wolffe, A.P. (1999) p300 stimulates transcription instigated by ligand-bound thyroid hormone receptor at a step subsequent to chromatin disruption. *Embo J*, **18**, 5634-5652.

Liang, T., Hoyer, S., Yu, R., Soltani, K., Lorincz, A.L., Hiipakka, R.A. and Liao, S. (1993) Immunocytochemical localization of androgen receptors in human skin using monoclonal antibodies against the androgen receptor. *J Invest Dermatol*, **100**, 663-666.

Liao, S. and Fang, S. (1969) Receptor-proteims for androgens and the mode of action of androgens on gene transcription in ventral prostate. *Vitam Horm*, **27**, 17-90.

Liaudet-Coopman, E.D., Berchem, G.J. and Wellstein, A. (1997) In vivo inhibition of angiogenesis and induction of apoptosis by retinoic acid in squamous cell carcinoma. *Clin Cancer Res*, **3**, 179-184.

Limtrakul Pn, P., Anuchapreeda, S., Lipigorngoson, S. and Dunn, F.W. (2001) Inhibition of carcinogen induced c-Ha-ras and c-fos proto-oncogenes expression by dietary curcumin. *BMC Cancer*, **1**, 1.

Lippman, S.M., Kessler, J.F., Al-Sarraf, M., Alberts, D.S., Itri, L.M., Mattox, D., Von Hoff, D.D., Loescher, L. and Meyskens, F.L. (1988) Treatment of advanced squamous cell carcinoma of the head and neck with isotretinoin: a phase II randomized trial. *Invest New Drugs*, **6**, 51-56. 52: Lippman SM et al. Retinoids as preventive and t...[PMID: 3552220]Related Articles, Links.

Lippman, S.M., Kessler, J.F. and Meyskens, F.L., Jr. (1987a) Retinoids as preventive and therapeutic anticancer agents (Part I). *Cancer Treat Rep*, **71**, 391-405.

Lippman, S.M., Kessler, J.F. and Meyskens, F.L., Jr. (1987b) Retinoids as preventive and therapeutic anticancer agents (Part II). *Cancer Treat Rep*, **71**, 493-515. 493: Lippman SM et al. Retinoids as preventive and t...[PMID: 3548957]Related Articles, Links.

Lippman, S.M. and Lotan, R. (2000) Advances in the development of retinoids as chemopreventive agents. *J Nutr*, **130**, 479S-482S.

Liu, M. and Freedman, L.P. (1994) Transcriptional synergism between the vitamin D3 receptor and other nonreceptor transcription factors. *Mol Endocrinol*, **8**, 1593-1604.

Liu, Y., Lyle, S., Yang, Z. and Cotsarelis, G. (2003a) Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. *J Invest Dermatol*, **121**, 963-968.

Liu, Y.J., Araujo, S., Recker, R.R. and Deng, H.W. (2003b) Molecular and genetic mechanisms of obesity: implications for future management. *Curr Mol Med*, **3**, 325-340.

Livera, G., Rouiller-Fabre, V., Pairault, C., Levacher, C. and Habert, R. (2002) Regulation and perturbation of testicular functions by vitamin A. *Reproduction*, **124**, 173-180.

Loening, T., Staquet, M.J., Schmitt, D. and Thivolet, J. (1982) Immunocytochemical and ultrastructural localization of keratin polypeptides in normal epidermal and mucosal cells and tissues. *J Invest Dermatol*, **78**, 44-47.

Lohnes, D., Kastner, P., Dierich, A., Mark, M., LeMeur, M. and Chambon, P. (1993) Function of retinoic acid receptor gamma in the mouse. *Cell*, **73**, 643-658.

Look, J., Landwehr, J., Bauer, F., Hoffmann, A.S., Bluethmann, H. and LeMotte, P. (1995) Marked resistance of RAR gamma-deficient mice to the toxic effects of retinoic acid. *Am J Physiol*, **269**, E91-98.

Lotan, R. (1993) Squamous cell differentiation markers in normal, premalignant, and malignant epithelium: effects of retinoids. *J Cell Biochem Suppl*, **17F**, 167-174.

Lotan, R. (1996) Retinoids and their receptors in modulation of differentiation, development, and prevention of head and neck cancers. *Anticancer Res*, **16**, 2415-2419.

Lozano, J., Xing, R., Cai, Z., Jensen, H.L., Trempus, C., Mark, W., Cannon, R. and Kolesnick, R. (2003) Deficiency of kinase suppressor of Ras1 prevents oncogenic ras signaling in mice. *Cancer Res*, **63**, 4232-4238.

Lubahn, D.B., Joseph, D.R., Sar, M., Tan, J., Higgs, H.N., Larson, R.E., French, F.S. and Wilson, E.M. (1988) The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. *Mol Endocrinol*, **2**, 1265-1275.

Luck, L.A., Barse, J.L., Luck, A.M. and Peck, C.H. (2000) Conformational changes in the human estrogen receptor observed by (19)F NMR. *Biochem Biophys Res Commun*, **270**, 988-991.

Lufkin, T., Lohnes, D., Mark, M., Dierich, A., Gorry, P., Gaub, M.P., LeMeur, M. and Chambon, P. (1993) High postnatal lethality and testis degeneration in retinoic acid receptor alpha mutant mice. *Proc Natl Acad Sci U S A*, **90**, 7225-7229.

Maas-Szabowski, N., Shimotoyodome, A. and Fusenig, N.E. (1999) Keratinocyte growth regulation in fibroblast cocultures via a double paracrine mechanism. *J Cell Sci*, **112 (Pt 12)**, 1843-1853.

Mackenzie, I.C. (1975) Ordered structure of the epidermis. *J Invest Dermatol*, **65**, 45-51.

Mackenzie, I.C. (1997) Retroviral transduction of murine epidermal stem cells demonstrates clonal units of epidermal structure. *J Invest Dermatol*, **109**, 377-383.

Mader, S., Chen, J.Y., Chen, Z., White, J., Chambon, P. and Gronemeyer, H. (1993) The patterns of binding of RAR, RXR and TR homo- and heterodimers to direct repeats are dictated by the binding specificites of the DNA binding domains. *Embo J*, **12**, 5029-5041.

Majewski, S., Marczak, M., Szmurlo, A., Jablonska, S. and Bollag, W. (1995) Retinoids, interferon alpha, 1,25-dihydroxyvitamin D3 and their combination inhibit angiogenesis induced by non-HPV-harboring tumor cell lines. RAR alpha mediates the antiangiogenic effect of retinoids. *Cancer Lett*, **89**, 117-124.

Majewski, S., Szmurlo, A., Marczak, M., Jablonska, S. and Bollag, W. (1993) Inhibition of tumor cell-induced angiogenesis by retinoids, 1,25- dihydroxyvitamin D3 and their combination. *Cancer Lett*, **75**, 35-39.

Mangelsdorf, D.J. and Evans, R.M. (1995) The RXR heterodimers and orphan receptors. *Cell*, **83**, 841-850.

Mangelsdorf, D.J., Umesono, K., Kliewer, S.A., Borgmeyer, U., Ong, E.S. and Evans, R.M. (1991) A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR. *Cell*, **66**, 555-561.

Mao-Qiang, M., Elias, P.M. and Feingold, K.R. (1993) Fatty acids are required for epidermal permeability barrier function. *J Clin Invest*, **92**, 791-798.

Marchese, C., Rubin, J., Ron, D., Faggioni, A., Torrisi, M.R., Messina, A., Frati, L. and Aaronson, S.A. (1990) Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: differentiation response distinguishes KGF from EGF family. *J Cell Physiol*, **144**, 326-332.

Marsh, N.L., Elias, P.M. and Holleran, W.M. (1995) Glucosylceramides stimulate murine epidermal hyperproliferation. *J Clin Invest*, **95**, 2903-2909.

Marshall, D., Hardman, M.J., Nield, K.M. and Byrne, C. (2001) Differentially expressed late constituents of the epidermal cornified envelope. *Proc Natl Acad Sci U S A*, **98**, 13031-13036.

Maruoka, Y., Harada, H., Mitsuyasu, T., Seta, Y., Kurokawa, H., Kajiyama, M. and Toyoshima, K. (1997) Keratinocytes become terminally differentiated in a process involving programmed cell death. *Biochem Biophys Res Commun*, **238**, 886-890.

Mathiasen, I.S., Colston, K.W. and Binderup, L. (1993) EB 1089, a novel vitamin D analogue, has strong antiproliferative and differentiation inducing effects on cancer cells. *J Steroid Biochem Mol Biol*, **46**, 365-371.

Matsuura, H., Adachi, H., Smart, R.C., Xu, X., Arata, J. and Jetten, A.M. (1999) Correlation between expression of peroxisome proliferator-activated receptor beta and squamous differentiation in epidermal and tracheobronchial epithelial cells. *Mol Cell Endocrinol*, **147**, 85-92.

McGowan, K.M. and Coulombe, P.A. (1998) Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. *J Cell Biol*, **143**, 469-486.

McGuire, T.F., Trump, D.L. and Johnson, C.S. (2001) Vitamin D(3)-induced apoptosis of murine squamous cell carcinoma cells. Selective induction of caspase-dependent MEK cleavage and up-regulation of MEKK-1. *J Biol Chem*, **276**, 26365-26373. 26364: Akutsu N et al. Amphiregulin is a vitamin D26363 ...[PMID: 11237771]Related Articles, Links.

McLane, J.A., Katz, M. and Abdelkader, N. (1990) Effect of 1,25-dihydroxyvitamin D3 on human keratinocytes grown under different culture conditions. *In Vitro Cell Dev Biol*, **26**, 379-387. 310: Matsunaga T et al. 371,324(R)-dihydroxyvitamin D373, ...[PMID: 2162363]Related Articles, Links.

McNamara, I.R., Muir, J. and Galbraith, A.J. (2002) Acitretin for prophylaxis of cutaneous malignancies after cardiac transplantation. *J Heart Lung Transplant*, **21**, 1201-1205.

Mendelsohn, C., Mark, M., Dolle, P., Dierich, A., Gaub, M.P., Krust, A., Lampron, C. and Chambon, P. (1994) Retinoic acid receptor beta 2 (RAR beta 2) null mutant mice appear normal. *Dev Biol*, **166**, 246-258.

Mester, J. and Baulieu, E.E. (1972) Nuclear estrogen receptor of chick liver. *Biochim Biophys Acta*, **261**, 236-244.

Metzger, D., Clifford, J., Chiba, H. and Chambon, P. (1995) Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. *Proc Natl Acad Sci U S A*, **92**, 6991-6995.

Metzger, D., Losson, R., Bornert, J.M., Lemoine, Y. and Chambon, P. (1992) Promoter specificity of the two transcriptional activation functions of the human oestrogen receptor in yeast. *Nucleic Acids Res*, **20**, 2813-2817.

Meyskens, F.L., Jr., Edwards, L. and Levine, N.S. (1986) Role of topical tretinoin in melanoma and dysplastic nevi. *J Am Acad Dermatol*, **15**, 822-825.

Michalik, L., Desvergne, B., Tan, N.S., Basu-Modak, S., Escher, P., Rieusset, J., Peters, J.M., Kaya, G., Gonzalez, F.J., Zakany, J., Metzger, D., Chambon, P., Duboule, D. and Wahli, W. (2001) Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J Cell Biol*, **154**, 799-814.

Middlebrook, J.L., Wong, M.D., Ishii, D.N. and Aronow, L. (1975) Subcellular distribution of glucocorticoid receptors in mouse fibroblasts. *Biochemistry*, **14**, 180-186.

Miesfeld, R., Okret, S., Wikstrom, A.C., Wrange, O., Gustafsson, J.A. and Yamamoto, K.R. (1984) Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature*, **312**, 779-781.

Milde, P., Hauser, U., Simon, T., Mall, G., Ernst, V., Haussler, M.R., Frosch, P. and Rauterberg, E.W. (1991) Expression of 1,25-dihydroxyvitamin D3 receptors in normal and psoriatic skin. *J Invest Dermatol*, **97**, 230-239.

Miles, S.A., Dezube, B.J., Lee, J.Y., Krown, S.E., Fletcher, M.A., Saville, M.W., Kaplan, L., Groopman, J., Scadden, D.T., Cooley, T., Von Roenn, J. and Friedman-Kien, A. (2002) Antitumor activity of oral 9-cis-retinoic acid in HIV-associated Kaposi's sarcoma. *Aids*, **16**, 421-429.

Mills, A.A., Zheng, B., Wang, X.J., Vogel, H., Roop, D.R. and Bradley, A. (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature*, **398**, 708-713.

Misago, N., Toda, S., Sugihara, H., Kohda, H. and Narisawa, Y. (1998) Proliferation and differentiation of organoid hair follicle cells co-cultured with fat cells in collagen gel matrix culture. *Br J Dermatol*, **139**, 40-48.

Miyashita, M., Baba, S. and Suzuki, H. (1992) Localized proliferative effect of preadipocytes on cultured human epidermal keratinocytes. *Acta Derm Venereol*, **72**, 413-417.

Miyata, K.S., McCaw, S.E., Patel, H.V., Rachubinski, R.A. and Capone, J.P. (1996) The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J Biol Chem*, **271**, 9189-9192.

Moll, R., Moll, I. and Franke, W.W. (1984) Differences of expression of cytokeratin polypeptides in various epithelial skin tumors. *Arch Dermatol Res*, **276**, 349-363.

Moon, R.C., Mehta, R.G. and Rao, K.J.V.N. (1994) *Retinoids and cancer in experimental animals*. Raven Press, New York.

Moon, T.E., Levine, N., Cartmel, B., Bangert, J.L., Rodney, S., Dong, Q., Peng, Y.M. and Alberts, D.S. (1997) Effect of retinol in preventing squamous cell skin cancer in moderate-risk subjects: a randomized, double-blind, controlled trial. Southwest Skin Cancer

Prevention Study Group. *Cancer Epidemiol Biomarkers Prev*, **6**, 949-956. 916: Agarwal R et al. Protection against malignant ...[PMID: 8876671]Related Articles, Links.

Moras, D. and Gronemeyer, H. (1998) The nuclear receptor ligand-binding domain: structure and function. *Curr Opin Cell Biol*, **10**, 384-391.

Morris, R.J. and Potten, C.S. (1994) Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro. *Cell Prolif*, **27**, 279-289.

Morris, R.J. and Potten, C.S. (1999) Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. *J Invest Dermatol*, **112**, 470-475.

Morris, R.J., Tryson, K.A. and Wu, K.Q. (2000) Evidence that the epidermal targets of carcinogen action are found in the interfollicular epidermis of infundibulum as well as in the hair follicles. *Cancer Res*, **60**, 226-229.

Mossner, R., Schulz, U., Kruger, U., Middel, P., Schinner, S., Fuzesi, L., Neumann, C. and Reich, K. (2002) Agonists of peroxisome proliferator-activated receptor gamma inhibit cell growth in malignant melanoma. *J Invest Dermatol*, **119**, 576-582.

Muller, R.E., Beebe, D.M., Bercel, E., Traish, A.M. and Wotiz, H.H. (1984) Estriol and estradiol interactions with the estrogen receptor in vivo and in vitro. *J Steroid Biochem*, **20**, 1039-1046.

Muramatsu, T., Kobayashi, N., Tada, H., Yamaji, M., Shirai, T., Mori, T. and Ohnishi, T. (1992) Induction and repair of UVB-induced cyclobutane pyrimidine dimers and (6-4) photoproducts in organ-cultured normal human skin. *Arch Dermatol Res*, **284**, 232-237.

Muscat, G.E., Burke, L.J. and Downes, M. (1998) The corepressor N-CoR and its variants RIP13a and RIP13Delta1 directly interact with the basal transcription factors TFIIB, TAFII32 and TAFII70. *Nucleic Acids Res*, **26**, 2899-2907.

Naar, A.M., Boutin, J.M., Lipkin, S.M., Yu, V.C., Holloway, J.M., Glass, C.K. and Rosenfeld, M.G. (1991) The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. *Cell*, **65**, 1267-1279.

Nagy, L., Kao, H.Y., Chakravarti, D., Lin, R.J., Hassig, C.A., Ayer, D.E., Schreiber, S.L. and Evans, R.M. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell*, **89**, 373-380.

Naito, M. and DiGiovanni, J. (1989) Genetic background and development of skin tumors. *Carcinog Compr Surv*, **11**, 187-212.

Nakamura, Y., Fukami, K., Yu, H., Takenaka, K., Kataoka, Y., Shirakata, Y., Nishikawa, S., Hashimoto, K., Yoshida, N. and Takenawa, T. (2003) Phospholipase Cdelta1 is required for skin stem cell lineage commitment. *Embo J*, **22**, 2981-2991.

Nakazawa, K., Nakazawa, H., Collombel, C. and Damour, O. (1995) Keratinocyte extracellular matrix-mediated regulation of normal human melanocyte functions. *Pigment Cell Res*, **8**, 10-18.

Nemanic, M.K., Whitney, J., Arnaud, S., Herbert, S. and Elias, P.M. (1983) Vitamin D3 production by cultured human keratinocytes and fibroblasts. *Biochem Biophys Res Commun*, **115**, 444-450.

Nicol, C.J., Yoon, M., Ward, J.M., Yamashida, M., Fukamachi, K., Peters, J.M. and Gonzalez, F.J. (2004) PPAR{gamma} influences susceptibility to DMBA-induced mammary, ovarian and skin carcinogenesis. *Carcinogenesis*.

Nikitakis, N.G., Hamburger, A.W. and Sauk, J.J. (2002) The nonsteroidal anti-inflammatory drug sulindac causes down-regulation of signal transducer and activator of transcription 3 in human oral squamous cell carcinoma cells. *Cancer Res*, **62**, 1004-1007.

Niles, R.M. (2000) Recent advances in the use of vitamin A (retinoids) in the prevention and treatment of cancer. *Nutrition*, **16**, 1084-1089.

Nilsson, M., Unden, A.B., Krause, D., Malmqwist, U., Raza, K., Zaphiropoulos, P.G. and Toftgard, R. (2000) Induction of basal cell carcinomas and trichoepitheliomas in mice overexpressing GLI-1. *Proc Natl Acad Sci U S A*, **97**, 3438-3443.

Nunneley, S.A., Wissler, E.H. and Allan, J.R. (1985) Immersion cooling: effect of clothing and skinfold thickness. *Aviat Space Environ Med*, **56**, 1177-1182.

Odell, J., Caimi, P., Sauer, B. and Russell, S. (1990) Site-directed recombination in the genome of transgenic tobacco. *Mol Gen Genet*, **223**, 369-378. 366: Sauer B et al. Targeted insertion of exogeno...[PMID: 2288914]Related Articles, Links.

O'Donnell, A.L. and Koenig, R.J. (1990) Mutational analysis identifies a new functional domain of the thyroid hormone receptor. *Mol Endocrinol*, **4**, 715-720.

Orban, P.C., Chui, D. and Marth, J.D. (1992) Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A*, **89**, 6861-6865. 6864: Russell SH et al. Directed excision of a transg...[PMID: 1495484]Related Articles, Links.

Oridate, N., Esumi, N., Lotan, D., Hing, W.K., Rochette-Egly, C., Chambon, P. and Lotan, R. (1996) Implication of retinoic acid receptor gamma in squamous differentiation and response to retinoic acid in head and neck SqCC/Y1 squamous carcinoma cells. *Oncogene*, **12**, 2019-2028.

Ouhtit, A., Nakazawa, H., Armstrong, B.K., Kricker, A., Tan, E., Yamasaki, H. and English, D.R. (1998) UV-radiation-specific p53 mutation frequency in normal skin as a predictor of risk of basal cell carcinoma. *J Natl Cancer Inst*, **90**, 523-531.

Owen, G.I. and Zelent, A. (2000) Origins and evolutionary diversification of the nuclear receptor superfamily. *Cell Mol Life Sci*, **57**, 809-827.

Owens, D.M., Romero, M.R., Gardner, C. and Watt, F.M. (2003) Suprabasal alpha6beta4 integrin expression in epidermis results in enhanced tumourigenesis and disruption of TGFbeta signalling. *J Cell Sci*, **116**, 3783-3791.

Panteleyev, A.A., Jahoda, C.A. and Christiano, A.M. (2001) Hair follicle predetermination. *J Cell Sci*, **114**, 3419-3431.

Panteleyev, A.A., Paus, R., Wanner, R., Nurnberg, W., Eichmuller, S., Thiel, R., Zhang, J., Henz, B.M. and Rosenbach, T. (1997) Keratin 17 gene expression during the murine hair cycle. *J Invest Dermatol*, **108**, 324-329.

Parkinson, E.K. (1985) Defective responses of transformed keratinocytes to terminal differentiation stimuli. Their role in epidermal tumour promotion by phorbol esters and by deep skin wounding. *Br J Cancer*, **52**, 479-493.

Parsa, R., Yang, A., McKeon, F. and Green, H. (1999) Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. *J Invest Dermatol*, **113**, 1099-1105.

Patel, L., Pass, I., Coxon, P., Downes, C.P., Smith, S.A. and Macphee, C.H. (2001) Tumor suppressor and anti-inflammatory actions of PPARgamma agonists are mediated via upregulation of PTEN. *Curr Biol*, **11**, 764-768.

Paus, R. (1998) Principles of hair cycle control. *J Dermatol*, **25**, 793-802.

Pavlovitch, J.H., Galoppin, L., Rizk, M., Didierjean, L. and Balsan, S. (1984) Alterations in rat epidermis provoked by chronic vitamin D deficiency. *Am J Physiol*, **247**, E228-233.

Pavlovitch, J.H., Rizk, M., Delecluse, C. and Balsan, S. (1981) [Effect of vitamin D on skin metabolism (author's transl)]. *Ann Endocrinol (Paris)*, **42**, 477-481. 476: Bittiner B et al. 471 alpha,425(OH)472 vitamin D473 in...[PMID: 2018729]Related Articles, Links.

Pavlovitch, J.H., Rizk-Rabin, M., Gervaise, M., Metezeau, P. and Grunwald, D. (1989) Cell subpopulations within proliferative and differentiating compartments of epidermis. *Am J Physiol*, **256**, C977-986.

Pazzaglia, S., Mancuso, M., Primerano, B., Rebessi, S., Biozzi, G., Covelli, V. and Saran, A. (2001) Analysis of c-Ha-ras gene mutations in skin tumors induced in carcinogenesis-susceptible and carcinogenesis-resistant mice by different two-stage protocols or tumor promoter alone. *Mol Carcinog*, **30**, 111-118.

Pekarsky, Y., Hallas, C., Palamarchuk, A., Koval, A., Bullrich, F., Hirata, Y., Bichi, R., Letofsky, J. and Croce, C.M. (2001) Akt phosphorylates and regulates the orphan nuclear receptor Nur77. *Proc Natl Acad Sci U S A*, **98**, 3690-3694.

Pellegrini, G., Dellambra, E., Golisano, O., Martinelli, E., Fantozzi, I., Bondanza, S., Ponzin, D., McKeon, F. and De Luca, M. (2001) p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci U S A*, **98**, 3156-3161.

Pence, B.C., Richard, B.C., Lawlis, R.S. and Kuratko, C.N. (1991) Effects of dietary calcium and vitamin D3 on tumor promotion in mouse skin. *Nutr Cancer*, **16**, 171-181.

Perlmann, T., Umesono, K., Rangarajan, P.N., Forman, B.M. and Evans, R.M. (1996) Two distinct dimerization interfaces differentially modulate target gene specificity of nuclear hormone receptors. *Mol Endocrinol*, **10**, 958-966.

Pessin, J.E. and Okada, S. (1999) Insulin and EGF receptors integrate the Ras and Rap signaling pathways. *Endocr J*, **46 Suppl**, S11-16.

Peters, J.M., Lee, S.S., Li, W., Ward, J.M., Gavrilova, O., Everett, C., Reitman, M.L., Hudson, L.D. and Gonzalez, F.J. (2000) Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta). *Mol Cell Biol*, **20**, 5119-5128.

Peterson, L.L. and Wuepper, K.D. (1984) Epidermal and hair follicle transglutaminases and crosslinking in skin. *Mol Cell Biochem*, **58**, 99-111.

Petkovich, M., Brand, N.J., Krust, A. and Chambon, P. (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature*, **330**, 444-450.

Pfister, R.R. and Renner, M.E. (1978) The corneal and conjunctival surface in vitamin A deficiency: a scanning electron microscopy study. *Invest Ophthalmol Vis Sci*, **17**, 874-883.

Picard, D., Kumar, V., Chambon, P. and Yamamoto, K.R. (1990) Signal transduction by steroid hormones: nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. *Cell Regul*, **1**, 291-299.

Picard, D., Salser, S.J. and Yamamoto, K.R. (1988) A movable and regulable inactivation function within the steroid binding domain of the glucocorticoid receptor. *Cell*, **54**, 1073-1080.

Picard, D. and Yamamoto, K.R. (1987) Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *Embo J*, **6**, 3333-3340.

Pillai, S., Bikle, D.D. and Elias, P.M. (1988a) 1,25-Dihydroxyvitamin D production and receptor binding in human keratinocytes varies with differentiation. *J Biol Chem*, **263**, 5390-5395.

Pillai, S., Bikle, D.D. and Elias, P.M. (1988b) Vitamin D and epidermal differentiation: evidence for a role of endogenously produced vitamin D metabolites in keratinocyte differentiation. *Skin Pharmacol*, **1**, 149-160. 144: Smith EL et al. Effect of 141 alpha,125-dihydrox...[PMID: 2423618]Related Articles, Links.

Pillai, S., Cho, S., Mahajan, M., Frew, L. and Rawlings, A.V. (1996) Synergy between vitamin D precursor 25-hydroxyvitamin D and short chain ceramides on keratinocyte proliferation and differentiation. *J Investig Dermatol Symp Proc*, **1**, 39-43.

Porter, W., Wang, F., Wang, W., Duan, R. and Safe, S. (1996) Role of estrogen receptor/Sp1 complexes in estrogen-induced heat shock protein 27 gene expression. *Mol Endocrinol*, **10**, 1371-1378.

Posner, G.H., Crawford, K.R., Peleg, S., Welsh, J.E., Romu, S., Gewirtz, D.A., Gupta, M.S., Dolan, P. and Kensler, T.W. (2001) A non-calcemic sulfone version of the vitamin D(3) analogue seocalcitol (EB 1089): chemical synthesis, biological evaluation and potency enhancement of the anticancer drug adriamycin. *Bioorg Med Chem*, **9**, 2365-2371.

Posner, G.H., Halford, B.A., Peleg, S., Dolan, P. and Kensler, T.W. (2002) Conceptually new low-calcemic oxime analogues of the hormone 1 alpha,25-dihydroxyvitamin D(3): synthesis and biological testing. *J Med Chem*, **45**, 1723-1730.

Posner, G.H., Lee, J.K., Wang, Q., Peleg, S., Burke, M., Brem, H., Dolan, P. and Kensler, T.W. (1998) Noncalcemic, antiproliferative, transcriptionally active, 24-fluorinated hybrid analogues of the hormone 1alpha, 25-dihydroxyvitamin D3. Synthesis and preliminary biological evaluation. *J Med Chem*, **41**, 3008-3014.

Potten, C.S. and Morris, R.J. (1988) Epithelial stem cells in vivo. *J Cell Sci Suppl*, **10**, 45-62.

Potten, C.S., Wichmann, H.E., Loeffler, M., Dobek, K. and Major, D. (1982) Evidence for discrete cell kinetic subpopulations in mouse epidermis based on mathematical analysis. *Cell Tissue Kinet*, **15**, 305-329.

Poumay, Y., Boucher, F., Leclercq-Smekens, M., Degen, A. and Leloup, R. (1993) Basal cell adhesion to a culture substratum controls the polarized spatial organization of human epidermal keratinocytes into proliferating basal and terminally differentiating suprabasal populations. *Epithelial Cell Biol*, **2**, 7-16.

Prockop, D.J. (1982) How does a skin fibroblast make type I collagen fibers? *J Invest Dermatol*, **79 Suppl 1**, 3s-6s.

Prudencio, J., Akutsu, N., Benlimame, N., Wang, T., Bastien, Y., Lin, R., Black, M.J., Alaoui-Jamali, M.A. and White, J.H. (2001) Action of low calcemic 1alpha,25-dihydroxyvitamin D3 analogue EB1089 in head and neck squamous cell carcinoma. *J Natl Cancer Inst*, **93**, 745-753. 743: McGuire TF et al. Vitamin D(743)-induced apoptosi...[PMID: 11331275]Related Articles, Links.

Quintanilla, M., Brown, K., Ramsden, M. and Balmain, A. (1986a) Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature*, **322**, 78-80.

Quintanilla, M., Brown, K., Ramsden, M. and Balmain, A. (1986b) Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature*, **322**, 78-80.

Ratnam, A.V., Bikle, D.D. and Cho, J.K. (1999) 1,25 dihydroxyvitamin D3 enhances the calcium response of keratinocytes. *J Cell Physiol*, **178**, 188-196.

Ray, M.K., Fagan, S.P., Moldovan, S., DeMayo, F.J. and Brunicardi, F.C. (1999) Beta cell-specific ablation of target gene using Cre-loxP system in transgenic mice. *J Surg Res*, **84**, 199-203.

Regan, J.D., Carrier, W.L., Smith, D.P., Waters, R. and Lee, W.H. (1978) Pyrimidine dimer excision in human cells and skin cancer. *Natl Cancer Inst Monogr*, 141-143.

Reichrath, J., Collins, E.D., Epple, S., Kerber, A., Norman, A.W. and Bahmer, F.A. (1996) Immunohistochemical detection of 1,25-dihydroxyvitamin D3 receptors (VDR) in human skin. A comparison of five antibodies. *Pathol Res Pract*, **192**, 281-289.

Reichrath, J., Kamradt, J., Zhu, X.H., Kong, X.F., Tilgen, W. and Holick, M.F. (1999a) Analysis of 1,25-dihydroxyvitamin D(3) receptors (VDR) in basal cell carcinomas. *Am J Pathol*, **155**, 583-589.

Reichrath, J., Kamradt, J., Zhu, X.H., Kong, X.F., Tilgen, W. and Holick, M.F. (1999b) Analysis of 1,25-dihydroxyvitamin D(3) receptors (VDR) in basal cell carcinomas. *Am J Pathol*, **155**, 583-589.

Reichrath, J., Mittmann, M., Kamradt, J. and Muller, S.M. (1997) Expression of retinoid-X receptors (-alpha,-beta,-gamma) and retinoic acid receptors (-alpha,-beta,-gamma) in normal human skin: an immunohistological evaluation. *Histochem J*, **29**, 127-133.

Reichrath, J., Munssinger, T., Kerber, A., Rochette-Egly, C., Chambon, P., Bahmer, F.A. and Baum, H.P. (1995) In situ detection of retinoid-X receptor expression in normal and psoriatic human skin. *Br J Dermatol*, **133**, 168-175.

Reichrath, J., Schilli, M., Kerber, A., Bahmer, F.A., Czarnetzki, B.M. and Paus, R. (1994) Hair follicle expression of 1,25-dihydroxyvitamin D3 receptors during the murine hair cycle. *Br J Dermatol*, **131**, 477-482. 473: Milde P et al. Expression of 471,425-dihydroxyv...[PMID: 1649228]Related Articles, Links.

Reifenberger, J., Knobbe, C.B., Sterzinger, A.A., Blaschke, B., Schulte, K.W., Ruzicka, T. and Reifenberger, G. (2004) Frequent alterations of Ras signaling pathway genes in sporadic malignant melanomas. *Int J Cancer*, **109**, 377-384.

Reiter, R.J. (1965) Uterine Keratinizing Metaplasia and Plasma Levels of Vitamin a in Rats Fed Vitamin a-Deficient Diets. *Tex Rep Biol Med*, **23**, 486-493.

Rochette-Egly, C. (2003) Nuclear receptors: integration of multiple signalling pathways through phosphorylation. *Cell Signal*, **15**, 355-366.

Rochette-Egly, C., Adam, S., Rossignol, M., Egly, J.M. and Chambon, P. (1997) Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. *Cell*, **90**, 97-107.

Rosario, G., Sachdeva, G., Okulicz, W.C., Ace, C.I., Katkam, R.R. and Puri, C.P. (2003) Role of progesterone in structural and biochemical remodeling of endometrium. *Front Biosci*, **8**, s924-935.

Rosenfield, R.L. and Deplewski, D. (1995) Role of androgens in the developmental biology of the pilosebaceous unit. *Am J Med*, **98**, 80S-88S.

Rosenfield, R.L., Deplewski, D. and Greene, M.E. (2000) Peroxisome proliferator-activated receptors and skin development. *Horm Res*, **54**, 269-274. 217: Michalik L et al. Nuclear hormone receptors and...[PMID: 11595815]Related Articles, Links.

Rougui, Z., Pavlovitch, J. and Rizk-Rabin, M. (1996) In vivo effects of vitamin D on the proliferation and differentiation of rat keratinocytes. *J Cell Physiol*, **168**, 385-394.

Rouiller, V., Gangnerau, M.N., Vayssiere, J.L. and Picon, R. (1990) Cholesterol side-chain cleavage activity in rat fetal gonads: a limiting step for ovarian steroidogenesis. *Mol Cell Endocrinol*, **72**, 111-120.

Rousseau, G.G., Higgins, S.J., Baxter, J.D., Gelfand, D. and Tomkins, G.M. (1975) Binding of glucocorticoid receptors to DNA. *J Biol Chem*, **250**, 6015-6021.

Rubin, J.S., Osada, H., Finch, P.W., Taylor, W.G., Rudikoff, S. and Aaronson, S.A. (1989) Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc Natl Acad Sci U S A*, **86**, 802-806.

Rundhaug, J.E., Gimenez-Conti, I., Stern, M.C., Budunova, I.V., Kiguchi, K., Bol, D.K., Coghlan, L.G., Conti, C.J., DiGiovanni, J., Fischer, S.M., Winberg, L.D. and Slaga, T.J. (1997) Changes in protein expression during multistage mouse skin carcinogenesis. *Mol Carcinog*, **20**, 125-136.

Runger, T.M., Epe, B., Moller, K., Dekant, B. and Hellfritsch, D. (1997) Repair of directly and indirectly UV-induced DNA lesions and of DNA double-strand breaks in cells from skin cancer-prone patients with the disorders dysplastic nevus syndrome or basal cell nevus syndrome. *Recent Results Cancer Res*, **143**, 337-351.

Russell, D.W. (1992) Cholesterol biosynthesis and metabolism. *Cardiovasc Drugs Ther*, **6**, 103-110.

Saade, M., Debahy, N.E. and Houjeily, S. (1999) Clinical remission of xeroderma pigmentosum-associated squamous cell carcinoma with isotretinoin and chemotherapy: case report. *J Chemother*, **11**, 313-317.

Saatcioglu, F., Lopez, G., West, B.L., Zandi, E., Feng, W., Lu, H., Esmaili, A., Apriletti, J.W., Kushner, P.J., Baxter, J.D. and Karin, M. (1997) Mutations in the conserved C-terminal sequence in thyroid hormone receptor dissociate hormone-dependent activation from interference with AP-1 activity. *Mol Cell Biol*, **17**, 4687-4695.

Saez, E., Rutberg, S.E., Mueller, E., Oppenheim, H., Smoluk, J., Yuspa, S.H. and Spiegelman, B.M. (1995) c-fos is required for malignant progression of skin tumors. *Cell*, **82**, 721-732.

Saitou, M., Sugai, S., Tanaka, T., Shimouchi, K., Fuchs, E., Narumiya, S. and Kakizuka, A. (1995) Inhibition of skin development by targeted expression of a dominant-negative retinoic acid receptor. *Nature*, **374**, 159-162.

Sakai, Y. and Demay, M.B. (2000) Evaluation of keratinocyte proliferation and differentiation in vitamin D receptor knockout mice. *Endocrinology*, **141**, 2043-2049. 2049: Sakai Y et al. Metabolic and cellular analys...[PMID: 11306599]Related Articles, Links.

Sakai, Y., Kishimoto, J. and Demay, M.B. (2001) Metabolic and cellular analysis of alopecia in vitamin D receptor knockout mice. *J Clin Invest*, **107**, 961-966. 910: Schuster I et al. Selective inhibition of vitam...[PMID: 11179750]Related Articles, Links.

Salbert, G., Fanjul, A., Piedrafita, F.J., Lu, X.P., Kim, S.J., Tran, P. and Pfahl, M. (1993) Retinoic acid receptors and retinoid X receptor-alpha down-regulate the transforming growth factor-beta 1 promoter by antagonizing AP-1 activity. *Mol Endocrinol*, **7**, 1347-1356.

Saldanha, G., Fletcher, A. and Slater, D.N. (2003) Basal cell carcinoma: a dermatopathological and molecular biological update. *Br J Dermatol*, **148**, 195-202.

Sande, S. and Privalsky, M.L. (1996) Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. *Mol Endocrinol*, **10**, 813-825.

Sanders, S.L. and Stouffer, R.L. (1997) Localization of steroidogenic enzymes in macaque luteal tissue during the menstrual cycle and simulated early pregnancy: immunohistochemical evidence supporting the two-cell model for estrogen production in the primate corpus luteum. *Biol Reprod*, **56**, 1077-1087.

Satake, K., Takagi, E., Ishii, A., Kato, Y., Imagawa, Y., Kimura, Y. and Tsukuda, M. (2003) Anti-tumor effect of vitamin A and D on head and neck squamous cell carcinoma. *Auris Nasus Larynx*, **30**, 403-412.

Sauer, B. and Henderson, N. (1989) Cre-stimulated recombination at loxP-containing DNA sequences placed into the mammalian genome. *Nucleic Acids Res*, **17**, 147-161. 148: Schwenk F et al. A cre-transgenic mouse strain...[PMID: 8559668]Related Articles, Links.

Savill, N.J. (2003) Mathematical models of hierarchically structured cell populations under equilibrium with application to the epidermis. *Cell Prolif*, **36**, 1-26.

Savill, N.J. and Sherratt, J.A. (2003) Control of epidermal stem cell clusters by Notch-mediated lateral induction. *Dev Biol*, **258**, 141-153.

Schmidt, A., Endo, N., Rutledge, S.J., Vogel, R., Shinar, D. and Rodan, G.A. (1992) Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol Endocrinol*, **6**, 1634-1641.

Schmuth, M., Haqq, C.M., Cairns, W.J., Holder, J.C., Dorsam, S., Chang, S., Lau, P., Fowler, A.J., Chuang, G., Moser, A.H., Brown, B.E., Mao-Qiang, M., Uchida, Y., Schoonjans, K., Auwerx, J., Chambon, P., Willson, T.M., Elias, P.M. and Feingold, K.R. (2004) Peroxisome Proliferator-Activated Receptor (PPAR)-beta/delta Stimulates Differentiation and Lipid Accumulation in Keratinocytes. *J Invest Dermatol*, **122**, 971-983.

Schrader, M., Muller, K.M., Nayeri, S., Kahlen, J.P. and Carlberg, C. (1994) Vitamin D3-thyroid hormone receptor heterodimer polarity directs ligand sensitivity of transactivation. *Nature*, **370**, 382-386.

Schreiber, M.M., Moon, T.E. and Bozzo, P.D. (1984) Chronic solar ultraviolet damage associated with malignant melanoma of the skin. *J Am Acad Dermatol*, **10**, 755-759.

Schulman, I.G., Shao, G. and Heyman, R.A. (1998) Transactivation by retinoid X receptor-peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimers: intermolecular synergy requires only the PPARgamma hormone-dependent activation function. *Mol Cell Biol*, **18**, 3483-3494.

Scott, G.A. and Haake, A.R. (1991) Keratinocytes regulate melanocyte number in human fetal and neonatal skin equivalents. *J Invest Dermatol*, **97**, 776-781.

Seery, J.P. and Watt, F.M. (2000) Asymmetric stem-cell divisions define the architecture of human oesophageal epithelium. *Curr Biol*, **10**, 1447-1450.

Seiberg, M., Paine, C., Sharlow, E., Andrade-Gordon, P., Costanzo, M., Eisinger, M. and Shapiro, S.S. (2000) The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp Cell Res*, **254**, 25-32.

Sengel, P. (1985) Role of extracellular matrix in the development of skin and cutaneous appendages. *Prog Clin Biol Res*, **171**, 123-135.

Sherman, J.A. and Partridge, M. (1997) Expression of retinoic acid receptors in normal, dysplastic and malignant oral epithelia. *Br J Oral Maxillofac Surg*, **35**, 260-266.

Sheu, M.Y., Fowler, A.J., Kao, J., Schmuth, M., Schoonjans, K., Auwerx, J., Fluhr, J.W., Man, M.Q., Elias, P.M. and Feingold, K.R. (2002) Topical peroxisome proliferator activated receptor-alpha activators reduce inflammation in irritant and allergic contact dermatitis models. *J Invest Dermatol*, **118**, 94-101. 119: Tan NS et al. Critical roles of PPAR beta/d...[PMID: 11751632]Related Articles, Links.

Shyamala, G. (1975) Glucocorticoid receptors in mouse mammary tumors: Specific binding to nuclear components. *Biochemistry*, **14**, 437-444.

Silver, A.F., Fleischmann, R.D. and Chase, H.B. (1977) The fine structure of the melanocytes of the adult mouse hair follicle during their amelanotic phase (telogen and early anagen). *Am J Anat*, **150**, 653-658.

Sistare, F.D., Thompson, K.L., Honchel, R. and DeGeorge, J. (2002) Evaluation of the Tg.AC transgenic mouse assay for testing the human carcinogenic potential of pharmaceuticals--practical pointers, mechanistic clues, and new questions. *Int J Toxicol*, **21**, 65-79.

Sivitz, W.I. and Pasley, A.M. (1995) Glucocorticoid effects on glucose transport and transporter gene expression in L6 muscle cells. *Biochem Biophys Res Commun*, **206**, 51-56.

Skerrow, C.J. (1978) Intercellular adhesion and its role in epidermal differentiation. *Invest Cell Pathol*, **1**, 23-37.

Smit, J.V., de Sevaux, R.G., Blokx, W.A., van de Kerkhof, P.C., Hoitsma, A.J. and de Jong, E.M. (2004) Acitretin treatment in (pre)malignant skin disorders of renal transplant recipients: Histologic and immunohistochemical effects. *J Am Acad Dermatol*, **50**, 189-196.

Smith, A.A., McLean, W.S., Hansson, V., Nayfeh, S.N. and French, F.S. (1975) Androgen receptor in nuclei of rat testis. *Steroids*, **25**, 569-586.

Smith, E.L., Walworth, N.C. and Holick, M.F. (1986) Effect of 1 alpha,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. *J Invest Dermatol*, **86**, 709-714. 705: Pavlovitch JH et al. [Effect of vitamin D on skin ...[PMID: 6803660]Related Articles, Links.

Smith, K. and Skelton, H. (2003) Overview of Merkel cell carcinoma and recent advances in research. *Int J Dermatol*, **42**, 749-751.

Smith, M.K., Trempus, C.S. and Gilmour, S.K. (1998) Co-operation between follicular ornithine decarboxylase and v-Ha-ras induces spontaneous papillomas and malignant conversion in transgenic skin. *Carcinogenesis*, **19**, 1409-1415.

Snellman, E., Strozyk, M., Segerback, D., Klimenko, T. and Hemminki, K. (2003) Effect of the spectral range of a UV lamp on the production of cyclobutane pyrimidine dimers in human skin in situ. *Photodermatol Photoimmunol Photomed*, **19**, 281-286.

Sonnenberg, A., Calafat, J., Janssen, H., Daams, H., van der Raaij-Helmer, L.M., Falcioni, R., Kennel, S.J., Aplin, J.D., Baker, J., Loizidou, M. and et al. (1991) Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J Cell Biol*, **113**, 907-917.

Sorensen, S., Solvsten, H., Politi, Y. and Kragballe, K. (1997) Effects of vitamin D3 on keratinocyte proliferation and differentiation in vitro: modulation by ligands for retinoic acid and retinoid X receptors. *Skin Pharmacol*, **10**, 144-152.

Spalding, J.W., Momma, J., Elwell, M.R. and Tennant, R.W. (1993) Chemically induced skin carcinogenesis in a transgenic mouse line (TG.AC) carrying a v-Ha-ras gene. *Carcinogenesis*, **14**, 1335-1341.

Spanjaard, R.A., Darling, D.S. and Chin, W.W. (1991) Ligand-binding and heterodimerization activities of a conserved region in the ligand-binding domain of the thyroid hormone receptor. *Proc Natl Acad Sci U S A*, **88**, 8587-8591.

Spencer, J.M., Kahn, S.M., Jiang, W., DeLeo, V.A. and Weinstein, I.B. (1995) Activated ras genes occur in human actinic keratoses, premalignant precursors to squamous cell carcinomas. *Arch Dermatol*, **131**, 796-800.

Spindler, B.J., MacLeod, K.M., Ring, J. and Baxter, J.D. (1975) Thyroid hormone receptors. Binding characteristics and lack of hormonal dependency for nuclear localization. *J Biol Chem*, **250**, 4113-4119.

Stallmeyer, B., Kampfer, H., Podda, M., Kaufmann, R., Pfeilschifter, J. and Frank, S. (2001) A novel keratinocyte mitogen: regulation of leptin and its functional receptor in skin repair. *J Invest Dermatol*, **117**, 98-105.

Steinert, P.M. and Marekov, L.N. (1995) The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. *J Biol Chem*, **270**, 17702-17711.

Steinert, P.M. and Marekov, L.N. (1997) Direct evidence that involucrin is a major early isopeptide cross-linked component of the keratinocyte cornified cell envelope. *J Biol Chem*, **272**, 2021-2030.

Steinert, P.M. and Marekov, L.N. (1999) Initiation of assembly of the cell envelope barrier structure of stratified squamous epithelia. *Mol Biol Cell*, **10**, 4247-4261.

St-Onge, L., Furth, P.A. and Gruss, P. (1996) Temporal control of the Cre recombinase in transgenic mice by a tetracycline responsive promoter. *Nucleic Acids Res*, **24**, 3875-3877. 3814: Feil R et al. Ligand-activated site-specifi...[PMID: 8855277]Related Articles, Links.

Strahl, B.D., Briggs, S.D., Brame, C.J., Caldwell, J.A., Koh, S.S., Ma, H., Cook, R.G., Shabanowitz, J., Hunt, D.F., Stallcup, M.R. and Allis, C.D. (2001) Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. *Curr Biol*, **11**, 996-1000.

Su, M.J., Bikle, D.D., Mancianti, M.L. and Pillai, S. (1994) 1,25-Dihydroxyvitamin D3 potentiates the keratinocyte response to calcium. *J Biol Chem*, **269**, 14723-14729. 14720: Bikle DD. 14721,14725(OH)14722D14723-regulated human k...[PMID: 7782932]Related Articles, Links.

Sugihara, H., Toda, S., Yonemitsu, N. and Watanabe, K. (2001) Effects of fat cells on keratinocytes and fibroblasts in a reconstructed rat skin model using collagen gel matrix culture. *Br J Dermatol*, **144**, 244-253.

Suzuki, A., Itami, S., Ohishi, M., Hamada, K., Inoue, T., Komazawa, N., Senoo, H., Sasaki, T., Takeda, J., Manabe, M., Mak, T.W. and Nakano, T. (2003) Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. *Cancer Res*, **63**, 674-681.

Szabowski, A., Maas-Szabowski, N., Andrecht, S., Kolbus, A., Schorpp-Kistner, M., Fusenig, N.E. and Angel, P. (2000) c-Jun and JunB antagonistically control cytokine-regulated mesenchymal-epidermal interaction in skin. *Cell*, **103**, 745-755.

Tajima, S. and Pinnell, S.R. (1981) Collagen synthesis by human skin fibroblasts in culture: studies of fibroblasts explanted from papillary and reticular dermis. *J Invest Dermatol*, **77**, 410-412.

Takeshita, A., Yen, P.M., Misiti, S., Cardona, G.R., Liu, Y. and Chin, W.W. (1996) Molecular cloning and properties of a full-length putative thyroid hormone receptor coactivator. *Endocrinology*, **137**, 3594-3597.

Tan, N.S., Michalik, L., Noy, N., Yasmin, R., Pacot, C., Heim, M., Fluhmann, B., Desvergne, B. and Wahli, W. (2001) Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes Dev*, **15**, 3263-3277. 3220: Smith KJ et al. Peroxisomes in dermatology. P...[PMID: 11685671]Related Articles, Links.

Tang, A., Eller, M.S., Hara, M., Yaar, M., Hirohashi, S. and Gilchrest, B.A. (1994) E-cadherin is the major mediator of human melanocyte adhesion to keratinocytes in vitro. *J Cell Sci*, **107**, 983-992. 986: Hara M et al. Endothelin-981 of keratinocyte ...[PMID: 7490466]Related Articles, Links.

Tarutani, M., Itami, S., Okabe, M., Ikawa, M., Tezuka, T., Yoshikawa, K., Kinoshita, T. and Takeda, J. (1997) Tissue-specific knockout of the mouse Pig-a gene reveals important roles for GPI-anchored proteins in skin development. *Proc Natl Acad Sci U S A*, **94**, 7400-7405.

Taylor, G., Lehrer, M.S., Jensen, P.J., Sun, T.T. and Lavker, R.M. (2000) Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell*, **102**, 451-461.

Tenchini, M.L., Morra, F., Soranzo, C. and Malcovati, M. (1995) Effects of keratinocyte-secreted soluble factors on spreading, number of dendrites and cell-cell contacts of human epidermal melanocytes and dermal fibroblasts: a quantitative analysis. *Epithelial Cell Biol*, **4**, 143-155.

Teng, D.H., Hu, R., Lin, H., Davis, T., Iliev, D., Frye, C., Swedlund, B., Hansen, K.L., Vinson, V.L., Gumpper, K.L., Ellis, L., El-Naggar, A., Frazier, M., Jasser, S., Langford, L.A., Lee, J., Mills, G.B., Pershouse, M.A., Pollack, R.E., Tornos, C., Troncoso, P., Yung, W.K., Fujii, G., Berson, A., Steck, P.A. and et al. (1997) MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. *Cancer Res*, **57**, 5221-5225.

Tennant, R.W., French, J.E. and Spalding, J.W. (1995) Identifying chemical carcinogens and assessing potential risk in short- term bioassays using transgenic mouse models. *Environ Health Perspect*, **103**, 942-950.

Tennant, R.W., Spalding, J. and French, J.E. (1996) Evaluation of transgenic mouse bioassays for identifying carcinogens and noncarcinogens. *Mutat Res*, **365**, 119-127.

Tennant, R.W., Stasiewicz, S., Eastin, W.C., Mennear, J.H. and Spalding, J.W. (2001) The Tg.AC (v-Ha-ras) transgenic mouse: nature of the model. *Toxicol Pathol*, **29**, 51-59.

Tennant, R.W., Tice, R.R. and Spalding, J.W. (1998) The transgenic Tg.AC mouse model for identification of chemical carcinogens. *Toxicol Lett*, **102-103**, 465-471.

Tennenbaum, T., Belanger, A.J., Quaranta, V. and Yuspa, S.H. (1996) Differential regulation of integrins and extracellular matrix binding in epidermal differentiation and squamous tumor progression. *J Investig Dermatol Symp Proc*, **1**, 157-161.

Tennenbaum, T., Lowry, D., Darwiche, N., Morgan, D.L., Gartsbein, M., Hansen, L., De Luca, L.M., Hennings, H. and Yuspa, S.H. (1998) Topical retinoic acid reduces skin papilloma formation but resistant papillomas are at high risk for malignant conversion. *Cancer Res*, **58**, 1435-1443.

Tennenbaum, T., Weiner, A.K., Belanger, A.J., Glick, A.B., Hennings, H. and Yuspa, S.H. (1993) The suprabasal expression of alpha 6 beta 4 integrin is associated with a high risk for malignant progression in mouse skin carcinogenesis. *Cancer Res*, **53**, 4803-4810.

Thiboutot, D., Jabara, S., McAllister, J.M., Sivarajah, A., Gilliland, K., Cong, Z. and Clawson, G. (2003) Human skin is a steroidogenic tissue: steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes, and an immortalized sebocyte cell line (SEB-1). *J Invest Dermatol*, **120**, 905-914.

Thompson, K.L., Rosenzweig, B.A., Honchel, R., Cannon, R.E., Blanchard, K.T., Stoll, R.E. and Sistare, F.D. (2001) Loss of critical palindromic transgene promoter sequence in chemically induced Tg.AC mouse skin papillomas expressing transgene-derived mRNA. *Mol Carcinog*, **32**, 176-186.

Thornton, M.J. (2002) The biological actions of estrogens on skin. *Exp Dermatol*, **11**, 487-502.

Thuillier, P., Anchiraico, G.J., Nickel, K.P., Maldve, R.E., Gimenez-Conti, I., Muga, S.J., Liu, K.L., Fischer, S.M. and Belury, M.A. (2000) Activators of peroxisome proliferator-activated receptor-alpha partially inhibit mouse skin tumor promotion. *Mol Carcinog*, **29**, 134-142.

Tiano, H.F., Loftin, C.D., Akunda, J., Lee, C.A., Spalding, J., Sessoms, A., Dunson, D.B., Rogan, E.G., Morham, S.G., Smart, R.C. and Langenbach, R. (2002) Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. *Cancer Res*, **62**, 3395-3401.

Toft, D. and Gorski, J. (1966) A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *Proc Natl Acad Sci U S A*, **55**, 1574-1581.

Tokuda, Y., Toda, S., Masaki, Z. and Sugihara, H. (1999) Proliferation and differentiation of rat dorsal prostatic epithelial cells in collagen gel matrix culture, focusing upon effects of adipocytes. *Int J Urol*, **6**, 509-519.

Tomic, M., Jiang, C.K., Epstein, H.S., Freedberg, I.M., Samuels, H.H. and Blumenberg, M. (1990) Nuclear receptors for retinoic acid and thyroid hormone regulate transcription of keratin genes. *Cell Regul*, **1**, 965-973.

Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. and Chambon, P. (1989) The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell*, **59**, 477-487.

Torma, H., Karlsson, T., Michaelsson, G., Rollman, O. and Vahlquist, A. (2000) Decreased mRNA levels of retinoic acid receptor alpha, retinoid X receptor alpha and thyroid hormone receptor alpha in lesional psoriatic skin. *Acta Derm Venereol*, **80**, 4-9.

Torma, H., Rollman, O. and Vahlquist, A. (1993) Detection of mRNA transcripts for retinoic acid, vitamin D3, and thyroid hormone (c-erb-A) nuclear receptors in human skin using reverse transcription and polymerase chain reaction. *Acta Derm Venereol*, **73**, 102-107.

Townson, S.M., Kang, K., Lee, A.V. and Oesterreich, S. (2004) Structure-function analysis of the ER alpha corepressor SAFB1: Identification of a potent transcriptional repression domain. *J Biol Chem*.

Trapman, J., Klaassen, P., Kuiper, G.G., van der Korput, J.A., Faber, P.W., van Rooij, H.C., Geurts van Kessel, A., Voorhorst, M.M., Mulder, E. and Brinkmann, A.O. (1988) Cloning, structure and expression of a cDNA encoding the human androgen receptor. *Biochem Biophys Res Commun*, **153**, 241-248.

Trempus, C.S., Mahler, J.F., Ananthaswamy, H.N., Loughlin, S.M., French, J.E. and Tennant, R.W. (1998) Photocarcinogenesis and susceptibility to UV radiation in the v-Ha-ras transgenic Tg.AC mouse. *J Invest Dermatol*, **111**, 445-451.

Treuter, E., Johansson, L., Thomsen, J.S., Warnmark, A., Leers, J., Pelto-Huikko, M., Sjoberg, M., Wright, A.P., Spyrou, G. and Gustafsson, J.A. (1999) Competition between thyroid hormone receptor-associated protein (TRAP) 220 and transcriptional intermediary factor (TIF) 2 for binding to nuclear receptors. Implications for the recruitment of TRAP and p160 coactivator complexes. *J Biol Chem*, **274**, 6667-6677.

Tsambaos, D., Mahrle, G. and Orfanos, C.E. (1980) Epidermal changes induced by oral excess of aromatic retinoid in guinea pigs. *Arch Dermatol Res*, **267**, 141-152.

Tsambaos, D., Stadler, R., Hilt, K., Zimmermann, B. and Orfanos, C.E. (1985) Effects of arotinoid ethyl ester on epithelial differentiation and proliferation. *Ciba Found Symp*, **113**, 97-116.

Tseng, L., Tang, M., Wang, Z. and Mazella, J. (2003) Progesterone receptor (hPR) upregulates the fibronectin promoter activity in human decidual fibroblasts. *DNA Cell Biol*, **22**, 633-640.

Tseng, S.C., Hatchell, D., Tierney, N., Huang, A.J. and Sun, T.T. (1984) Expression of specific keratin markers by rabbit corneal, conjunctival, and esophageal epithelia during vitamin A deficiency. *J Cell Biol*, **99**, 2279-2286.

Tsou, H.C., Xie, X.X., Yao, Y.J., Ping, X.L. and Peacocke, M. (1997) Expression of retinoid X receptors in human dermal fibroblasts. *Exp Cell Res*, **236**, 493-500.

Tsujita-Kyutoku, M., Kiuchi, K., Danbara, N., Yuri, T., Senzaki, H. and Tsubura, A. (2003) p63 expression in normal human epidermis and epidermal appendages and their tumors. *J Cutan Pathol*, **30**, 11-17.

Tur, E. (1997) Physiology of the skin--differences between women and men. *Clin Dermatol*, **15**, 5-16.

Uribe, P., Wistuba, II and Gonzalez, S. (2003) BRAF mutation: a frequent event in benign, atypical, and malignant melanocytic lesions of the skin. *Am J Dermatopathol*, **25**, 365-370.

Utomo, A.R., Nikitin, A.Y. and Lee, W.H. (1999) Temporal, spatial, and cell type-specific control of Cre-mediated DNA recombination in transgenic mice. *Nat Biotechnol*, **17**, 1091-1096. 1017: Tronche F et al. Disruption of the glucocortic...[PMID: 10471508]Related Articles, Links.

Valyi-Nagy, I.T. and Herlyn, M. (1991) Regulation of growth and phenotype of normal human melanocytes in culture. *Cancer Treat Res*, **54**, 85-101.

Valyi-Nagy, I.T., Hirka, G., Jensen, P.J., Shih, I.M., Juhasz, I. and Herlyn, M. (1993) Undifferentiated keratinocytes control growth, morphology, and antigen expression of normal melanocytes through cell-cell contact. *Lab Invest*, **69**, 152-159.

Valyi-Nagy, I.T., Murphy, G.F., Mancianti, M.L., Whitaker, D. and Herlyn, M. (1990) Phenotypes and interactions of human melanocytes and keratinocytes in an epidermal reconstruction model. *Lab Invest*, **62**, 314-324.

van de Kerkhof, P.C. (1995) Biological activity of vitamin D analogues in the skin, with special reference to antipsoriatic mechanisms. *Br J Dermatol*, **132**, 675-682. 618: Bollag WB et al. Biphasic effect of 671,625-dihyd...[PMID: 7706369]Related Articles, Links.

van den Bemd, G.J., Pols, H.A., Birkenhager, J.C., Kleinekoort, W.M. and van Leeuwen, J.P. (1995) Differential effects of 1,25-dihydroxyvitamin D3-analogs on osteoblast- like cells and on in vitro bone resorption. *J Steroid Biochem Mol Biol*, **55**, 337-346.

van der Schroeff, J.G., Evers, L.M., Boot, A.J. and Bos, J.L. (1990) Ras oncogene mutations in basal cell carcinomas and squamous cell carcinomas of human skin. *J Invest Dermatol*, **94**, 423-425.

Varani, J., Nickoloff, B.J., Dixit, V.M., Mitra, R.S. and Voorhees, J.J. (1989) All-trans retinoic acid stimulates growth of adult human keratinocytes cultured in growth factor-deficient medium, inhibits production of thrombospondin and fibronectin, and reduces adhesion. *J Invest Dermatol*, **93**, 449-454.

Vasioukhin, V., Degenstein, L., Wise, B. and Fuchs, E. (1999) The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proc Natl Acad Sci U S A*, **96**, 8551-8556.

Verma, A.K., Shapas, B.G., Rice, H.M. and Boutwell, R.K. (1979) Correlation of the inhibition by retinoids of tumor promoter-induced mouse epidermal ornithine decarboxylase activity and of skin tumor promotion. *Cancer Res*, **39**, 419-425.

Verma, A.K., Slaga, T.J., Wertz, P.W., Mueller, G.C. and Boutwell, R.K. (1980) Inhibition of skin tumor promotion by retinoic acid and its metabolite 5,6-epoxyretinoic acid. *Cancer Res*, **40**, 2367-2371.

vom Baur, E., Zechel, C., Heery, D., Heine, M.J., Garnier, J.M., Vivat, V., Le Douarin, B., Gronemeyer, H., Chambon, P. and Losson, R. (1996) Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *Embo J*, **15**, 110-124.

Wahli, W., Devchand, P.R., A, I.J. and Desvergne, B. (1999) Fatty acids, eicosanoids, and hypolipidemic agents regulate gene expression through direct binding to peroxisome proliferator-activated receptors. *Adv Exp Med Biol*, **447**, Fajas L et al. PPARgamma3 mRNA: a distinct P...[PMID: 9821958]Related Articles, Links.

Wang, D., Russell, J., Xu, H. and Johnson, D.G. (2001) Deregulated expression of DP1 induces epidermal proliferation and enhances skin carcinogenesis. *Mol Carcinog*, **31**, 90-100.

Wang, X.J., Greenhalgh, D.A., Jiang, A., He, D., Zhong, L., Medina, D., Brinkley, B.R. and Roop, D.R. (1998) Expression of a p53 mutant in the epidermis of transgenic mice accelerates chemical carcinogenesis. *Oncogene*, **17**, 35-45.

Watt, F.M. (1988) Epidermal stem cells in culture. *J Cell Sci Suppl*, **10**, 85-94.

Watt, F.M. (2002) Role of integrins in regulating epidermal adhesion, growth and differentiation. *Embo J*, **21**, 3919-3926.

Watts, R.G., Ben-Ari, E.T., Bernstein, L.R., Birrer, M.J., Winterstein, D., Wendel, E. and Colburn, N.H. (1995) c-jun and multistage carcinogenesis: association of overexpression of introduced c-jun with progression toward a neoplastic endpoint in mouse JB6 cells sensitive to tumor promoter-induced transformation. *Mol Carcinog*, **13**, 27-36.

Weaver, T.E., Na, C.L. and Stahlman, M. (2002) Biogenesis of lamellar bodies, lysosome-related organelles involved in storage and secretion of pulmonary surfactant. *Semin Cell Dev Biol*, **13**, 263-270.

Weedon, D. and Strutton, G. (1984) The recognition of early stages of catagen. *Am J Dermatopathol*, **6**, 553-555.

Weeks, C.E., Slaga, T.J., Hennings, H., Gleason, G.L. and Bracken, W.M. (1979) Inhibition of phorbol ester-induced tumor promotion in mice by vitamin A analog and anti-inflammatory steroid. *J Natl Cancer Inst*, **63**, 401-406.

Wei, S.J., Trempus, C.S., Cannon, R.E., Bortner, C.D. and Tennant, R.W. (2003) Identification of Dss1 as a 12-O-tetradecanoylphorbol-13-acetate- responsive gene expressed in keratinocyte progenitor cells, with possible involvement in early skin tumorigenesis. *J Biol Chem*, **278**, 1758-1768.

Westergaard, M., Henningsen, J., Svendsen, M.L., Johansen, C., Jensen, U.B., Schroder, H.D., Kratchmarova, I., Berge, R.K., Iversen, L., Bolund, L., Kragballe, K. and Kristiansen, K. (2001) Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. *J Invest Dermatol*, **116**, 702-712. 720: Billoni N et al. Expression of peroxisome prol...[PMID: 11200828]Related Articles, Links.

Whitehead, S.N.a.S. (2001) Endocrinology. An Integrated Approach.

Wilhelm, B., Meinhardt, A. and Seitz, J. (1996) Transglutaminases: purification and activity assays. *J Chromatogr B Biomed Appl*, **684**, 163-177.

Willson, T.M. and Moore, J.T. (2002) Genomics versus orphan nuclear receptors--a half-time report. *Mol Endocrinol*, **16**, 1135-1144.

Willy, P.J., Umesono, K., Ong, E.S., Evans, R.M., Heyman, R.A. and Mangelsdorf, D.J. (1995) LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev*, **9**, 1033-1045.

Wirth, P.J., Yuspa, S.H., Thorgeirsson, S.S. and Hennings, H. (1987) Induction of common patterns of polypeptide synthesis and phosphorylation by calcium and 12-O-tetradecanoylphorbol-13-acetate in mouse epidermal cell culture. *Cancer Res*, **47**, 2831-2838.

Wisner, J.R., Jr. and Gomes, W.R. (1975) Comparative ability of rat seminiferous tubules, interstitium, and whole testes to utilize cholesterol-1,5-3H as a substrate for testosterone synthesis and the intratesticular distribution of cholesterol side-chain cleavage enzyme. *Steroids*, **26**, 769-783.

Wong, C.S., Strange, R.C. and Lear, J.T. (2003) Basal cell carcinoma. *Bmj*, **327**, 794-798.
Wong, C.W. and Privalsky, M.L. (1998) Transcriptional repression by the SMRT-mSin3 corepressor: multiple interactions, multiple mechanisms, and a potential role for TFIIB. *Mol Cell Biol*, **18**, 5500-5510.
Wood, A.W., Chang, R.L., Huang, M.T., Uskokovic, M. and Conney, A.H. (1983) 1 alpha, 25-Dihydroxyvitamin D3 inhibits phorbol ester-dependent chemical carcinogenesis in mouse skin. *Biochem Biophys Res Commun*, **116**, 605-611.
Wrange, O. and Gustafsson, J.A. (1978) Separation of the hormone- and DNA-binding sites of the hepatic glucocorticoid receptor by means of proteolysis. *J Biol Chem*, **253**, 856-865.
Wu, H., Goel, V. and Haluska, F.G. (2003) PTEN signaling pathways in melanoma. *Oncogene*, **22**, 3113-3122.
Wu, X. and Pandolfi, P.P. (2001) Mouse models for multistep tumorigenesis. *Trends Cell Biol*, **11**, S2-9.
Xie, Z., Komuves, L., Yu, Q.C., Elalieh, H., Ng, D.C., Leary, C., Chang, S., Crumrine, D., Yoshizawa, T., Kato, S. and Bikle, D.D. (2002) Lack of the vitamin D receptor is associated with reduced epidermal differentiation and hair follicle growth. *J Invest Dermatol*, **118**, 11-16.
Xu, J., Thompson, K.L., Shephard, L.B., Hudson, L.G. and Gill, G.N. (1993) T3 receptor suppression of Sp1-dependent transcription from the epidermal growth factor receptor promoter via overlapping DNA-binding sites. *J Biol Chem*, **268**, 16065-16073.
Xu, W., Cho, H., Kadam, S., Banayo, E.M., Anderson, S., Yates, J.R., 3rd, Emerson, B.M. and Evans, R.M. (2004) A methylation-mediator complex in hormone signaling. *Genes Dev*, **18**, 144-156.
Xu, X.C., Wong, W.Y., Goldberg, L., Baer, S.C., Wolf, J.E., Ramsdell, W.M., Alberts, D.S., Lippman, S.M. and Lotan, R. (2001) Progressive decreases in nuclear retinoid receptors during skin squamous carcinogenesis. *Cancer Res*, **61**, 4306-4310.
Yamamoto, H., Ochiya, T., Takeshita, F., Toriyama-Baba, H., Hirai, K., Sasaki, H., Sakamoto, H., Yoshida, T., Saito, I. and Terada, M. (2002) Enhanced skin carcinogenesis in cyclin D1-conditional transgenic mice: cyclin D1 alters keratinocyte response to calcium-induced terminal differentiation. *Cancer Res*, **62**, 1641-1647.
Yang, L., Mao-Qiang, M., Taljebini, M., Elias, P.M. and Feingold, K.R. (1995) Topical stratum corneum lipids accelerate barrier repair after tape stripping, solvent treatment and some but not all types of detergent treatment. *Br J Dermatol*, **133**, 679-685.
Yao, T.P., Ku, G., Zhou, N., Scully, R. and Livingston, D.M. (1996) The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc Natl Acad Sci U S A*, **93**, 10626-10631.
Yen, P.M., Sugawara, A., Forgione, M., Spanjaard, R.A., Macchia, E., Cheng, S.Y. and Chin, W.W. (1993) Region-specific anti-thyroid hormone receptor (TR) antibodies detect changes in TR structure due to ligand-binding and dimerization. *Mol Cell Endocrinol*, **97**, 93-99.
Yoshizawa, T., Handa, Y., Uematsu, Y., Takeda, S., Sekine, K., Yoshihara, Y., Kawakami, T., Arioka, K., Sato, H., Uchiyama, Y., Masushige, S., Fukamizu, A., Matsumoto, T. and Kato, S. (1997) Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet*, **16**, 391-396.
You, M.J., Castrillon, D.H., Bastian, B.C., O'Hagan, R.C., Bosenberg, M.W., Parsons, R., Chin, L. and DePinho, R.A. (2002) Genetic analysis of Pten and Ink4a/Arf interactions in the suppression of tumorigenesis in mice. *Proc Natl Acad Sci U S A*, **99**, 1455-1460.
Yu, J., Papavasiliou, V., Rhim, J., Goltzman, D. and Kremer, R. (1995) Vitamin D analogs: new therapeutic agents for the treatment of squamous cancer and its associated hypercalcemia. *Anticancer Drugs*, **6**, 101-108.
Yu, V.C., Delsert, C., Andersen, B., Holloway, J.M., Devary, O.V., Naar, A.M., Kim, S.Y., Boutin, J.M., Glass, C.K. and Rosenfeld, M.G. (1991) RXR beta: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell*, **67**, 1251-1266.
Yudoh, K., Matsuno, H. and Kimura, T. (1999) 1alpha,25-dihydroxyvitamin D3 inhibits in vitro invasiveness through the extracellular matrix and in vivo pulmonary metastasis of B16 mouse melanoma. *J Lab Clin Med*, **133**, 120-128.
Yuspa, S.H. (1998) The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J Dermatol Sci*, **17**, 1-7.
Yuspa, S.H., Dlugosz, A.A., Denning, M.F. and Glick, A.B. (1996) Multistage carcinogenesis in the skin. *J Investig Dermatol Symp Proc*, **1**, 147-150.
Yuspa, S.H., Hennings, H., Roop, D., Strickland, J. and Greenhalgh, D.A. (1990) The malignant conversion step of mouse skin carcinogenesis. *Environ Health Perspect*, **88**, 193-195.
Zechel, C., Shen, X.Q., Chambon, P. and Gronemeyer, H. (1994) Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. *Embo J*, **13**, 1414-1424.
Zelent, A., Krust, A., Petkovich, M., Kastner, P. and Chambon, P. (1989a) Cloning of murine alpha and beta retinoic acid receptors and a novel receptor gamma predominantly expressed in skin. *Nature*, **339**, 714-717. 711: Mattei MG et al. Mapping of the human retinoic...[PMID: 2844649]Related Articles, Links.
Zelent, A., Krust, A., Petkovich, M., Kastner, P. and Chambon, P. (1989b) Cloning of murine alpha and beta retinoic acid receptors and a novel receptor gamma predominantly expressed in skin. *Nature*, **339**, 714-717.
Zettersten, E.M., Ghadially, R., Feingold, K.R., Crumrine, D. and Elias, P.M. (1997) Optimal ratios of topical stratum corneum lipids improve barrier recovery in chronologically aged skin. *J Am Acad Dermatol*, **37**, 403-408.
Zhang, X.K., Hoffmann, B., Tran, P.B., Graupner, G. and Pfahl, M. (1992) Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature*, **355**, 441-446.
Zhou, X.F., Shen, X.Q. and Shemshedini, L. (1999) Ligand-activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-Jun/c-Fos dimerization. *Mol Endocrinol*, **13**, 276-285.
Zile, M.H. (2001) Function of vitamin A in vertebrate embryonic development. *J Nutr*, **131**, 705-708.
Zinser, G.M., Sundberg, J.P. and Welsh, J. (2002) Vitamin D(3) receptor ablation sensitizes skin to chemically induced tumorigenesis. *Carcinogenesis*, **23**, 2103-2109.