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Role of the p53 family in chemotherapy-related side effects on the enteric nervous system and skeletal muscle homeostasis

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ABBREVIATIONS

A.

ACH	Acetylcholine
AD	Alzheimer Disease
ADP	Adenosine diphosphate
AEN	Apoptosis enhancing nuclease
AIDS	Acquired immunodeficiency syndrome
AIF	Apoptosis inducing factor
AKT	Serine threonine kinase
ALS	Amyotrophic lateral sclerosis
AMPk	AMP-activated protein kinase
APAF1	Apoptotic peptidase activating factor 1
ATF4	Activating transcription factor 4
ATP	Adenosine triphosphate
ARB	Angiotensin receptor blocker

B.

BAX	Bcl-2–associated X protein
BCL-2	B-cell lymphoma 2
BGP-15	O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime

C.

CAC	Cancer-associated cachexia
C/EBPd	CAAT/enhancer-binding protein delta
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COX2	Cyclo-oxygenase 2
CRL	Cullin-RING E3 ligase
CTCAE	Common Terminology Criteria for Adverse Events

CTD	C-terminal regulatory domain
CTGF	Connective tissue growth factor
CUL1	Cullin protein 1
CYR61 (CCN1)	Cellular communication network factor 1

D.

DBD	DNA binding domain
DNA	Desoxy ribonucleic acid

E.

EDN3	Endothelin3
EDNRB	EDN3 receptor
EGCs	Enteric glial cells
EN	Enteric neurons
ENCCs	Enteric neural crest-derived cells
ENS	Enteric nervous system
EPA	Ecosapentaneoic acid
ERK	Extracellular regulated MAP kinase
ESCs	Embryonic stem cells
ESMO	European society for medical oncology

F.

FCCP	Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone
FoxO	Forkhead box class O
5-FU	5-Fluorouracil

G.

GAP43	Growth-associated-protein-43
GDF15	Growth differentiation factors
GDNF	Glial-derived neurotrophic factor

GFAP	Glial fibrillary acid protein
GI	Gastrointestinal
GIT	Gastrointestinal tract
GTSE1	G2 And S-Phase Expressed 1

H.

HIV	Human immunodeficiency virus
5-HT	5-hydroxytryptamin
HSP70	Heat shock protein 70
HSP90	Heat shock protein 90

I.

IBA1	Ionized calcium binding adaptor molecule 1
IFNγ	Interferon gamma
IGF-1	Insulin-like growth factor 1
IL-1	Interleukin 1
IL-6	Interleukin 6
ISL1	Insulin gene enhancer protein
ITCH	Itchy, E3 ubiquitin protein ligase

J.

JAK	Janus kinase

K.

KO	Knock-out

L.

LATS1/2	Large tumor suppressor 1/2
LDH	Lactate dehydrogenase

LKB1	Liver kinase B1
LIF	Leukemia inhibitory factor

M.

MAFbx	Muscle Atrophy F-box, Atrogin-1
MCK	Muscle creatine kinase
MDM2	Mouse double minute 2 homolog
MDMX	Mouse double minute 4
MLC2V	Myosin regulatory light chain 2
MRFs	Myogenic regulatory factors
mRNA	Messenger ribonucleic acid
MST1/2	Mammalian Sterile 20-like kinase 1/2
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
MuRF1	Muscle Ring Finger protein 1
MYF5	Myogenic factor 5
MyHC	Myosin heavy chain
MYOD	Myoblast determination protein
MYOG	Myogenin

N.

NAC	N-acetylcystein
NER	Nucleotide excision repair
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NKX2.5	NK2 Homeobox 5
NOS	Nitric oxide synthase
NOXA (PMAIP)	Phorbol-12-myristate-13-acetate-induced protein 1
NPCs	Neuroprogenitor cells

O.

OD	Oligomerization domain

P.

PARP	Poly-(ADP-ribose) polymerase
PARP1	Poly-(ADP-ribose) polymerase 1
PAX3	Paired box protein 3
PAX7	Paired box protein 7
PD	Parkinson disease
PGP9.5	Protein gene product 9.5
PI3K	Phosphatidyl-inositol 3-kinase
PNI	Perineural invasion
PNS	Peripheral nervous system
PRD	Prolin-rich domain
PSRC1	Proline And Serine Rich Coiled-Coil 1
PUMA	p53 upregulated modulator of apoptosis

R.

RB	Retinoblastoma tumor suppressor protein
RING	Really interesting new gene
RIP3	Receptor-interacting protein kinase 3
ROM	Reactive oxygen metabolite
ROS	Reactive oxygen species

S.

Sirt1	Sirtuin 1
SKP1	S-Phase Kinase Associated Protein 1
SMARs	Selective androgen receptor modulators
SNE	Système nerveux entérique
SNP	Single nucleotide polymorphism

SOD1	Superoxide dismutase 1
SOX10	SRY (sex determining region Y)-box 10
STAT	Signal transducers and activators of transcription

T.

TAD	Transactivation domain
TAZ	Tafazzin
TBX5	Tbox 5
TCA	Tricarboxylic acid
TEAD1-4	TEA domain family members 1-4
TGFB	Transforming growth factor beta
TLR-4	Toll Like Receptor 4
TNF-α	Tumor necrosis factor alpha
TRIM63	Tripartite Motif Containing 63
TWEAK	TNF-related weak inducer of apoptosis
TYA	Teenagers and young adult

U.

UPS	Ubiquitin-proteasome system

V.

VIP	Vasointestinal peptide

Y.

YAP	Yes-associated protein

RESUME DE THESE

Rôle de la famille p53 dans l'impact des drogues anti-cancéreuses sur l'homéostasie du système nerveux entérique et du muscle lisse.

-Introduction-

Une des problématiques majeures de santé publique actuelle est le cancer, plus particulièrement les thérapies anticancéreuses associées. De nombreux agents de chimiothérapies actuels, tels que les dérivés de platine (ex. cisplatine, oxaliplatine) ou les anthracyclines (ex. doxorubicine), provoquent respectivement des effets secondaires sur le tractus gastro-intestinal, induisant ainsi des diarrhées/vomissements, et sur les muscles squelettiques, induisant des faiblesses musculaires sévères associées au cancer. Ces toxicités liées aux thérapies anticancéreuses impactent significativement la qualité de vie des patients et conduisent même parfois à un arrêt forcé du traitement, mettant en jeu leur chance de guérison.

Pour expliquer ces altérations fonctionnelles, des études indépendantes ont montrées d'une part, qu'une exposition répétée au cisplatine réduit l'activité motrice intestinale ainsi que le nombre de neurones et la taille des ganglions du système nerveux entérique (SNE), entité contrôlant l'activité du tractus gastro-intestinal [1]. De même que le cisplatine, l'oxaliplatine induit également une perte de cellules nerveuses entériques corrélée à une motilité réduite du côlon. D'autre part, la doxorubicine a été décrite comme étant un facteur responsable de l'atrophie musculaire ; mécanismes résultant d'un déséquilibre entre la synthèse et la dégradation des protéines ; observée après traitement. Certains mécanismes moléculaires sous-jacents à l'atrophie musculaire induite par la doxorubicine ont été identifiés et mettent en jeu des facteurs pro-atrophiques tels que Trim63 et Atrogin-1 [2]. La littérature suggère également qu'une activation des membres de la famille p53 [3] connus entre autres pour leur rôle dans la prolifération et mort des cellules musculaires, via une potentielle interaction avec la voie Hippo (YAP/TAZ), serait impliquée dans ce mécanisme.

Malheureusement, les stratégies thérapeutiques développées visant à contrer ces effets secondaires restent rarement efficaces et les mécanismes sous-jacents mal connus. En effet, aucune information ne permet actuellement d'expliquer les mécanismes impliqués dans la

perte neuronale du SNE, qu'il s'agisse du type de mort cellulaire engagée (apoptose, autophagie) ou du processus cellulaire affecté (altération du renouvellement ou de la différenciation des cellules souches). De même, l'identité exacte des cellules impactées (neurones, cellules gliales, cellules souches) n'a pas encore été clarifiée. Il en est de même pour la perte musculaire associée à l'administration de doxorubicine pour laquelle les éléments connus de la littérature ne permettent pas de totalement expliquer ce processus atrophique, ni même d'y remédier.

De manière intéressante, ces deux types de toxicité liées aux sels de platine et aux anthracyclines convergent en un point commun : la famille p53. La famille p53 est une famille de facteurs de transcription incluant trois membres : p53, p63 et p73. Tous trois jouent un rôle dans de nombreux processus cellulaires, en particulier, dans la réponse aux dommages à l'ADN, donc en réponse aux traitements anticancéreux (cf. cisplatine, doxorubicine). Ils sont également impliqués dans le muscle où ils régulent la différenciation des cellules musculaires mais aussi au sein du système nerveux central où ils contrôlent la différenciation et la balance mort/survie des cellules neuronales.

Les travaux du laboratoire ont précisément montré que les membres de la famille p53 participent à la mort neuronale dans le système nerveux central en réponse à l'utilisation de drogues anticancéreuses [4][5], et que leur expression est déterminante dans l'apparition de l'atrophie musculaire observée dans la sclérose latérale amyotrophique (SLA), une pathologie neurodégénérative [6]. Il a en particulier été établit dans ce contexte pathologique que p63 régule l'expression du facteur pro-atrophique Trim63.

C'est donc dans ce contexte que s'inscrit mon projet de thèse qui consiste à comprendre le **rôle de la famille p53 dans les effets secondaires des drogues anticancéreuses** selon deux axes précis : **I)** Identifier le rôle de la famille p53 parmi les mécanismes moléculaires sous-jacents à la perte neuronale induite par le cisplatine en identifiant précisément le types de cellules affectées (neurones, cellules gliales, cellules souches) ; **II)** Comprendre la régulation complexe de Trim63 par la famille p53 dans l'atrophie musculaire en réponse à la doxorubicine. Par ailleurs, dans chacun de ces contextes j'ai utilisé une approche « Omique »

sans apriori (RNA sequencing) pour déterminer l'importance de la famille p53 dans l'ensemble des voies de signalisation dérégulées et identifier des mécanismes inattendus.

I) Effets secondaires du cisplatine sur le SNE et rôle de la famille p53

La difficulté de ce projet réside dans la procédure longue et techniquement difficile d'isolement du SNE chez la souris ainsi que dans la faible quantité de matériel obtenu.

-Modèles utilisés-

Pour déterminer le rôle des membres de la famille p53 dans la perte neuronale du SNE, j'ai utilisé deux modèles expérimentaux.

- **Modèle *ex vivo*** : D'une part, en collaboration avec l'équipe du Pr. Schäfer (Zweibrücken, Allemagne), j'ai développé au laboratoire une culture *ex vivo* de plexus myentériques purifiés contenant les neurones entériques et les cellules gliales associées dans une structure ganglionnaire préservée et pouvant être traités par du cisplatine.

- **Modèles *in vivo*** : Les animaux utilisés sont des souris Wild-Type (WT) ou génétiquement modifiées pour p63 (KO-p63, TAp63-Tomato). Ces modèles consistent à traiter directement des souris par du cisplatine avant d'analyser le SNE. Chacun des modèles peut être analysé pour l'expression d'ARNm (RT-qPCR) et de protéines d'intérêt (Western blot ou immunofluorescence.

-Résultats-

Analyse transcriptomique : Afin d'élucider l'impact du cisplatine sur l'expression des gènes dans le système nerveux entérique chez les souris traitées, une approche transcriptomique non biaisée de RNA sequencing associée à une analyse bioinformatique a été réalisée sur trois réplicats biologiques d'échantillons de SNE à la suite d'une expérience *in vivo* de traitement au cisplatine de 1 ou 7 jours. Ces analyses ont montré une forte dérégulation des gènes impliqués dans le cycle cellulaire, l'apoptose et les dommages à l'ADN à 1 jour, parmi lesquels il est intéressant de noter que beaucoup sont des gènes cibles connus de p53, tels que *Aen*, *Mdm2*, *Psrc1*, *p21* ou *Gtse1*, suggérant un potentiel rôle de la famille p53 dans l'effet du cisplatine sur la mort des cellules du SNE. De manière intéressante il a été observé à 7 jours de traitement un switch de signalisation en faveur de l'inflammation et la plasticité neuronale

à travers respectivement l'induction des gènes comme des interleukines/chimiokines et des facteurs déterminants dans l'identité neuronale.

Activation de l'apoptose : Afin de déterminer le processus de mort cellulaire engagée lors de la perte neuronale du SNE décrite dans la littérature nous nous sommes tout d'abord intéressés à la mort cellulaire par apoptose en suivant le clivage de la caspase-3 et la condensation/fragmentation du noyau à 1 jour de traitement au cisplatine. Mes expériences *ex vivo* et *in vivo* ont révélées que le cisplatine induit à la fois le clivage de la caspase-3 et une condensation nucléaire de manière dose et temps-dépendante, corrélés dans les mêmes temps à l'induction de AIF (un marqueur d'apoptose) relocalisé dans les noyaux des neurones, dont l'expression diminue à 7 jours. Ces résultats sont cohérents avec l'augmentation des ARNm des gènes pro-apoptotiques *Noxa* et *Puma* observée à 1 jour, ainsi que la diminution de l'expression de *Bcl-2* qui est un gène pro-survie. Cela suggère que la perte neuronale observée pourrait être causée par l'apoptose. Aussi, le stress cellulaire induit par le cisplatine et retrouvé dans le SNE est confirmé par l'augmentation de l'expression de la GFAP (Glial fibrillary acid protein), un marqueur de stress spécifique des cellules gliales.

Analyse fonctionnelle : Dans le but de confirmer l'implication des membres de la famille p53 dans l'effet cytotoxiques du cisplatine dans le SNE, et d'affirmer que l'expression des gènes pro-apoptotiques est effectivement dépendante d'une signalisation p53, des expériences fonctionnelles ont été réalisées. L'utilisation ; dans notre modèle *ex vivo* ; de la pifithrine, un inhibiteur pharmacologique de p53/p73, a révélé une réduction considérable de l'induction des gènes cibles de p53 par le cisplatine, notamment *Noxa*, confirmant ainsi le rôle potentiel de p53/p73 dans ce contexte.

Régulation de l'expression des membres de la famille p53 : La mort cellulaire par apoptose est un des mécanismes caractéristiques de la famille p53, c'est pourquoi leur implication dans ce processus, plus particulièrement le profil d'expression des différents membres de la famille a été investigué, d'autant que leur rôle dans le SNE n'a jamais été décrit.

• **Changement différentiel de l'expression de p53 :** De manière surprenante l'analyse de l'expression de p53 indique seulement une diminution de son niveau d'expression protéique *in vivo*, mais indique une relocalisation nucléaire de p53 dans le modèles *ex vivo.* Il nous reste donc encore à préciser les changements d'expression de p53 avec d'autres outils.

• **Induction de p73 par le cisplatine :** De façon intéressante, l'étude de p73 dans le SNE a permis d'observer une augmentation du nombre de cellules exprimant la protéine p73 après 1 jour de traitement au cisplatine, et qui diminue dès 7 jours. Les taux d'ARNm de TAp73 ; isoforme pro-apoptotique ; sont également retrouvés induits à la fois *ex vivo* et *in vivo*. Afin de déterminer la localisation précise de p73 au sein du SNE, des expériences de co-localisation entre un marqueur nucléaire (DAPI), p73 et des marqueurs neuronaux tels que la synaptobrévine et HuC/D ont montré que l'expression de p73 se situe dans les neurones, plus particulièrement dans le noyau (Figure A). En effet, HuC/D étant un marqueur neuronal dont l'expression est cytoplasmique il a pu être observé que l'expression de p73 au sein du SNE est nucléaire (Figure A).

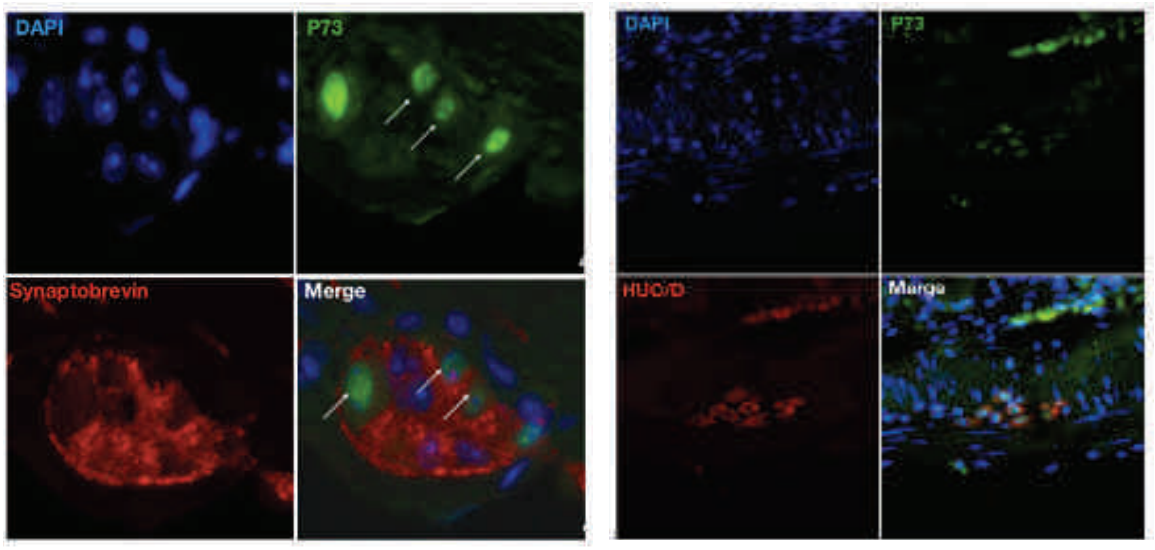

***Figure A**. Mise en évidence de l'expression de p73 dans les noyaux de neurones entériques en réponse au cisplatine.*

• **Modulation de la réponse au cisplatine par p63 :** L'étude de l'expression de p63 s'est avérée plus délicate puisqu'en dépit des nombreux anticorps utilisés, il n'a pas été possible de détecter de marquage protéique de p63 dans nos deux modèles. Cela s'explique certainement par le faible taux d'ARNm de TAp63 observé. C'est pourquoi grâce à l'utilisation d'un modèle de souris « rapportrice » TAp63-Tomato nous avons visualisé l'expression de la protéine fluorescente rouge Tomato pilotée par l'activation du promoteur « p2 » contrôlant l'expression des isoformes TAp63 dans le gène *Tp63*. Ceci nous a donné le moyen d'affirmer en association avec des marqueurs neuronaux que le promoteur de TAp63 est activé en réponse au cisplatine au sein des neurones entériques. Cet outil cependant ne permet pas de quantifier le signal d'expression de la Tomato, ni même de conclure quant à des différences d'expression avec ou sans traitement au cisplatine. Nous avons donc eu recourt à l'utilisation de souris déplétées pour les variants TAp63 permettant de définir le rôle de ce dernier dans le SNE en réponse au cisplatine. Ce modèle nous a permis d'établir que la perte de TAp63 résulte d'une augmentation de l'expression de TAp73 après 1 jour de traitement, mais également que les gènes pro-apoptotiques exprimés transitoirement à 1 jour étaient maintenus chez ces souris transgéniques. Cela indique clairement que TAp63 participe à la régulation des gènes de mort et survie cellulaire dans le SNE en réponse au cisplatine.

-Conclusion-

Mon travail démontre pour la première fois l'implication des membres de la famille p53 dans le SNE, plus particulièrement associée à la réponse stress-dépendante induite par le cisplatine. De nombreux points restent néanmoins à éclaircir, comme notamment la chronologie évènementielle moléculaire exacte suivant l'administration du cisplatine. Il n'est encore pas connu si l'homéostasie des cellules souches est affectée lors du traitement, ni même l'implication exacte des isoformes pro-survie de la famille p53 telles que ΔNp63 et ΔNp73. Aussi, l'effet inhibiteur de la pifithrine reste à confirmer dans un modèle murin.

II) Atrophie musculaire, famille p53 et voie Hippo

-Modèles utilisés-

- **Modèle *in vitro*** : Une lignée cellulaire murine myoblastique nommée C2C12 a été utilisée dans des conditions expérimentales de traitement à la doxorubicine afin d'induire une mort cellulaire.

- **Modèles *in vivo*** : Ce modèle consiste à traiter des souris à la doxorubicine (18mg/kg) par injection afin de développer un modèle d'atrophie musculaire que nous analysons sur le muscle squelettique gastrocnémien.

-Contexte-

Au moment où j'ai débuté ma thèse des travaux non publiés du laboratoire indiquaient que TAp63 était également induit dans le muscle en réponse au traitement à une drogue anticancéreuse, la doxorubicine. Dans ce contexte, TAp63 semblait aussi pouvoir réguler l'expression de Trim63, mais présentait des différences entre les résultats *in vitro* et *in vivo* qui reflètent sans doute la complexité de la régulation de l'expression de Trim63. En effet, l'expression de Trim63 est contrôlée par plusieurs facteurs de transcription tels que FOXO3 ou encore ceux de la voie Hippo : YAP et TAZ. Ainsi, j'ai continué ce projet en essayant de mieux comprendre comment l'expression de Trim63 pouvait être induite par la doxorubicine.

-Résultats-

Analyse transcriptomique : Afin de mieux caractériser l'implication de la voie Hippo dans le phénomène d'atrophie musculaire lié à la doxorubicine une analyse de RNAseq a aussi été réalisée sur des échantillons de muscle squelettique de souris après traitement à la doxorubicine. Cette analyse nous a permis de confirmer ce que nous avions déjà pu montrer, à savoir l'implication de TAp63 et la dérégulation de ses gènes cibles spécifiques du muscle squelettique. L'analyse des données est actuellement en cours afin de précisément déterminer le rôle de la voie Hippo au sein de ce contexte.

Analyse de la signalisation Hippo : La voie Hippo est une voie de méchanotransduction qui a un rôle clé dans la régulation de la croissance des organes via notamment le méchanosenseur YAP. Dans le muscle, YAP est une protéine qui module la myogenèse et la régénération. Des études ont montrées que la surexpression de YAP se traduit par une hypertrophie tissulaire [7] et une diminution de l'expression génique de Trim63 et donc de l'activité pro-atrophique associée. Cependant la littérature est controversée à son sujet, car il est décrit par Judson et al., 2013 [8], qu'une surexpression d'un variant de YAP chez des souris entraine une atrophie en augmentant la dégradation musculaire via Trim63 et Atrogin1. De plus, les voies Hippo et p53 interagissent ensemble, notamment YAP et p73, en réponse à des dommages à l'ADN [9]. Il est également décrit que YAP est un médiateur de la fonction de p73 en régulant son activité transcriptionnelle [10] ou en le stabilisant par compétition avec ITCH (ubiquitine ligase spécifique de p73), empêchant ainsi sa dégradation [11]. A partir de ces données, nous avons émis l'hypothèse que la voie Hippo et la famille p53 pourraient interagir physiquement ou fonctionnellement pour finement réguler l'expression de Trim63 dans le muscle, et en particulier en réponse à la doxorubicine.

L'expression des facteurs de transcription YAP et TAZ et de certains de leur gènes cibles a été caractérisé dans nos deux modèles où il apparaît dans les muscles de souris traitées à la doxorubicine, une diminution de l'expression des ARNm de YAP, corrélée à une diminution de l'expression des ARNm d'un de ces gènes cibles, Ctgf, mais pas d'un autre, Cyr61. La diminution de Ctgf suggère une diminution de l'activité de YAP, ce qui corrobore l'augmentation de l'expression de Trim63 (normalement réprimée par YAP). Cependant, des résultats contradictoires *in vitro* montrent que la doxorubicine augmente l'expression des ARNm de Ctgf **et** Cyr61, pertinent cette fois avec la diminution de la phosphorylation de YAP observée par immunofluorescence. Aussi, la caractérisation de l'expression protéique de YAP en réponse à la doxorubicine est difficile et reste à confirmer. En effet, de futures analyses complémentaires nous permettront d'expliquer ces différences entre nos deux modèles, sans omettre la possibilité que des mécanismes de spécificité cellulaire ou de méchanotransduction soient impliqués.

-Conclusion-

Dans ce deuxième projet il a été confirmé la modulation de l'expression de Trim63 par la famille p53 en réponse à la doxorubicine précédemment montré par des résultats antérieurs au sein du laboratoire. Toutefois, de manière intéressante, il a nouvellement été mis en évidence que les membres de la voie de méchanotransduction Hippo prennent part à cette modulation de Trim63 par une potentielle interaction, qu'elle soit directe avec Trim63 ou indirecte via la famille p53. Globalement, nos données suggèrent que la doxorubicine induit l'atrophie musculaire par suractivation de Trim63 au travers d'une régulation complexe entre l'activation de la voie dépendante de p53 et YAP/TAZ et l'induction de ROS et de céramides. Cependant, diverses questions restent ouvertes, notamment la suggérée interaction entre p73/YAP décrite dans la littérature, ainsi que la possibilité que d'autres facteurs de transcription encore non connus interagissent directement avec le promoteur de Trim63. D'autre part, il reste évidemment à établir clairement le profil d'expression de YAP au sein de ces deux modèles et d'investiguer si des gènes cibles communs à la voie Hippo et la famille p53 peuvent être induits dans le modèle atrophique.

-Conclusion globale-

L'ensemble des travaux réalisés sur ces deux projets nous a permis de mettre en évidence le rôle important que joue la famille p53 dans les effets secondaires induits par les drogues anticancéreuses. Ils nous ont également permis de trouver d'autres relais moléculaires acteurs parfois plus inattendus dans les processus de toxicité liés aux traitements anticancéreux, à la fois sur le système nerveux entérique et sur le muscle. Il reste maintenant à déterminer lesquels seront des cibles thérapeutiques les plus intéressantes pour le développement de molécules protectrices.

INTRODUCTION

I. The controversial role of anti-cancer treatments

Now-a-day, each of us knows a family member, a friend, a colleague or simply a neighbor that is; or has been; concerned by cancer.

Cancer represents one of the major world-wide cause of death whose incidence is continuously increasing. More than 18.1 millions of people were diagnosed for cancer in 2018, with 9.6 millions of death [12]. Among them, in France, more than 382 000 people were affected (204 600 men for 177 400 women) according the Institut National du Cancer (INCa), representing the first cause of premature cause of death. Furthermore, the World Cancer Report estimate for 2030 more than 26.4 millions of cancers with up to 17 millions of associated-death in the all world [13]. All the complexity of this public health problem lies in its symptom's diagnosis, localization, advanced stage or not, but also possible and/or available treatments. Hundreds approved anticancer treatments such as chemotherapies, targeted therapies and radiotherapies exist without mentioning possible combination of them. However, despite their benefits, they possess important disadvantages. In particular, the main issue concerns anticancer therapy-induced side effects.

Among all, the anticancer treatment accused to be one of the worst toxicity inducers is cytotoxic chemotherapy. Cytotoxic chemotherapies are the oldest anticancer treatment and are still used in clinic as first line of treatment for multiple types of cancers. In addition, their use is still evolutive because of their association with modern therapies such as targeted therapies representing today a new tool to personalize anticancer treatments [14]. Their toxicity often lies in their cumulative effects, which, unfortunately, are further increased when combined with other anticancer therapies, including targeted therapies that are supposed to be less toxic for the healthy tissues. In addition, resistances against chemotherapies can be developed notably with platinum-based compounds as cisplatin. Their manifestations appear under different mechanisms called pre-target, on-target and off-target resistances [15]. However, resistances to cytotoxic chemotherapies are less critical than those of other anticancer treatment, that also are less effective than chemotherapies, such as targeted therapies resistances. Indeed, mutation of the single target of a given targeted therapy causes

a complete loss of activity of the therapy [16] and subsequently impair the anticancer treatment efficiency.

Anticancer toxicity has to be highlighted because of its major importance and influence on patient's survival, recovery and quality of life. Since 2009, side effects have been graded according to the Common Terminology Criteria for Adverse Events (CTCAE) [17], on a 1-to-5 scale: 1=mild, 2=moderate, 3 = severe, 4 = life-threatening, 5 = toxicity-related death (National Cancer Institute 2009). They can affect all the body, such as phanera (hair, nails) or vital organs as well as kidney, liver or heart (Figure 1).

Based on the severity of the side effects, doses can be de-escalated up to a stop in the treatment impacting severely the chance of remission for the patient.

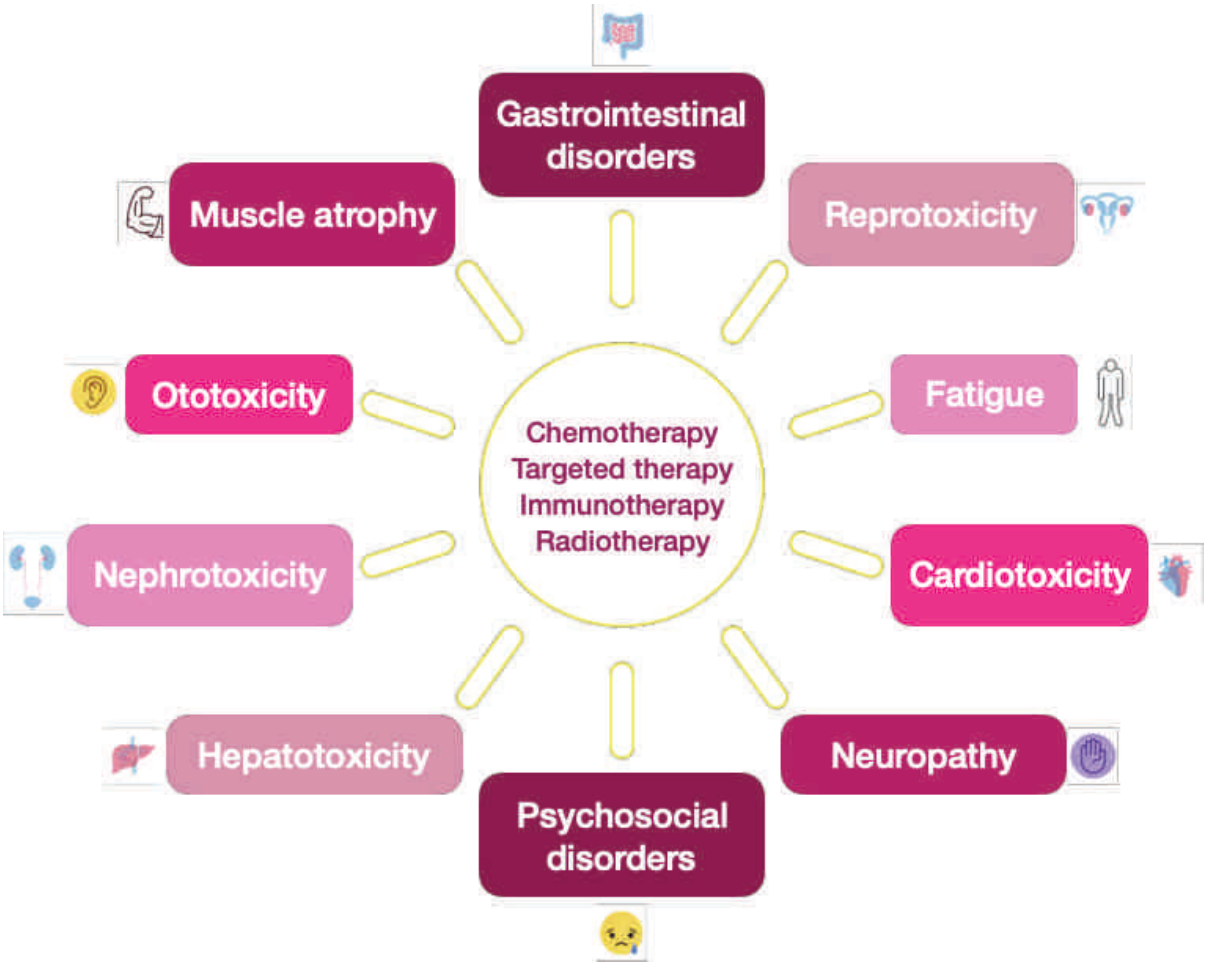

Fig.1 Illustration of the numerous anticancer treatment-induced side effects.

All anticancer treatments do not have the same mode of action and therefore they may display different types of toxicities towards the healthy tissues. The cytotoxic chemotherapy remains the main therapy used because of its high efficacy, the variety of chemotherapeutic agents and its low cost.

Chemotherapeutic agents include several categories, such as **anti-microtubule** molecule which inhibit cell proliferation by acting on microtubules, **antimetabolites** (5-Fluorouracile) impairing DNA synthesis, but also **cytotoxic antibiotics** (doxorubicin) characterized for their interruption of cell division and **alkylating agents** (cisplatin, oxaliplatin) that induce DNA damages and block its repair.

Among antibiotics, we find anthracycline compounds such as **Doxorubicin**, which mainly acts via topoisomerase II inhibition which subsequently induces double stranded DNA breaks, mitochondrial biogenesis defects and ROS production due to oxidative stress [18]. Indeed, topoisomerase enzyme regulates DNA repair, explaining why its inhibition drives to replication and transcription impairments [19].

About cytotoxic agents, **platinum-based compounds** are often considered as alkylating agents due to their mode of action. They are one of the most used chemotherapeutic agents. Platinum compounds, such as cisplatin, oxaliplatin and carboplatin are described to induce DNA damages by their binding into adjacent N-7 position of guanine and form DNA adducts [20]. Normally, DNA damages are recognized by specific protein involved in the Nucleotid Excision Repair process (NER) able to detect defective bases. Then helicases enzymes intervene to open the DNA helix and concomitantly with endonuclease recruitment, to remove nucleotides and synthetize neo-strand [21][22]. **However, platinum salts interfere with this DNA repair process and drives to cell death through the activation of the p53 signaling pathway, notably with activation of caspases cleavage and pro-apoptotic target genes expression.**

A. Nephrotoxicity

Kidneys are in the top of the organs impacted by anticancer drug administration and nephrotoxicity is one reason of treatment discontinuation [23]. Antibiotics used as anticancer treatments, such as gentamicin or amphotericin, have been reported to alter kidneys. However, among all therapies, cisplatin is the anticancer agent the most described to induce nephrotoxicity, especially when associated with radiotherapy, due its dose-limiting effect [24].

Kidneys accumulate cisplatin to a higher proportion than other organs, because of their filtration function. Up to 90% of cisplatin-treated patients are affected by acute renal failure [23]. Furthermore, nephrotoxicity risk is totally proportional to cisplatin-doses and chemotherapy cycles but can also simply occur even after a single exposure. It has been demonstrated that within the first hour of administration, kidneys rapidly excreted cisplatin through an important glomerular filtration process. No cisplatin reabsorption by tubules for urinary elimination has been reported neither, suggesting an important accumulation into kidneys [24]. Nephrotoxicity is detected by serum analysis when kidney serum components such as urea, and creatinine are observed as significantly increased, in correlation with a decrease of creatinine clearance level [23][25].

At the cellular level, renal failure is described to be caused by reactive stress characterized by lipid peroxidation and reactive oxygen metabolite production (ROM) in kidney tissue leading to distal nephron apoptosis or necrosis, depending on the cisplatin dose [24]. Platinum-based compounds are known to induce an activation of the p53 signaling pathway which in turn provoke cell death response. Interestingly, it was shown that cisplatin-associated ROS production induced early activation of p53 and its apoptotic target genes Bax (Bcl-2–associated X protein) and Puma (p53 upregulated modulator of apoptosis), but also activated caspases expression, altogether responsible of nephron apoptosis [26].

B. Hepatotoxicity

Hepatotoxicity is also directly linked to cisplatin but also to oxaliplatin use and impacts up to 50% of treated patients [23]. Conversely to nephrotoxicity, a variety of anticancer drugs are toxic for liver. It has been listed that cytotoxic (platin) and alkylating agents (cyclophosphamide), as well as, antimetabolites (5-fluorouracil), anti-tumor antibiotics (doxorubicin) and hormone therapy cause liver alterations [27]. Because of liver plays a key

role in drug metabolization, its functioning is importantly impaired. Indeed, several liver damages have been reported in the literature, notably necrosis mediated by platinum-based compounds use, but also many other such as sinusoidal obstructive syndrome, pseudocirrhosis, portal vein thrombosis and steatosis [27]. These alterations induce abdominal pain and swelling as principal symptoms [23], which are concomitant with an important serum increase of liver specific proteins release such as LDH (Lactate dehydrogenase), bilirubin and albumin but also an elevation of enzymes as transaminases [25].

At the molecular level, this elevation of liver enzymes is associated to increase of the level of malondialdehyde and glutathione in response to cisplatin suggesting oxidative stress response. However, the pathway involved in response to anticancer treatment hepatotoxicity are not completely understood and discussed. Indeed, it has been reported that the p53 signaling pathway would **contribute** to liver injury [28] **but could also attenuate** chemotherapy-induced hepatotoxicity [29].

C. Muscle atrophy

Muscles are another body component impacted by antitumor treatments. The most described compounds accused to induce this toxicity are antibiotics ones, more precisely doxorubicin which has been documented to provoke muscle atrophy when combined with cyclophosphamide and 5-fluorouracil (5-FU) [30]. Muscle atrophy is often related to the cardiac tissue and is even stronger with platinum-based compounds that increase muscle protein degradation, even worst when combined Dexamethasone; dexamethasone which is prescribed to counteract emetic effect of cisplatin molecule [32][33]. This part will be further described in a specific chapter bellow.

D. Cardiotoxicity

Indeed, more than its muscle mass loss, cardiac tissue is a vulnerable organ susceptible to be impacted by other anticancer side effects. Cancer-mediated cardiotoxicity has various form such as hypertension, ventricular failure or coronary artery disease [33], and appears almost in every case of anticancer treatment. For example, chemotherapy, radiotherapy and

targeted therapy can be at the origin of cardiotoxicity [34]. More precisely, it has been proved that anthracyclines drugs such as doxorubicin, used since the sixties, are the main inducer of cardiac failure, notably through their atrophic effect described before. Their use is able to provoke acute toxicity; reversible in a week; but more importantly, chronic toxicity occurring within the first year or up to 20 years after treatment arrest. Studies have warned the medical core about the fact that up to 65% of patients display cardiac issue 6 years after anthracyclines arrest [36][37].

Associated interindividual factors such as female sex, radiotherapeutic antecedent or age, can increase the risk of developing cardiac problems. In this sense, antimetabolites substances (5-Fluorouracil, capecitabin) represent the second cause of cardiotoxicity chemotherapeutically induced, where in 8% of cases, thoracic symptoms appear within the first 72 hours [34]. Platin salts such as cisplatin, oxaliplatin or carboplatin, have also been documented to increase the risk of late cardiac deficiency since ischemic cardiotoxicity or stroke have been noticed, explaining why patients must be placed under prolonged surveillance through bidimensional cardiac echography. In addition, monoclonal antibodies used in targeted therapy such as the trastuzumab is known to decrease cardiomyocytes contractility, fortunately, not by inducing cardiac cells apoptosis, meaning that this is a reversible cytotoxic effect.

On the contrary to platinum-based compounds, Doxorubicin drives cardiomyocytes to apoptosis. Indeed, anthracycline molecules act by inhibiting the DNA replication, thus creating DNA damages and ROS responsible of cardiac cell injury. It has been demonstrated in rats that the accumulation of doxorubicin-induced ROS was responsible of mitochondrial biogenesis defects associated with an activation of the apoptotic signaling pathway notably through the activation of p53 target genes as Bax, Mdm2 or Apaf-1 [19][38][39]. Furthermore, increase caspase-3 and PARP expression have been observed during cardiomyopathies, responsible of apoptosis [39]. However, p53 is not the only actor of cardiotoxicity and is linked to the ERK pathway in order to inhibit cell growth and provoke cardiac cell death, in association with the activation of NF-kB dependent-PUMA pathway [40]. In this sense, ERK and p53 pathway could represent interesting target for therapeutic approaches [41].

E. Gastrointestinal disorders

Associated to platinum-based compounds, digestive tract side effects emerged because of an attack of gastrointestinal cells due to the hyperproliferative property of the intestinal epithelium. Through their important cell cycle process, epithelial cells integrate more platin-related DNA (Desoxy ribonucleic acid) damages and subsequently are dying.

These cellular alterations lead to different symptoms such as mucus composition changes [42], constipation which is a major source of morbidity and distress, diarrhea, but nausea and vomiting. Indeed, cisplatin is considered as a high-risk emetic drug, where more than 90% of treated patients that experience dyspepsia, nausea and vomiting in opposition to carboplatin or oxaliplatin whose emetic rate is lower (between 30 and 90%) [23]. However, these symptoms are also found with monoclonal antibodies use, such as ipilimumab with or without nivolumab association [43].

F. Ototoxicity

Other organs, especially sensory organs such ears are targeted by anticancer treatment administration, especially cisplatin. Ototoxicity is defined as hearing loss in up to 30% of cisplatin-treated patients [44] while oxaliplatin-related ototoxicity remains rare. It impacts the two ears by cisplatin accumulation within the cochlea, leading to reactive oxygen species (ROS) production and driving to cell apoptosis. Indeed, ROS production promotes the expression of STAT1 (signal transducer and activator of transcription 1), which when associated with activated p53 and caspases-1 and -3 expressions, is responsible of inflammation and apoptosis of cochlear cells. This ototoxicity appears through ears pain manifestations or otalgia, tinnitus (noises or ringing in the ears), and vestibular alterations (balance problems) [23] and can really be unpleasant, particularly difficult for children victims of brain tumors [45].

G. Reprotoxicity

Reprotoxicity of antitumor drugs has to be highlighted as an important side effect, especially in women patients. Indeed, their ability to give birth to a baby is importantly impaired, notably by chemotherapeutic agents such as cisplatin and doxorubicin. However, the underlying mechanisms of cancer-associated fertility are not described because of their dependence of the chemotherapeutic drug used [46]. However, it is described that, due to their deleterious effects on proliferating cells (i.e ovarian cells for example), cisplatin and doxorubicin affect follicles and ovarian stroma driving to direct oocytes alterations [47]. It is explained by a cascade of events such as impairment of ovarian vessels preventing correct angiogenesis and vascular growth. In this context, pre-antral follicles pool is depleted driving to an absence of proliferation and hormone production. In another case, blood vessels can be obstructed and provoke ischemia and ovarian cortex destruction with a loss of primordial follicles. Unfortunately, fertility issues are not only characteristic of cytotoxic agents, but also of antibiotics drugs. Doxorubicin has been demonstrated to specifically affect granulosa cells while cisplatin mostly targets oocytes.

Next to fecundation issues, fetus implantation and placentation are also defective after radiotherapeutic treatment. It has been demonstrated that pelvis radiations, in addition to impairing ovarian reserve, damage endometrial tissue, affecting the nidation and thus driving to pregnancy complications [47]. More importantly, delayed side effects have also been described such as an increased risk of premature menopause five years after treatments, proportionally correlated with patient ages [33]. At the molecular level, these alterations are explained, in part, by the involvement of the p53 family, which is well known to be an important actor in male spermatogenesis and female germ cell survival [48]. Indeed, p53 associated with LIF is essential for implantation and eggs fertilization, as well as TAp73 and TAp63 (its homologs) are crucial for the conservation of follicle pool, ovulation process and quality of oocytes. **Unfortunately, due to p53 mutations associated with cancers or anticancer-treatment that induce cellular stress, which in turn activate p53-dependent apoptosis, the p53 status is defective and participate to the reprotoxicity and fertility of cancer patients [49].**

H. Fatigue & Psychosocial effects

Firstly, fatigue is a common system side effect from whose depend many other symptoms such as loss of appetite or headache for example [43]. Interestingly, cancer-related fatigue has been linked to "distressing, persistent, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning" [33], playing a major role in the decrease of patient quality of life.

Excepted the fatigue, it was pointed-out the proper cancer and anticancer treatment influence on human psychological aspects. Indeed, an important and interesting point was highlighted by the study of Ahmad and colleagues that pointed-out the distress of women patients after the loss of their fertile capacities. Unfortunately, cancer and anticancer treatment effects on the psychological dimension are rarely evoked, however, it represents a necessary element to take into consideration.

Despite the difficulty to distinguish which psychological effects are related to sequelae of cancer from those linked to therapy side effects, it appears essential to consider physical and psychological changes. Indeed, an interesting study: AYA HOPE, has demonstrated that of 100 cancer patients, 61 of them get a negative body image because of their disease and anticancer treatment [50].

In addition, self-esteem problems and social skills acquisition was described as altered capacities, more importantly in teenagers and young adults (TYA). Indeed, TYA are victims of stress, anxiety and depression symptoms, that have been clearly linked with an alteration of life goals, relationship and self-esteem. Radiotherapy used for kidney, neuroblastoma or bone tumors has been particularly linked to psychosocial disorders and neurocognitive deficits suggesting a potential alteration of central nervous neurons. In addition, chemotherapeutic drug as anthracyclines or alkylating agents are also reported to significantly induce more psychological distress [51]. More interestingly, an Australian work has shown that almost 50% of the 15-25 years old cancer survivors were diagnosed for post-traumatic stress disorder [52]

as well as 42% of their parents, warning about the necessity to understand and manage psychological and psychosocial cancer-related issues.

I. Neuropathy

Psychosocial effects can also be related to neuropathy which is an unpleasant and irritant side effect impacting the peripheral nervous system (PNS) and reported to importantly impair patient's quality of life because of hyper-sensibilities. Peripheral nervous system toxicity or neuropathy is the main limiting dose-effect and therapy discontinuation of platinum-based compounds, more precisely oxaliplatin which induces two types of sensory neuropathies: acute peripheral one, appearing during drug administration or in an early time point, and chronic neuropathy caused by cumulative drug dose [45]. From 60 to 95% of oxaliplatin treated patients experienced neuropathic effects, and about 20% of them are graded between 3 and 4, principally altering hands and feet sensibility, also as perioral and pharyngo-laryngeal areas through paresthesia manifestations [23].

Although less neurotoxic, cisplatin and carboplatin are also known to cause PNS issues. Cisplatin induces neurotoxicity through peripheral neurons death in almost 50% of receiving patients while carboplatin is associated with an increased risk of induced toxicity when used at a high dosage or in combination [45].

Numerous studies have highlighted the role of platinum salts in neuropathy. **This severe side effect is explained by the fact that peripheral nervous cells does not possess an efficient system to remove and repair DNA damages caused by platins [22].** Subsequently it causes DNA alterations and oxidative stress. Indeed, cisplatin and oxaliplatin particularly, induced neuropathy through various cellular damages, more importantly mitochondrial dysfunction in dorsal root ganglia neurons [53] where it has been showed that cisplatin accumulates p53 after mitochondria DNA damages leading to apoptosis. Platinum compounds also affect receptor and ion channels of neurons impairing signaling and neurotransmission and leading to DNA damages and degeneration of axons [21]. Oxaliplatin for example has been described to alter NA+ channel function of sensory neurons that provoke their hyperexcitability explaining why patients display ectopic discharges [53]. Furthermore, it was proved that

platinum drugs also impact microglial and astrocyte cell populations, participating to the establishment of neurotoxicity. Interestingly, oxaliplatin-associated neuropathy could be detected as early as the treatment begins, thus allowing to spot patients with a higher risk to develop moderate or severe neuropathy. Even if the precise molecular pathway responsible of cisplatin-induced neuropathy, it has been clearly suggested that p53 is mainly involved in the associated alterations, and that its chemical inhibitor: pifithrin, prevent cisplatin-mediated allodynia [54][55].

J. Supportive care

You now have a global overview of major side effects of anticancer treatment showing the need to counteract their happening in order to improve patient's quality of life, survival and recovery. Some side effects tend to improve in response to treatment arrest, as for oxaliplatin administration due to its cumulative effects [34]. However, when it is not as simple as that, strategies are developed to prevent or resolve them, such as hydration which is the supportive care protocol used to reduce nephrotoxicity [44]. Other solutions consist to interrupt permanently or totally the treatment, but also to implement precautions about the use of nephrotoxic medicines (ESMO: European society for medical oncology).

Therapeutic approaches to solve neuropathic problems remain rare too. Indeed, neuroprotective agents as thiol compounds or vitamin E are used to counteract neuropathy [45]. Ketamine was previously used as opioid adjuvant, however without reporting qualitative effects. Hence, non-opioid and opioid analgesics treatment are now combined with tricyclic antidepressant or anticonvulsant to improve their effects. In case of nerve compression, it has also been reported that steroid use is efficient (ESMO).

In some cases, such as for cardiotoxicity, many strategies are thought and tested to resolve anticancer side effects and prevent its related-death risk. Carvedilol for example, is able to prevent doxorubicin-related toxicity through its antioxidant property (ESMO), such as angiotensin receptor blocker names (ARB) and Dexrazoxane, an iron-chelating agent which reduces anthracycline-related side effects [56]. Angiotensin-converting enzyme inhibitors and beta-blocking agents use have also showed highly efficient results in patients. In addition, in

the case of immunotherapy-related cardiotoxicity, patients are placed under (methyl)prednisone in order to decrease myocarditis (ESMO), treatment which is also advocated for hepatitis linked to the immune checkpoint inhibitor use.

Other approaches are uncommon and delicate, as for example in the case of reprotoxicity. In order to act for the fertility preservation, it is suggested to women, before their cancer treatment to cryopreserve their embryo or oocytes. In addition, chemotherapy and radiotherapy-induced sterility can be prevented through harvesting and freezing their ovaries, which has been reported to be successful, notably with the 28 pregnancies that happened since [57].

The necessity to consider anticancer treatment effects on psychosocial dimension and also to help patient and their family was pointed-out before. This is what is highlighted in the study of Koll and colleagues [58] that reported that services such as caregiver or financial help for example, are key components of supportive care therapy, more precisely for elderly patients [59]. Emotional and social help is also considered as essential to improve the well-being and quality of life of cancer patients, who also represent a supportive care to each other in every social situation [60].

Gastrointestinal disorders are mainly reported in response to anticancer-treatment and occur under various symptoms. In this respect, to counteract them, numerous therapeutic strategies are established. For example, the clinical guidelines on diagnosis of EMSO in 2018, have considered that constipation was a non-recognized and poorly treated problem. Supportive care approaches consist to use non-pharmacological strategy through an increase of physical activity, when possible, and increase of fluid intake. When no efficiency is observed, the use of laxatives and suppositories is recommended in addition to a dietetic support (ESMO). Diarrhea is also commonly reported after anticancer administration and is managed according its complexity. When not severe, diarrhea is managed by hydration and dietary modifications, more precisely through an increase of caloric and fluid intake. On the contrary, when diarrhea is considered as complicated (i.e associated with fever and vomiting), its therapeutic approach is the use of opioid such as loperamide, somatostatin analog, steroids and/or antibiotics.

About vomiting, a combination of 3 drugs is required: 5-HT3 (5-hydroxytryptamin)-receptor antagonist, dexamethasone and aprepitant and administered in prevention of chemotherapy treatment. Indeed, the food drug administration has approved the use of two new neurokinin receptor antagonists: netupitant and rolapitant for their positive effects showing a complete response rate (no vomiting and no rescue treatment) in 2247 cancer patients submitted to cisplatin-based chemotherapy [61].

However, the effect of aprepitant, once administered, can be different across the time, and depending of the chemotherapeutic agents used. Also, its "miraculous" effect is not reported by all studies, suggesting that patients population, interaction with chemotherapeutic agents and metabolization of aprepitant has to be considered [62].

However, muscle atrophy remains today an important side effect without therapeutic solutions even if a recent study showed that Fucoidan administration has an interesting potential against cisplatin or gemcitabin-associated muscle atrophy by suppressing NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) related inflammation and myostatin synthesis [63]. Indeed, Fucoidan is composed of sulfated hetereopolysaccharide group, conferring numerous biological activities, such as anti-inflammatory or anti-coagulant function by affecting neoplasic and antineoplasic cells. More interestingly, Fucoidan also possesses anti-cancer activity where its efficiency against many cancers has been reported, notably in prostate and lung cancer [64]. Its activity depends on its molecular weight, for example, more it is lower, better the anticancer activity is. Furthermore, it has been noticed that fucoidan would have synergistic effect with cytotoxic drug, such as cisplatin in breast cancer cells. However, the way Fucoidan counteracts muscle atrophy remain unknown.

Unfortunately, it happens sometimes that no effective strategy exists, as it is the case for ototoxicity or chronic constipation, highlighting the need to produce remedy against all these unwanted and toxic side effects. To reach this goal, we have, as researchers, to study, to find and to understand the complex underlying mechanisms behind each induced toxicity to hope to develop therapeutic tools.

This challenge represents the main aspect of my thesis project which will be discussed in future pages.

Among all anticancer treatment-induced toxicities, chemotherapies are those that generate the most important and acute ones, in particular platinum compounds such as cisplatin and oxaliplatin that especially provoke gastrointestinal issues. Their effect on the gastrointestinal tract has been mostly studied and is reported to impact the proper nervous component of the digestive tract: the enteric nervous system (ENS). Indeed, enteric nervous system alterations are one of the most frequent, important and long-term existing effects associated to chemotherapies. Surprisingly, taking into consideration the importance of this side effect and all described strategies to tend to counteract it, intestinal toxicity still remains an unsolved major patient health problem. Indeed, the precise involved mechanisms are not known, for example, the way that ENS is impacted by platinum-based compounds is now-a-days absolutely not understood and represents to us an essential point to investigate.

II. The enteric nervous system: our intestinal brain

The enteric nervous system (ENS) is the most complex division of the peripheral nervous system (PNS) proper to the gastrointestinal tract (GIT). The ENS is the only part of the PNS able to function independently of the central nervous system (CNS) innervation (Figure 2).

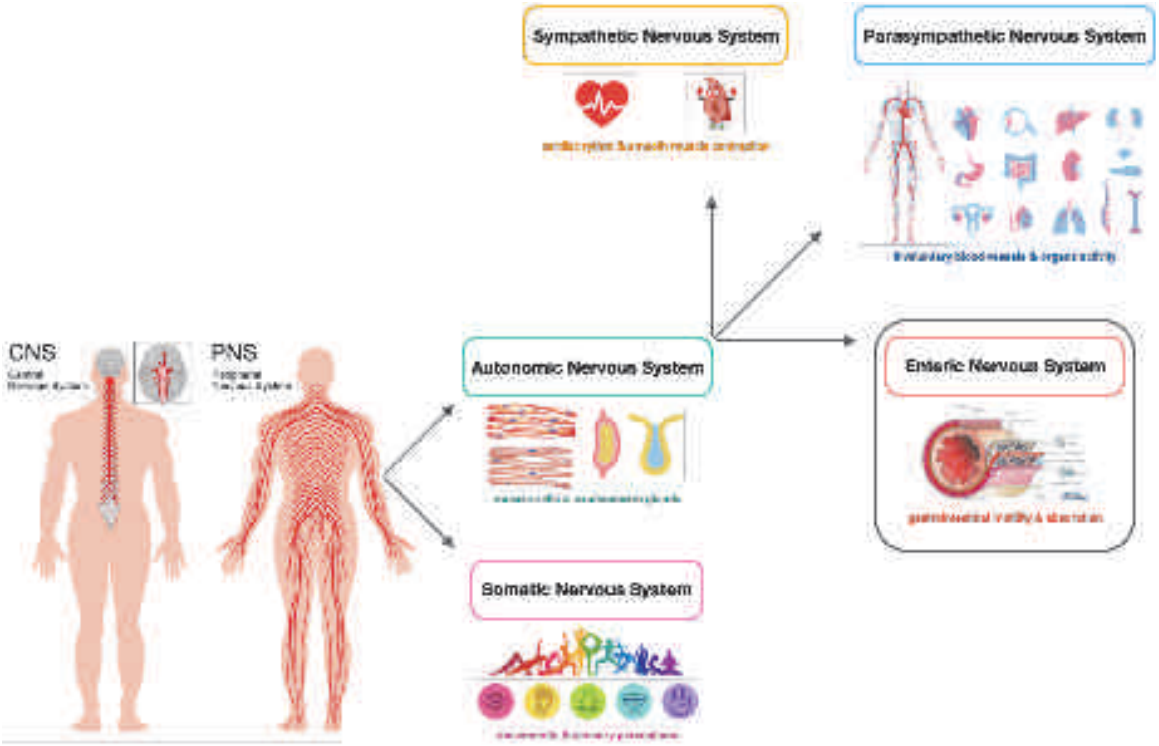

Fig.2 Body central nervous system and peripheral nervous system divisions.
Central nervous system comprises brain and spinal cord nerves.
Peripheral nervous system is divided into somatic and autonomic nervous systems itself giving sympathetic and parasympathetic nervous systems but also enteric nervous system.

This is a sensory and endocrine organ composed of enteric neurons (EN) and enteric glial cells (EGCs) that provide support to neurons but also regulate synaptic transmission and maintenance of the epithelio-intestinal barrier.

EN and EGCs are encompassed within ganglionic structures in the gut wall according two main plexuses: the Auerbach/myenteric plexus at the surface of the gut between the longitudinal and circular muscle layers and the Meissner/submucosal plexus, deeper, between the circular muscle layer and the mucosa (Figure 3).

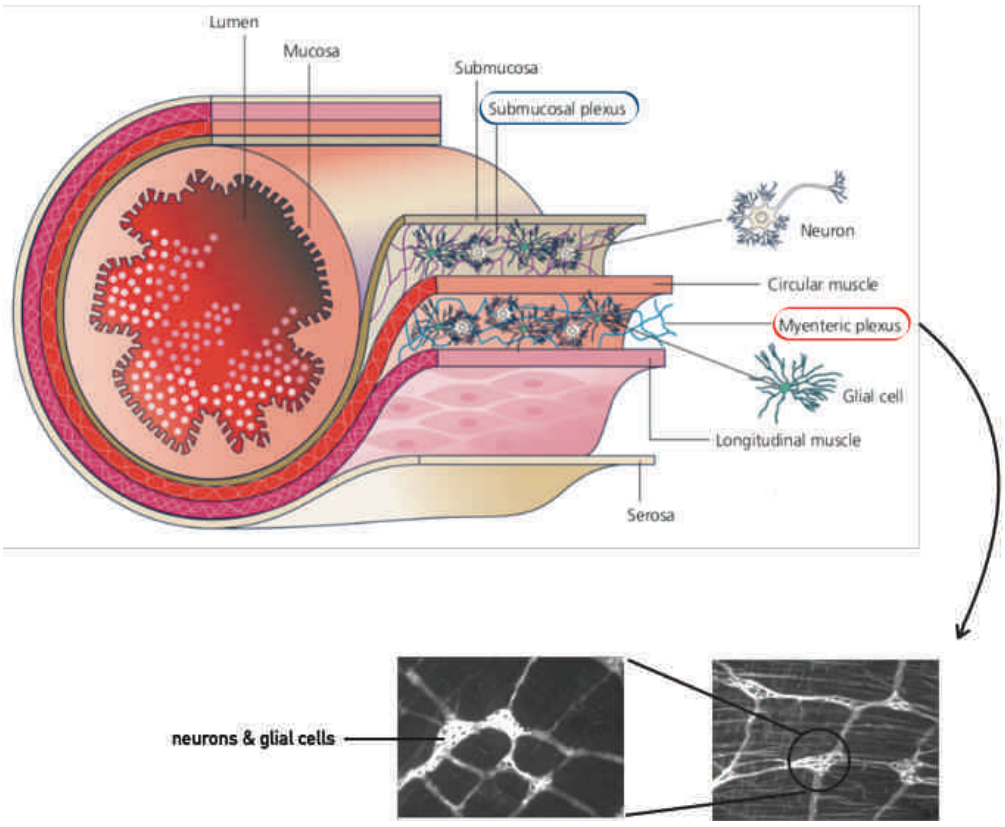

Fig.3 Schematic representation of the Enteric Nervous System structure within the gastrointestinal tract with its two plexuses: Submucosal or Auerbach and Myenteric or Meissner plexus.

Auerbach plexus is located between the submucosa and the circular muscle layer and control fluid absorption and secretion while Meissner plexus between circular and longitudinal muscle layers controls contractile property of the gut. Neurons and glia are encompassed into ganglia nodes and give to ENS its network architecture.

Adapted from Grubisic et al., 2017.

Each plexus owns a specific function. The myenteric plexus controls the gastrointestinal motility whereas the submucosal one is involved in the fluid absorption and secretion such as electrolytes and water [65]. Basically, the enteric nervous system is now-a-days also known as our second brain due to its amazing complexity, function and involvement in our body homeostasis and well-being.

A. ENS Development

The ENS development takes its origin into the neural tube, more precisely the enteric neural crest-derived cells (ENCCs). It is orchestrated according specific signaling pathway staging. Indeed, glial-derived neurotrophic factor (GDNF) and its receptor, *Ret*, are the most critical players in this process. Ret plays an essential role in the survival, proliferation, migration and differentiation of ENCCs [66]. Other factors are essential for Ret expression, such as sonic hedgehog (Shh) and its receptor (Ptc); Sox 10; Phox2b, Mash-1 [67]. In addition, the mesenchyme influence ENS development by secreting additional factors such as endothelin 3 (EDN3) which interacts with its receptors (EDNRB) from ENCCs.

All EN and EGCs are derived from the neuronal crest cells (Figure 4). During development and under GDNF/Ret signalization, neural crest cells, at the vagal and sacral levels, come off the neural tube at embryonic day 8.5 in mice (week 3 in human), invade the gut wall, become enteric neural crest-derived cells (ENCCs). They undergo their migration along the gut wall at day 9.5 (week 3-4 in human), progressively integrating into the entire length of the gut. At the same time, many cytoskeletal filament rearrangements occur such as nestin and peripherin, useful to detect neural stem cells, positive for nestin marker and developing neurons through peripherin expression [68].

During their migration process, some ENCCs initiate their differentiation into neurons and glial cells, detectable as early as embryonic day 10.5 to 11.5 in mice (weeks 4-6 in human) by expressing Sox10 in neuron progenitors and HuC/D in immature neurons. Progenitors form first the myenteric plexus at day 13.5 (week 7 in human). Then, they establish the submucosal plexus through a process called the “radial migration” at day 14.5. When migration ends, ENCCs have given birth to Glial fibrillary acid protein (GFAP)-immunoreactive EGCs also S100B positive [69] and differentiated neurons expressing, among many other neurotransmitters, the pan-neuronal marker: PGP9.5 (protein gene product 9.5).

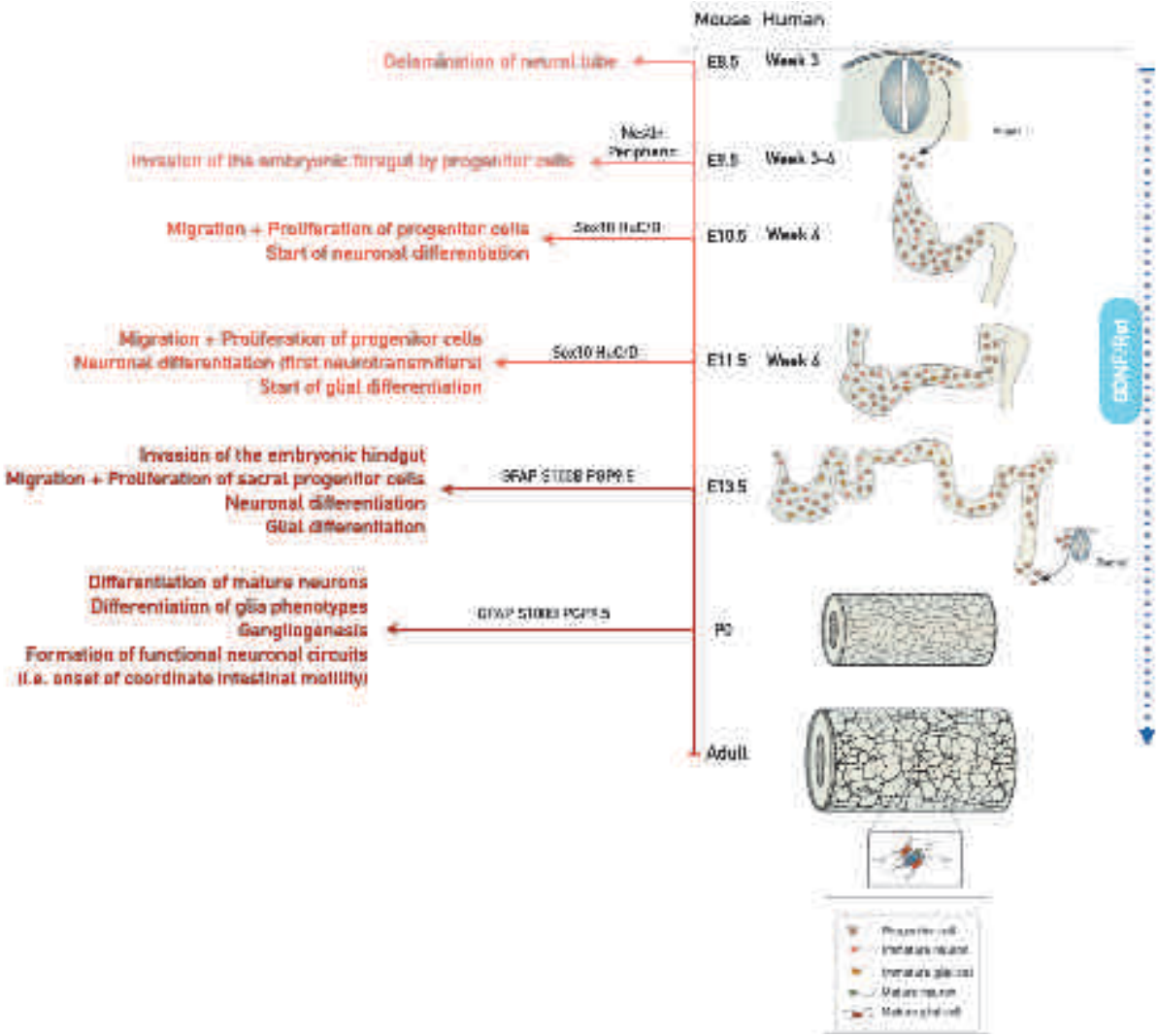

Fig.4 Schematic representation of the Enteric Nervous System development

B. ENS neuron subtypes

When completely developed, ENS counts no less than 16 subtypes neurons classes. Enteric neurons are classified according their morphology and their electrophysiologic properties (Figure 5). Indeed, it has been proposed that enteric neurons with dendrites but only a unique axon will be called Dogiel I neurons. This class of neurons possesses a high excitability potential and generally is composed by inhibitory and excitatory motoneurons. In comparison, the Dogiel II group owns neurons with numerous axons characterized by a strong hyperpolarization potential.

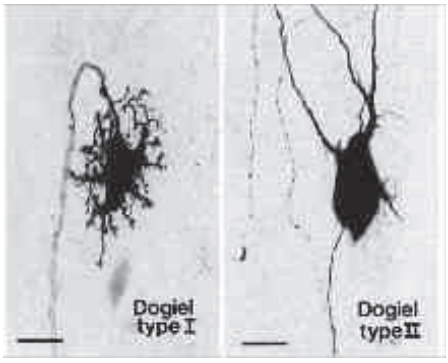

Fig.5 Dogiel I and II enteric neurons subtypes
Clerc et al., 1997

EN are also subtyped according to the neurotransmitters or specific proteins they expressed, which are characteristic of their origin within the ENS (Figure 6). For example, neurons expressing the 5-hydroxytryptamin (5-HT) are the first appearing during embryogenesis while those expressing the calcitonin gene related peptide (CGRP) are generated during the post-natal period. Some of them are shared with the central nervous system such as neurons expressing either CGRP (Calcitonin gene-related peptide), neuropeptide Y, peptide YY, while other are specific to mature enteric neurons such the co-expression of NOS (nitric oxide synthase) and VIP (Vasointestinal peptide) [69].

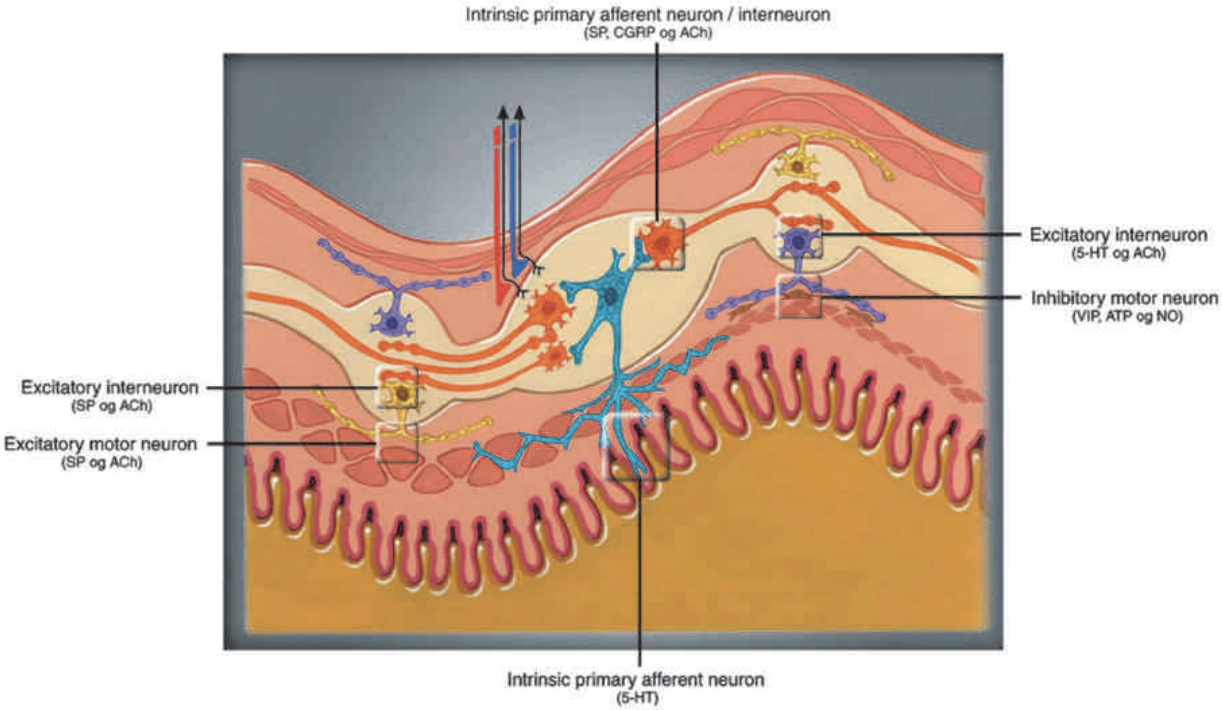

Fig.6 Enteric neurons subtypes according their connections
Hansen 2003

C. Neurotransmitters & ENS functions

Neurotransmitters or specific proteins allow to classify EN, however, they are also interesting by the functions they possess within the ENS. For example, the synaptobrevin which belongs to the vesicle-associated membrane protein-2 is responsible of the synaptic transmission while nitric oxide synthase enzyme (NOS) acts for intestine relaxation by producing nitric oxide (NO).

The control of gut motility by the ENS lies in the secretion of 5-HT provoking peristaltic reflex through the direct stimulation of intestinal primary afferent neurons. This excitability induces the release of acetylcholine (Ach) and substance P, responsible of smooth muscle contractions. On the contrary, gut peristaltis is inhibited by the secretion of NO and VIP that relax the descending proportion of the gut and allow the luminal gut content propagation [70][71]. In a short way, Ach and substance P increase gut permeability when VIP reduces it. Despite they have opposite effects, when released, these three neurotransmitters, as well as 5-HT, play a role in epithelial cell proliferation, even if VIP antiproliferative effects has also been reported [71].

D. Enteric glia and neurons plasticity

Neuronal plasticity has been defined as every change both within neuronal structures and functions in response to stimulus [72]. Since years it is known that the central nervous system (CNS) possess plastic properties characterizing the ability of high vertebrates to adapt to environment changes when no regeneration exists. Indeed, specific CNS regions, as hippocampus and olfactive bulb area, neurons are still produced during adulthood.

Interestingly, it seems that equal process happens within the ENS. Indeed, the ENS is an extremely plastic organ that undergoes changes throughout the life, via the highly dynamic property of the gastrointestinal tract.

As neurons, enteric glial cells represent a heterogeneous cell lineage possessing plasticity properties. It has been demonstrated by numerous studies that EGCs are able to adapt their phenotype in response to ‘reactive’ stimulus, and more interestingly, that they not only suffer of their phenotype change, but also participate in the inflammatory response by pro-inflammatory cytokines release [73]. Another interesting study also demonstrated the

capacity of EGCs to revert into enteric neural crest cell-derived glial populations. It was confirmed by lineage tracing showing that in response to ENS damages, EGCs generate by their own new enteric neurons [73].

However, not only EGCs own plastic characteristics. As mentioned before, some enteric neurons, more precisely the CGRP positives, appear during post-natal phases suggesting that neurogenesis continues throughout later developmental time periods and persists even after the postnatal period. This process was particularly described during lifespan, where the gastrointestinal tract is, one of all body components, the most submitted to size changes. It was found that its circumference increase would be caused by mucosa thickness evolution, as well as those of muscle layers. As the circumference, gastrointestinal length also undergoes changes by modification of the different gut layers, thought to impact on enteric plexuses organization [74]. Indeed, some EN subtypes, more precisely intrinsic sensory neurons and long-axonned interneurons are involved during this process to maintain contact with their targets.

This maintenance of neurogenesis at later stage of life allows plasticity and repair potential that provides interesting properties to use the ENS as source of neuronal stem cells more precisely in response to pathologic context or damages. In this sense, ENS plasticity was highlighted after intestinal surgical procedures where it was observed that EN played a key role during regeneration of neuronal circuits and permitted to restore the correct intestinal functioning [72]. To go further, they proved that ENS, and more precisely intrinsic neurons, were able to adapt to extrinsic denervation and to restore digestive functions. In addition, ENS plasticity is also mentioned during intestinal inflammation where EN excitability and synaptic transmission were reported to be modified in animal models [75]. In addition, EGCs showed their trans-differentiation capacity within animal models myenteric plexus area bordering injury sites, suggesting that injury-induced inflammation might play a role in their trans-differentiation [76].

Unfortunately, despite a common development between different species (mouse, chicken, human and zebrafish) and a considerable plasticity, ENS is not an invincible organ and is vulnerable to the decrease of certain genes expression leading to important gut pathologies.

E. ENS-associated pathologies

By its essential function in the nutrient absorption via the control of gastrointestinal (GI) motor and secretory activity, deregulations of ENS functions have been linked to severe human pathologies without clearly demonstrating whether it involves the ENS and whether deregulations of the ENS are causative. However, there are few examples, such as the following description in which neuronal losses seems to play a major role.

1. Congenital diseases

Down syndrome [77], Hirschsprung's [78] and Chagas's diseases [65] and other syndromes with GI functional abnormalities (i.e. esophageal achalasia, gastroparesis) have been linked with alterations in the ENS. These pathologies can be linked to genetic abnormalities (i.e. chromosome 21 defect for Down syndrome) or to a direct interaction of pathological parameters with the ENS (IBS, diabetes), but also be associated to alteration within the most important pathway for ENS establishment: the GDNF/Ret signaling. In particular, Hirschsprung's disease display mutations of GDNF/Ret leading to pathologic phenotypes characterized by a megacolon phenotype located in the upper part where ENS is missing (Figure 7).

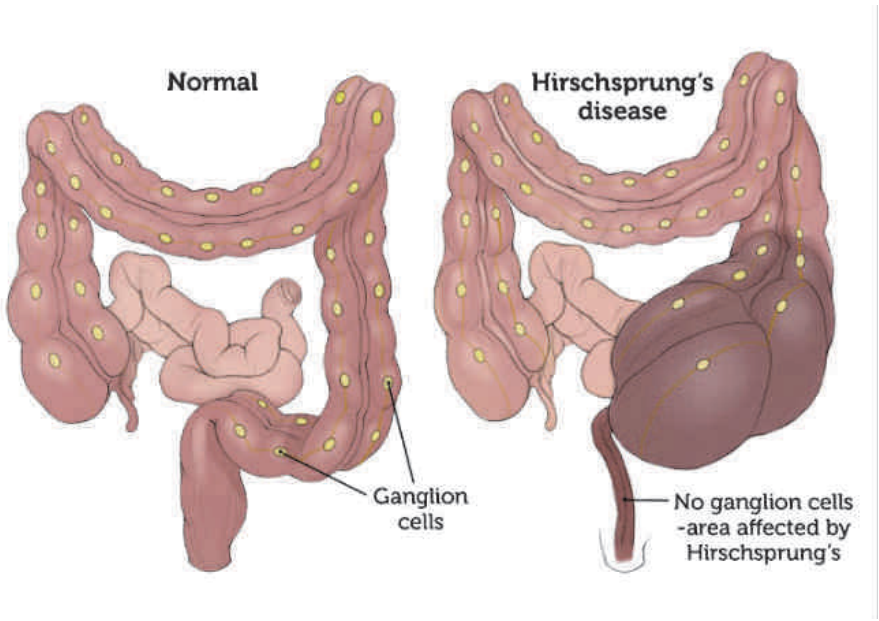

Fig.7 Illustration of normal and Hirschprung's disease intestine
https://www.childrenhospital.org

This absence of ENS is represented by an aganglionosis in the terminal part of the colon due to defects of the proliferation, migration and survival of ENCCs progenitors, however that does not result in neuron number reduction [67]. These dysfunctions cause tonic contractions and obstructions accompanied by an absence of peristaltis. Mutation in Ret account for approximately 50% of familial Hirschsprung cases and mice with null mutations in either Ret or GDNF have complete intestinal aganglionosis. Unfortunately, the low penetrance of the Ret/GDNF mutations suggests that other genes are involved, and the predisposing gene mutations causative of several GI abnormalities remains to be identified. Later, Hirschsprung's disease can degenerate into Chagas' disease because of bacterial infection and deficiency into NOS transmission [65].

2. ENS inflammation

ENS-related pathologies can also be due to inflammation that is the cause and the consequence of alteration within the gut wall. Indeed, defects in EN density increase the severity of gut inflammation. Moreover, ENS is the source of inflammation regulation through its secretions of neurotransmitters. Some enteric markers such as VIP or GDNF are reported to counteract inflammation. Indeed, they act by inhibiting pro-inflammatory cytokines (IL-12, IL-6, INFγ) and stimulating anti-inflammatory ones (VIP), in addition to decreasing gut permeability and promoting neuronal survival [79][80]. Inversely, when substance P, 5-HT and neuropeptide Y are released by EN, inflammation is potentialized through pro-inflammatory cytokine activation and permeability increase.

Macrophages are important regulators of inflammation through their chemotactic property that drives them on the inflammation site in order to eat cell debris. Interestingly, the ENS seems to be involved in their development (homeostasis, polarization), but inversely, EN activity is also regulated by muscularis macrophages that interact with them. Indeed, muscularis macrophage–ENS communication depend on gut microbiota and allows ENS-immune system interaction improvement [81]. To go further, a recent study showed that ageing induces macrophages phenotype changes, from non-inflammatory to pro-inflammatory macrophages lineage. This shift of macrophages polarization is associated to an

important increase of cytokine and immune cells within the ENS driving to EN apoptosis and loss that subsequently provoke intestinal disorders [82].

In addition, EGCs are also involved during intestinal inflammation due to their similarities to macrophages in response to inflammatory stimulus. Indeed, in response to vagal nerve stimulation, which is the only connection between the CNS and the ENS, EGCs play an anti-inflammatory and neuroprotective role [83].

3. *Neurodegenerative diseases*

In addition to these pathologies impacting predominantly the GI function, ENS deregulations have been early observed in neurodegenerative diseases such as Parkinson and Alzheimer's diseases (AD), sharing common characteristics, notably the accumulation of unfolded proteins/aggregates.

Parkinson disease (PD) or Lewy body disease is defined as a brain pathology characterized by inclusion of a-synuclein proteins provoking Lewy bodies appearing in neurons. Although considered as specific to the CNS, it has also been attributed as ENS pathology since enteric neuronal alterations have been characterized and seemed to precede those observed in the CNS [84]. It was proved that a-synuclein aggregates were localized within *in vivo* EN [85]. Interestingly, in 2015, the role of EGCs during PD initiation and development was highlighted. It was well demonstrated that, more than their involvement in gastrointestinal disorders such as inflammatory bowel disease and chronic constipation, EGCs function was altered in PD patients [86][87]. Their GFAP expression was strongly correlated to the presence of EGCs activation-associated pro-inflammatory cytokines release such as IL-6, IL-1, TNFα and INFγ during the PD [88].

Alteration in the ENS activity has also been proposed in Alzheimer's disease which is characterized by accumulation of beta-amyloid protein aggregates within CNS neurons [85][89]. Here again, it was demonstrated, in particular by our collaborator Dr. Schäfer, that in an animal model that the accumulation of beta-amyloid peptide in enteric neurons correlates with reduction of neuronal density and bowel motility [89].

These alterations are time and location dependent. Indeed, mice presented a strong expression of beta-amyloid protein exclusively in EN, and amyloid plaques within ENS ganglia. This is responsible of significant gut motility reduction explained by neuronal loss and smooth muscle atrophy during the development of the disease, as well as impressive alterations in their enteric neuronal protein patterns. Indeed, changes in neuronal markers expression such as GFAP, Nestin, TLR-4 (Toll-like receptor) and synaptobrevin were observed during the onset of the disease in AD mice, as well as deregulation of neurogenesis and neuroinflammation molecules such as GAP-43 (growth-associated-protein-43) and TLR-4. Moreover, the amount and time of appearance of the Alzheimer precursor protein was higher and earlier in the ENS than in the brain, consistent with literature suggesting that Alzheimer's disease origin would takes place in the ENS.

4. Cancer & chemotherapy-induced toxicity

In addition to genetic and neurodegenerative pathologies, ENS alteration has been described in cancer patients, more precisely patients affected by gastric and colorectal cancer. Enteric neuronal loss was also observed in more than 50% cases of cancer-associated inflammation. On the contrary, when inflamed because of bacteria infection or microbiome changes for example, the gastrointestinal mucosa secretes pro-inflammatory factors such as TNF-a (Tumor necrosis factor alpha), IL-1 and IL-6 (Interleukin 1 and 6) that have been noticed to participate in colorectal cancer initiation [90].

Furthermore, it was demonstrated that colorectal and prostate cancer aggressiveness and diagnosis outcome were dependent of perineural invasion (PNI). Perineural invasion is described as the colonization of nerve structures by tumor cells. When present, PNI is associated with a worst survival. Finally, it was suggested that ENS would be associated to tumor development susceptibility since study showed that vagal denervation between stomach and ENS considerably reduced risk of tumor development. Indeed, denervation procedure clearly demonstrated that tumor number and cancer progression were decreased, suggesting the involvement of the ENS [91].

Hence, the interrelation between cancer and the nervous system, including the ENS, seems to be important for cancer initiation and development. It is therefore likely that this dynamic interaction is influenced by the anticancer treatment.

For instance, as evoked at the beginning of this manuscript, cancer is not the only guilty factor of organs and systems alterations, indeed, their treatments are responsible too. Alterations of GI functions have been described in the context of the side effects of anticancer chemotherapies, such as in the case of cisplatin and oxaliplatin, which cause frequently diarrhea, nausea and constipation [92][93][94].

As remainder, cisplatin and oxaliplatin are chemotherapeutic drugs that are the most frequently used to treat a large variety of cancers in combination of several other therapeutic protocols, including targeted therapies and immunotherapies [20][94]. These platinum-containing drugs induce single and double stranded DNA breaks. It is now proposed that the GI side effects caused by these drugs might be mediated by enteric neuronal degradation.

For instance, it has been shown that a repeated exposure of cisplatin or oxaliplatin reduces motor activity throughout the intestines. Cisplatin further reduces neuron number and ganglion size in the colon, potentially causing reduced colonic motility [1][95] (Figure 8).

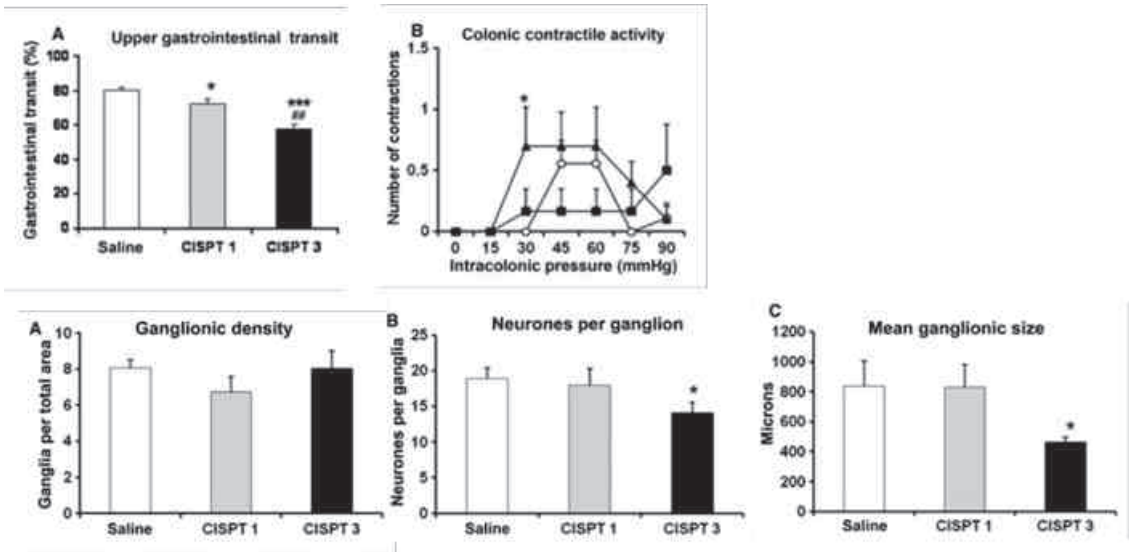

Fig.8 Alteration of enteric motility after platinum administration and neuronal loss
Vera et al., 2011

Similarly, oxaliplatin induces EN cell loss, which correlates with a reduced colonic motility [95][96]. In a surprisingly way, Carbone and colleagues provided the contradictive evidence that human enteric neurons showed hyperexcitable properties with increased soma size and a higher proportion of Hu-positive neurons, protein involves in neuronal plasticity, maintenance and survival [98].

However, these opposite results have to be carefully considered because of the considerable diversity of human samples used. Indeed, ratio of age, sex and treatment-antecedent of patients' samples are not homogeneous which could strongly explain their data.

In addition to the fact that enteric neurons are impaired, enteric glial cells are also impacted by platinum treatment [99]. Indeed, observed decrease of GFAP marker showed a clear disruption of EGCs consistent with S100b-positive cells proportion, another glial marker of cell damages and apoptosis. In addition to platinum salts, other anticancer treatments, such as 5-FU, are also reported to ENS impairments. For instance, 5-FU also impacts the gastrointestinal motor function in mice, beginning first by an increase of the transit associated with an acute inflammatory response that decreases later [100]. Indeed, the migration of colonic motor complexes where inhibited driving to abnormal short and fragmented bowel contractions, associated with neuronal loss and a significative smaller proportion of acetylcholine transferase immunoreactive neurons, enzyme responsible of the acetylcholine neurotransmitter synthesis.

However, the ENS appears not to be only impacted through neurotoxic side effects. In this respect, other studies described alimentary mucositis, another important chemotherapeutic-induced side effect occurring in 40% case of standard dose and 100% in high dose chemotherapy. Alimentary mucositis is linked with mucin expression and secretion, physiologically involved in the protection of the gastrointestinal tract from stress, bacteria as well as penetration or digestion of mucosa. Interestingly, they suggest that ENS may be clearly involved in mucositis development through an abnormal neurotransmitter secretion [101]. They notably demonstrated that chemotherapy administration induces changes in the enteric neurotransmitters vasointestinal peptide (VIP) expression but also that VIP was significantly associated with increased mucus secretion, suggesting the involvement of the ENS. This is consistent with two previous studies where it was shown that 5-Fluorouracil (5-FU) related mucositis in rats led to the decrease of the mucin secretion [101][102]. Moreover, VIP is known to be altered in other gastrointestinal dysfunction, especially in Crohn's disease or ulcerative colitis. These studies highlight that enteric nervous system side effects can appear according different way.

In each of these pathological situations, the exact processes or the signaling pathways involved are still not clearly established. In particular, it remains to establish what cell death processes are responsible for the neuronal loss and whether alterations in the EN stem cell or glial cell physiology are involved. Moreover, there is evidence that not all neurons respond in the same way, so that a certain pathological condition might affect only a subset of neurons or glial cells [104].

To counteract these important toxic side effects, therapeutic approaches have been developed such as PARP (Poly-(ADP-ribose) polymerase) inhibition using BPG-15 (O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime) that showed interesting enteric neuroprotective effects in mice and improved gastrointestinal functions when combined with platinum derivatives [105]. The poly (ADP-ribose) polymerase 1 (PARP1) is a critical enzyme that intervenes in response to platinum-induced DNA strand breaks repair by being cleaved. Its advantage lies in the lifespan enhancement by 160% when combined with cisplatin, but also in its cytoprotective and dose-limiting side effect properties. Its neuroprotective effect is translated by the reduction of symptoms such as temperature-related hyperalgesia and mechanical allodynia, however, despite its promising property, they did not investigate the cleaved profile of PARP, giving not information about the way apoptosis. However, this study clearly evidenced that BGP-15 (O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime) alleviated oxaliplatin-induced mitochondria defects and improved enteric neurons survival. Moreover, colonic motility and pellet formation were restored in BGP-15/Oxaliplatin group.

However, despite the great progress about side-effect prevention, the mechanisms responsible for the neuronal damage of ENS by platinum derivatives are still poorly understood and need to be. It strongly justifies our interest to resolve this essential problematic, because these side effects lead to a treatment dose reduction or arrest, but also induce an important decrease of patients' quality of life, strongly impacting the clinical outcome [106]. Platinum-based compounds act through DNA damages, especially in a p53 family-dependent pathway. The p53 family is widely described to be activated in response to cellular damages, and more interestingly, previous studies in the lab showed its function within the cell-death process of brain neurons. These informations let us suggest that the p53 family may also play a role during platinum-related toxicity on ENS.

III. The p53 family: A maestro of cellular functions

A. Family members & functions

Potential regulators of neuronal loss and stem cell alterations in the ENS could involve transcription factors encoded by genes of the p53 family: p53 and its two homologs, p63 and p73.

Their structures are based on a transactivation domain (TAD), a prolin-rich domain (PRD), a DNA-binding domain (DBD), a nuclear localization domain, an oligomerization domain (OD) and a C-terminal regulatory domain (CTD) [107]. All members share a high level of protein sequence similarity, particularly in the DNA-binding domain. It is noteworthy that the three genes encode for many isoforms. Alternative splicing produces carboxy-terminal variations (α, -β, -γ, -δ, -ε, -ζ, -η) while at the N-terminus, alternative promoter produces either full-length (TA-) or truncated (ΔN-) isoforms [107][108] (Figure 9). Since the ΔN-isoforms lack the N-terminal transactivation domain, they are often considered as dominant-negative isoforms thought to inhibit TA-isoform.

Unlike what was initially thought, p53 possesses also numerous isoforms. Its 11 exons generate up to 12 isoforms. For example, full-length p53 also corresponds to the p53α isoform, also known as FLp53 or TAp53α [110]. Among all, three major isoforms are particularly reported in human, through alternative promoter activation: Δ40p53, Δ160p53 and Δ133p53 [111]. This last is the most p53 conserved isoform found in zebrafish, drosophila, and mouse, showing its importance in the p53 signal pathway.

P63 and p73 also possess numerous isoforms, whose most described ones are TAp63, TAp73 and ΔNp63, ΔNp73 (Figure 9).

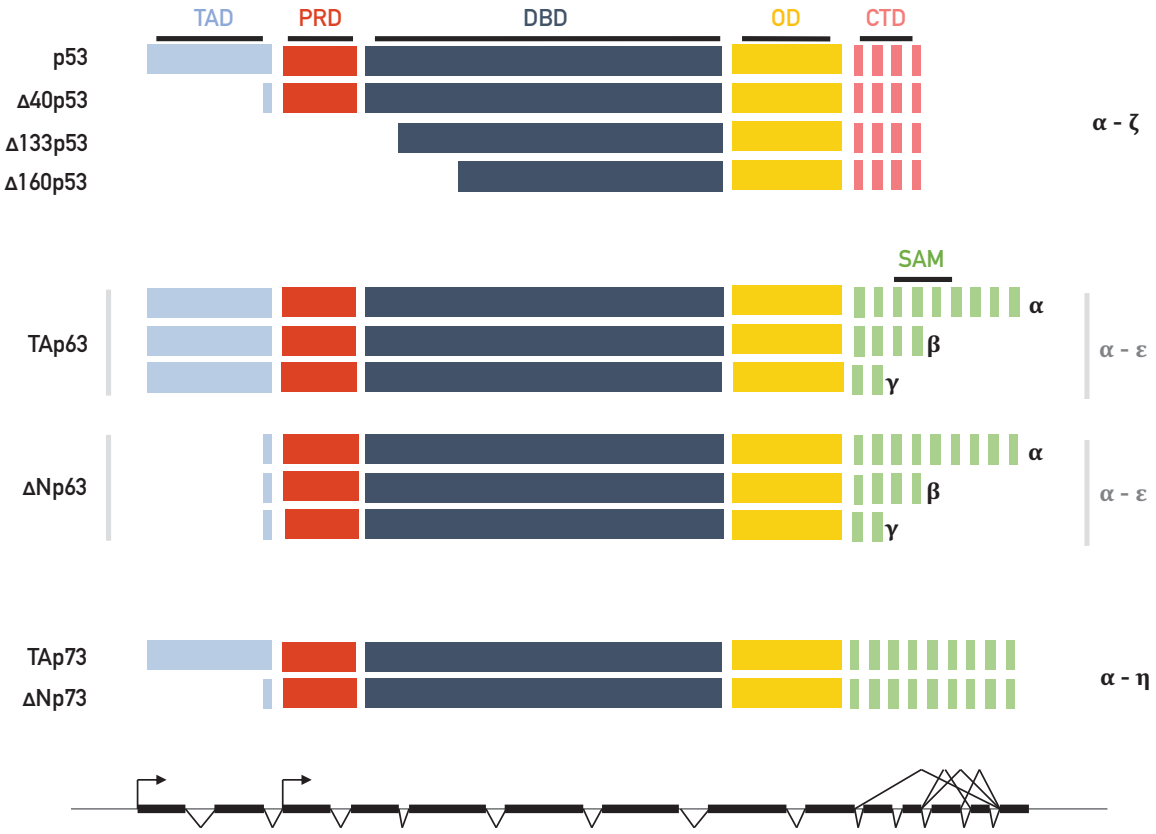

Fig.9 Structure of the p53 family members: p53, p63 & p73 and their different isoforms sharing structural homology with p53.
Inspired from Zhang et al., 2019

These 3 transcription factors are targeting sets of common genes [111][112]. Several of these target genes have been identified and account for the biological function of the p53 family by playing a role in diverse cellular functions. Cell cycle inhibition is in part mediated by *p21* and *p57*, apoptosis through *noxa*, *bax* and *puma* expression [114], cell differentiation via epidermal-related genes and metabolism with *sirt1*, *ampk* (AMP-activated protein kinase) and *tiger* activation (Figure 10). However, the common or specific p53 family targets genes are not all known, notably because it also depends on physiologic parameters such as cell type or stress able to modify the regulated genes.

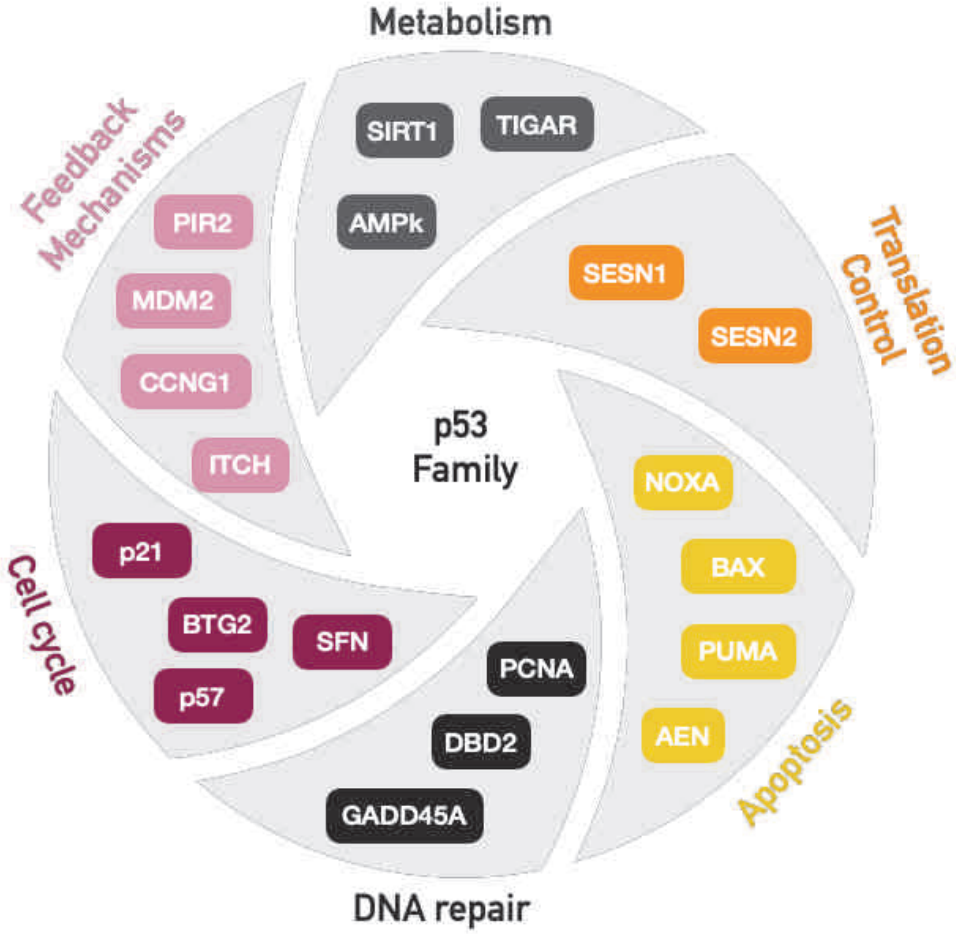

Fig.10 Non-exhaustive cellular role of the p53 family and its specific target genes
Adapted from Fischer 2007, Nature

However, p53, p63, and p73 do also have their specific target genes. Indeed, the unique functions of p53, p63 and p73 have been demonstrated in many studies through animal model knock-out [111]. P53 is known as a major tumor suppressor gene, whose misregulations drive to cancer in 50% of cases. P53 null embryos are viable with correct development, but are reported develop tumor at a later stage [111]. Knock-out of p63 and p73 also have been investigated and showed that total p63 null mice embryos are not viable once born because of severe skin problems (ectodermic dysplasia, absence of teeth). Specific p63 isoform knock-out induces limbs malformations associated to epithelial organization defects in ΔNp63 null mice [115] and cardiac malformations in TAp63 null embryos while adult display advanced ageing. TAp73 and total p73 knock-out mice display similar defects such as fertility, hippocampal dysgenesis and tumorigenesis, while cortical thickness and neurodegeneration in ΔNp73 null mice have been reported [116]. It clearly shows that p53 is mostly involved in response to DNA damage while p63 is acting during development and p73 in both functions. More precisely, among their numerous functions, the p53 family members have been linked to CNS development.

B. P53 family & central nervous system

Among their variety of functions, the p53 family has been shown to be importantly involved in central nervous system (CNS) development notably p63 and p73 (Figure 11). Indeed, both isoform deficient $\Delta N^{-/-}$ and $TAp73^{-/-}$ mice produce non-overlapping nervous defects, such as abnormalities in brain formation with cortex defects and hippocampus dysgenesis explained by an unusual organization [116][117]. Other studies also found that embryonic $p63^{-/-}$ mice display a deregulated sympathetic neuronal apoptosis, although other papers do not find altered neurogenesis [118][119].

The disturbed CNS development in $p63^{-/-}$ and $p73^{-/-}$ mice could be explained by deregulation of neuronal apoptosis. Indeed, the p53 family is strongly implicated in neuronal survival of the CNS. During hippocampal neurogenesis, p53 negatively inhibits neuroprogenitor cells (NPCs) proliferation and survival [121]. Conversely, by inhibiting p53 apoptotic actions, ΔNp63 and ΔNp73 are key survival proteins for both embryonic and adult NPCs [117][121][122]. By acting as pro-survival protein, ΔNp73 is suggested to also play a role

in the long-term maintenance of adult neurons [123], whereas TAp73 intervenes in the maintenance of neural stem cells and progenitor cells self-renewal [118].

Next to their involvement in neurons development and survival, the p53 family is also implicated in their differentiation. By using human neuroblastoma cells (SH-SY5Y) it was shown that p73 is directly involved in neurons because an augmented endogenous p73 protein expression by retinoic acid was determinant for SH-SY5Y differentiation [124], correlated by p73 overexpression experiment. More precisely TAp73 was driving this differentiation whereas ΔNp73 is able to negatively autoregulate p53 and TAp73 in this cell line [125]. The role of p63 and p73 in neuronal differentiation was further supported by *in vivo* observations, notably in murine hippocampal neurogenesis, where the deletion of TAp63 in adult NPCs was shown to lead to NPC pool depletion [121]. Furthermore, TAp63 was shown to induce cell cycle arrest and inhibit differentiation in these NPC. Likewise p63, p73 is essential in the maintenance of the NPCs pool, during both embryonic as adult neurogenesis [125][126]. Indeed, NPCs from $p73^{-/-}$ mice cultured *ex vivo* as neurospheres contain less cells compared to control, and after redifferentiation, the quality of neurons is poorer [116][125]. In addition, TAp73 was also described to intervene during oligodendrocytes precursor cells differentiation [118].

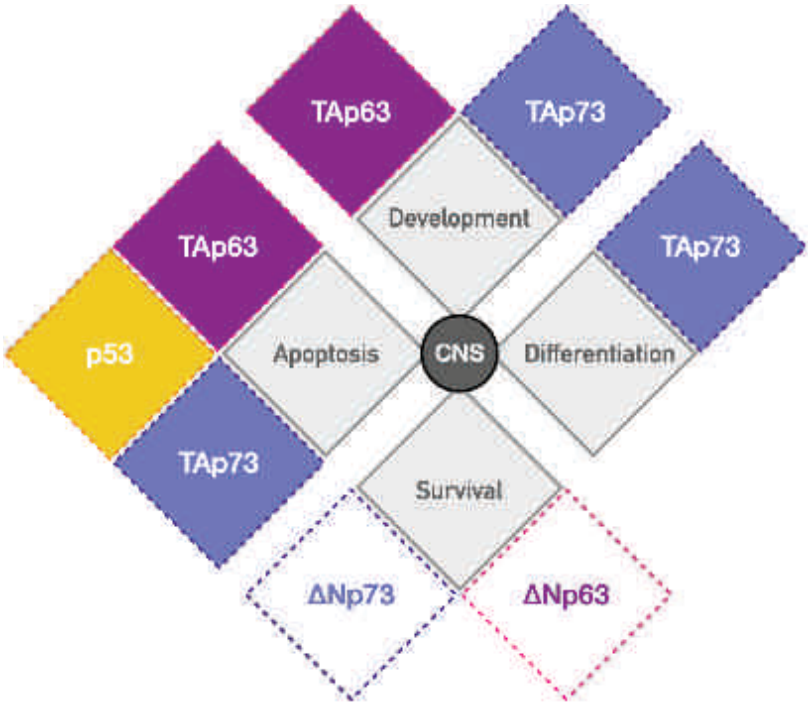

Fig.11 Involvement of the p53 family within the central nervous system

Next to their numerous roles in the CNS embryonic development and physiology the involvement of the p53 family, more specifically p73, was also pointed out in neurodegenerative pathologies. Among these neurodegenerative diseases, the p53 family is mostly described in Alzheimer's disease which is characterized by two different nervous lesions: the amyloid beta aggregate and fibrillary degeneration by phosphorylated-Tau protein accumulation. The loss of one allele of p73 make mice more susceptible to have a fibrillary neurodegeneration [128]. Indeed, p73 haplo-insufficient mice have a decrease of motor and cognitive functions and accumulation of phosphorylated Tau proteic filaments was noticed. In this sense, Mattson et al., correlated these results by demonstrating in aged mice that a reduced ΔNp73 expression induced the same phenotype [129], confirmed with ΔNp73 deficient model where neuronal degeneration and cognitive impairments was also observed. The neuroprotective effect of ΔNp73 was also demonstrated in the other AD phenotype, where its expression prevents from amyloid-beta-induced neuronal degeneration.

Moreover, p53 was also observed during AD. Indeed, it was shown that its expression was increased both in neuronal cells bodies and neurites containing abnormal Tau protein level, as well as in neurons associated with amyloid-beta aggregates [130]. In addition, Turnquist and colleagues demonstrated that Δ133p53 and p53β isoforms had influence on neurotoxic or neuroprotective effects of CNS astrocytes [131]. Indeed, Δ133p53 acts as a neuroprotectors because of its prevention effects on astrocytes against senescence while p53β is mostly described in neurodegenerative disease where its expression is increased, inversely to those of Δ133p53.

Next to its involvement during neurodegenerative disease, the p53 family also intervenes in response to neurotoxic stresses, notably after cytotoxic agent use such as platinum-derived compounds. In this context, our laboratory previously described that cisplatin administration induced the p53 family in cortical neurons, more precisely p53, through a deregulation of its negative regulator MDMX (Mouse double minute 4) [4]. In addition, our laboratory also showed an upregulation of TAp73 and downregulation of ΔNp73 expression, resulting in specific target genes induction as well as neuronal apoptosis [5].

Although the p53 family roles in the central nervous system are well described since years, it is still not known if we could attribute the same p53 family functions in other nervous systems, more precisely in enteric nervous system. Indeed, the ENS is also a collateral victim of chemotherapeutic agents, allowing the hypothesis that the p53 family members would also have a role in response to neurotoxic stresses in ENS, especially because it was proved that p63 is involved in ectoderm development, tissue from where ENS derived, and that $p63^{-/-}$ mice show malformation in ectodermal tissue development suggesting that p63 may be involved in the ENS establishment [132].

IV. Muscle physiology, anatomy and myogenesis

As indicated precedingly, muscles are negatively impacted by cancer and anticancer therapies, leading to muscle atrophy and loss of strength, which ultimately reduced patient' quality of life, and may lead to death if it causes a stop in the treatment. The absence to efficient therapy that may be used to prevent muscle atrophy in patient highlight the complexity of this organ and the pathologies associated, that are often lethal.

This complexity lies in their essential role in organism's survival (respiration, digestion, motility…), diversity, high plasticity, and complex interactivity with the metabolism.

For instance, all movement capacity of living organisms necessarily requires muscles. Muscles are essential body components through their abundant and energy-consuming characteristics. Muscle structure is based on fibers composed by actin and myosin protein filaments allowing the contraction. Furthermore, muscle tissue is classified in two categories: the smooth and the striated muscles; itself divided in cardiac and skeletal muscles; according the specific needed tasks.

A. Smooth muscles

As indicated in their name, smooth muscles are devoid of striations and contain less myosin than the striated ones. They are specialized in slow and involuntary movements, such as those involved in the digestive tract, urinary bladder, uterus, blood and lymphatic vessels contractions.

B. Striated muscles

Adversely to smooth muscle, striated ones are characterized by their own filament organization giving the striated look.

1. Cardiac muscle

Cardiac muscle or myocardium consists in branched individual cardiomyocytes involuntarily controlled, generating contraction responsible of heart-beats. In order to continuously perform blood pumping through arteries, the cardiac tissue must contract permanently and in a coordinated manner. This is managed by cardiac cells or cardiomyocytes joined in a syncytium tissue by intercalated discs structures, while gap junctions assure electrical and molecular communication from cell to cell and form a network that allows a synchronized and efficient contraction [133].

2. Skeletal muscle

Adversely to cardiac muscle tissue, skeletal muscles are involved in voluntary movements notably because of their localization: attached to the bones by tendons, allowing body movements. Skeletal muscles are composed of multinucleated cells, also called muscle fibers or myofibers, themselves containing several myofibrils wrapped in a membrane: the sarcolemma. Myofibers are divided in two categories depending whether they play a role during slow and fast twitches, respectively classified as slow muscle fibers (type I) and fast muscle fibers (type II) [134]. Muscle fibers type II are required for rapid and powerful contractions due to the continuous oxygen-to-energy transformation ability through an aerobic metabolism. It explains why they possess numerous mitochondria and a richer blood supply compared to slow muscle fibers that use an anaerobic metabolism and are more quickly tired. Indeed, fibers type II are important for example during athletes' marathons or in case of bursts of speed and strength [135]. The heterogeneity of fibers' composition confers to skeletal muscle its own contractile property.

C. Muscle development

As the majority of other organs and tissues, muscle development occurs during embryogenesis and mostly derived from mesodermal germ layer. Muscle development, especially those of skeletal muscles comes from the neural tube giving the mesoderm, itself divided in blocks called somites that will give birth to all skeletal muscles in the body [136].

1. Myogenesis

Myogenesis occurs when transcription factors such as Pax7 and Pax3 (Paired box protein 7 and 3) or Myf5 (Myogenic factor 5) and MyoD (Myoblast determination protein) induce differentiation of embryonic progenitor cells, which are either in a quiescent undifferentiated status (satellite cells) or in a differentiated cells lineage such as myoblasts (Figure 12). Skeletal muscle progenitors can also be differentiated into myocytes, which are myoblast-derived cells, characterized by their contractile units (myofibers), through the activation of MyoG (Myogenin) and Mrf4 (Myogenic regulatory factor 4) (Figure 12) [137].

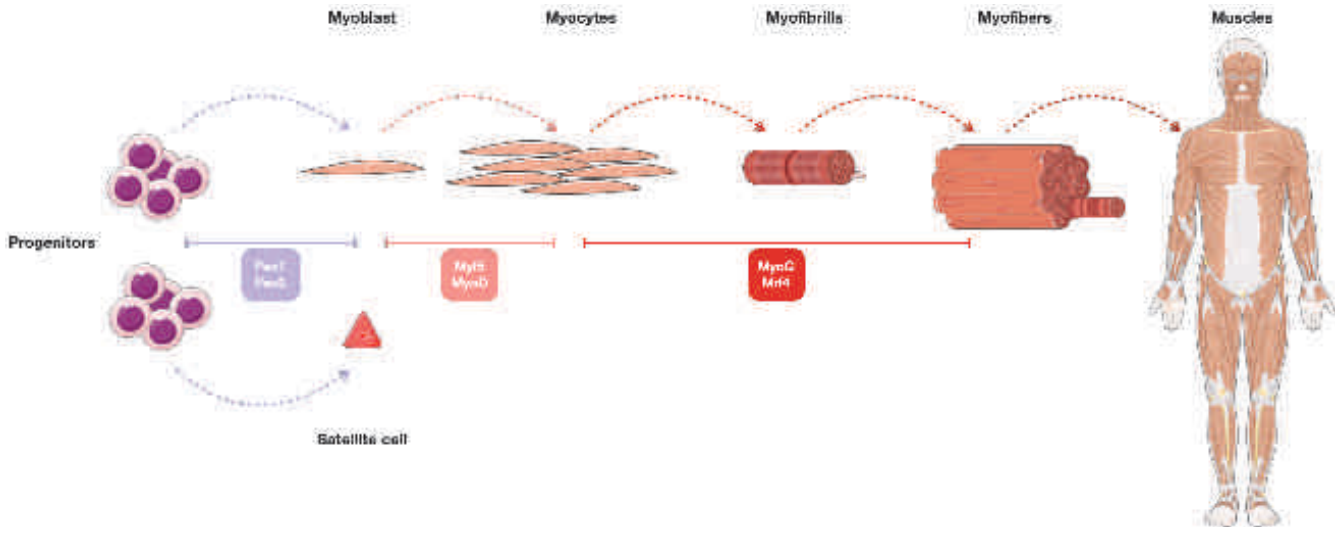

Fig.12 Schema of muscle myogenesis and differentiation in skeletal muscle.
Chronologic representation of specific transcription factors involvement according each step.
Inspired by Zammit et al., 2006, Bentzinger et al., 2012 and So-ichiro Fukada et al., 2018.

2. *Muscle regeneration*

In general, muscles are remarkable plastic tissue because of their ability to regenerate after damages, pathologies, or lack of muscle activity. All these processes can alter muscle functions causing weakness, contraction defects, body instability and poorer quality of life. Fortunately, somatic stem cells or satellite cells own regenerative properties, sharing common features with the embryonic myogenesis process [138]. Regeneration process is carried out according two coordinated phases: 1) the muscle fibers necrosis inducing the invasion of inflammatory cells allowing 2) the regeneration via satellite cells that activate and induce myoblast proliferation which will differentiate into myofiber [135][137].

D. Hypertrophy or atrophy

Muscles are composed of proteins, and their protein quantity vary according to the needs by an adjustment of the protein synthesis and protein degradation processes. This is a balance tightly regulated notably by satellite cells proliferation called hyperplasia, which is the main process to increase skeletal muscle mass during embryogenesis or adulthood [140]. However, skeletal muscle mass can also be increased through another process named hypertrophy which requires growth factors and nutrients that act on existing myofibers. Hypertrophy occurs when the protein regulation balance is in favor of synthesis. In contrast, muscle loss or atrophy occurs when the protein degradation rate is higher than synthesis, what is frequently observed in pathologic conditions where muscle atrophy can really have a vital dimension.

E. Muscle atrophy in pathological conditions

Skeletal muscle atrophy happens during different diseases such as denervation, immobilization, starvation, muscular dystrophy, ALS (Amyotrophic lateral sclerosis), sepsis, diabetes, heart failure, HIV (human immunodeficiency virus) or cancer. It also during physiological processes for example in ageing where skeletal muscle atrophy is called sarcopenia. In every case, skeletal muscle loss leads to an important weakness which strongly impairs people quality of life and participates to an increase mortality.

1. *Sarcopenia*

Sarcopenia occurs during ageing and is characterized by a progressive loss of skeletal muscle mass [141]. This is an age-related disease that has been considered as a major health issue due to the increasing life-span. Indeed, sarcopenia consequences are the decrease of muscle mass which cause important fatigue and muscle weakness that are major recurrent problems affecting more than 3% of the global world population [142]. The muscle recovery of these elderly patients is diminished because of the impaired regenerative capacities of satellite cells that may be due to their age-related decrease of growth factors release and slowed down physical activities [143]. The involved mechanisms are still controversial but suggest that the ubiquitin proteasome system may have a role [142][143]. However, to brake this age-related disease, exercise and nutrition care are the most effective and used approaches.

2. *Denervation*

Denervation also takes place in muscle atrophy category and is defined as the loss of nerve and muscle connection which induce muscle paralysis as a direct consequence. It can also be linked to physical damages like in spinal cord injury but also occur as consequence of disease as in ALS [146]. Amyotrophic lateral sclerosis is a progressive neurodegenerative disease characterized by the degeneration of motoneurons leading to muscle denervation and consequently strong muscle atrophy [147]. This is a progressive muscle waste that drives slowly to death through respiratory muscle paralysis and for which the overall survival after diagnosis is about 3 years [148]. The principal cause of ALS is SOD1 (superoxide dismutase 1) gene mutation found in 20% of the inherited ALS cases, that cause impairments in the ROS-to-oxygen transformation leading to toxic accumulation of ROS in muscle [149][148][149].

3. *Cachexia*

The third type of skeletal muscle atrophy is called cachexia. Cachexia is defined as a multifactorial complex syndrome associated with a large number of chronic diseases such AIDS (Acquired Immuno Deficiency Syndrome) [152], cancer [153], chronic renal failure [154], chronic obstructive pulmonary disease (COPD) [155], rheumatoid arthritis [156] and heart

failure [157]. Skeletal muscle wasting is accompanied by additional symptoms such as adipose tissue loss, fatigue, weakness, anemia, loss of appetite and alterations in carbohydrate, lipid and protein metabolism, but also impacts other organs such as heart, intestine, kidney and liver [156][157]. Physiologically, skeletal muscle homeostasis requires autophagy-mediated cell death to remove damaged components (proteins, organelles), however, during cachexia, this process is upregulated, leading to an excessive activation of skeletal tissue breakdown [160]. Furthermore, it was shown by Sabourin and colleagues [161] that satellite cells normally expressing Pax7 were lacking its expression and were not able to provide regenerative capacities because of the absence of the key myogenic factors MyoD1 and myogenin. It leads to the failure of muscle stem cells to undergo differentiation in impaired muscle.

This is a serious pathology due to the proportion of the skeletal muscle tissue within the body (around 40%) explaining why cachexia is correlated with bad prognosis for the pathologies that are causing it. Indeed, skeletal muscle wasting early signs are very difficult to recognized. To solve this problem, several international specialists gathered by the Society for Cachexia and Wasting Disorders have developed in 2006 the term of “pre-cachexia” to established standards that characterize early signs to facilitate diagnosis. Pre-cachexia is defined as an event occurring with chronic diseases and provoking inflammation and 5% or less of body weight loss within 6 months [162]. Furthermore, in order to improve treatment approaches, cachexia was scored in different groups from mild (0–25), moderate (26–50), severe (51–75) to terminal (76–100) cachexia based on diverse characteristics such as body weight loss and lean body mass, inflammation, metabolic disturbances, immunosuppression, physical performance, anorexia, and quality of life [162].

Despite effort to optimize patients’ diagnosis, cachexia is often detected when already established. Indeed, cachexia is diagnosed according to three “detectable” parameters such as the presence of systemic inflammation profile, or more than 10% of the total body loss, or decrease in food intake [163]. However, decrease in food intake is a major parameter to consider, notably in a specific cachexia type called cancer-associated cachexia (CAC), where calorie deficits can reach up to 1200kcal and where energy intake rate is lower than resting energy expenditure promoting negative energy balance and can be related to tumor

metabolism. Indeed, we know that tumor can competes with other body components such as organs or tissue for energy and substrates [160].

4. Cancer-associated cachexia

Hippocrate is known to have said “Walking is man’s best medicine”. In this respect, regular physical exercise has been proved to prevent up to 40% from several cancers and to be associated with lower tumor recurrence risks [164]. Indeed, cancer is the principal cause of death worldwide with more than 8.2 million cases according to the World Health Organization, in 2012, estimated to 22 million cases in 2030. Cancer-associated pathology also play a decisive role in cancer survival such as cancer-associated cachexia, another important health issue that cannot be ignored.

Cancer-associated cachexia is an important and relevant systemic syndrome whose specific underlying molecular pathways are still not completely known. It is characterized by the loss of body weight by skeletal muscle with or without adipose tissue wasting accompanied by systemic inflammation with the consequences of the decrease in quality of life and reduction in patient’s physical activity [157][161]. Up to 80% of patients with cancer are impacted [165] by cancer-related cachexia which is responsible of more than 20% of cancerous patients’ death. Muscular atrophy in cancer cachexia is a product of several cumulative factors: abnormal metabolism (insulin resistance and lipolysis increase), reduction of physical activity and food intake, activation of proteolytic pathways (ubiquitin-proteasome pathway), increase on myostatin expression, elevated levels of pro-inflammatory cytokines, testosterone decrease as well as tumor and chemotherapy influence.

Nevertheless, variable aspects like age and co-existing morbidities also linked to cancer associated cachexia, contribute to the severity and heterogeneity of muscle atrophy and complicate treatments [166].

In addition, it has been demonstrated that predisposing parameters such as single nucleotide polymorphisms (SNP) increase the susceptibility of developing cancer-associated cachexia. In 2012, it was highlighted in animal and patients that a SNP within the gene Selectin P was significantly associated with weight loss and muscle atrophy [167]. Selectin P is known to be increased in response of early catabolic process of cachexia mediated by inflammation.

Interestingly, cancer patients possessing a specific SNP present a low expression of Selectin P that correlates with a lower risk to develop CAC [168].

However, some cancers are more susceptible to be associated with cachexia than others, for example, patients with prostate, gastric, lung or pancreatic cancers are more vulnerable, and their overall survival is strongly dependent of cancer-associated cachexia and is worst when it occurs [160]. More than 80% of patients with an upper gastrointestinal tract cancer are impacted by cancer-associated cachexia which has a significant influence on their survival [165]. Indeed, patient's weight loss is directly proportional to morbidity and mortality. In addition, of 198 pancreatic cancerous' patients in Bachmann and colleagues' paper, up to 70% presented weight loss, within 40% exceeding 10% [165].

5. *Tumor involvement*

Tumor presence has to be taken in consideration in CAC because of its ability to release factors that would act as potential muscle atrophy inductors. Indeed, tumor cells secretes pro-inflammatory cytokines as IL-6 which is a key regulator of skeletal muscle, immune cells, eicosanoids, but also specific molecules such as HSP70 and HSP90 (Heat shock protein 70 and 90, or members of the TGF-B family specifically targeting skeletal muscle tissue [160]. Among these specific released factors, we also find TNF-α (Tumor necrosis factor alpha), IL-1, and INF-γ (Interferon gamma), LIF (Leukemia inhibitory factor), GDF15 (Growth differentiation factors 15) and TWEAK (TNF-related weak inducer of apoptosis) that stimulate the host systemic inflammatory response and actively participate in CAC through activation of selective transcription factors by their respective cell surface receptor [153][167][168]. Indeed, it was shown that animals with administered TNF-α significantly showed decrease in food intake and muscle loss [171]. Indirectly, they also participate to the CAC establishment with their actions in the central nervous system by reducing appetite through the release of the "satiety hormone" or leptin [172]. Reduced food intake is an important parameter of the CAC physiopathology explained by the serious protein synthesis impairment associated with insufficient available nutrients rate [169].

6. *Anticancer agents' role in cachexia*

Cancer associated cachexia affects anticancer treatment efficacy and strongly influence survival outcomes [159]. However, this is a real crosstalk that exists between CAC and chemotherapeutics because we know that anticancer treatments cause loss of appetite [173] but also that they generate side effects that might contribute to cachexia such as anorexia, nausea and diarrhea. In addition, chemotherapeutic agents have a direct negative influence on the protein metabolism, notably through the activation of catabolic processes [172][173].

Cisplatin, for example, has been proved to reduce muscle size and mass, both in *in vitro* and *in vivo* models through the NF-κB signaling pathway [174][175]. Even more recent and supposedly more specific therapies, such as sorafenib (a tyrosine kinase inhibitor) or glucocorticoids (used in symptoms palliation) have been described for their catabolic effects on skeletal muscle tissue [160]. It was also described in mice that drugs combination such as cyclophosphamide, doxorubicin and 5-fluorouracil provoke a significant loss of skeletal muscle mass through an inflammatory response [30].

Among all these anticancer drugs involvement in CAC, doxorubicin remains the most virulent agent. This anthracycline compound has to function to interact with topoisomerase II and subsequently to induce double stranded DNA breaks associated with mitochondria alteration and reactive oxygen species production [18]. Doxorubicin has notably been described to produce cardiotoxic side effects through oxidative stress [178]. In this respect, it was shown in rodents and dogs that anterior administration of an antioxidant glutathione precursor: NAC (N-acetylcystein), protected animals from doxorubicin-induced cardiotoxicity and myocardial lesions [177][178]. However, these positive effects were discussed one year after Doroshow and colleagues' study by Dresdale and his team, showing that NAC treatment had no effect on doxorubicin-induced cardiomyopathy in patient, and even more, that no changes in ventricular ejection were observed between the control and treated group [181].

Moreover, two studies demonstrated that doxorubicin also stimulates systemic inflammatory, in particular by inducing the cytokine TNF-α [182] which is able to increase ROS level in striated muscle. This fact was correlated by inhibiting TNF-α which have demonstrated

doxorubicin-induced cardiotoxicity counteraction [183]. In this sense it was also discovered that ROS production can be linked to apoptosis-related cell and participate to muscle catabolism but also that subsequently doxorubicin is an inducer if cardiac myocytes and differentiated myoblasts apoptosis. It is now commonly considered that ROS could be a promising therapeutic target for muscle atrophy [176][182].

In addition to cardiotoxicity, doxorubicin was mostly described to generate skeletal muscle loss notably in rats, primates and humans [183][184][187]. More precisely, the ubiquitin-proteasome signaling pathway composed of Atrogin-1 and Trim63 was pointed-out to play a role during doxorubicin-induced muscle atrophy.

F. Molecular pathways of muscle atrophy

During distinct catabolic conditions, proteins are mobilized from the muscle cells, which can be associated with remodeling of mitochondrial and sarcoplasmic networks and loss of myonuclei. These complex processes caused muscle atrophy and involve different molecular pathways that are also specific to the cause of the atrophy (Figure 16). Because of the various and complex origins of muscle atrophy, it remains difficult to identify the mediators of protein degradation.

To solve that problem, in 2011, a scientists group compared the gene expression profile of numerous and different muscle atrophy models (diabetes, cancer cachexia, chronic renal failure, fasting, and denervation) and identified a sub-set of genes frequently mis-regulated in these models. They particularly identified two E3 ubiquitin ligases (E3), muscle-specific: atrogin-1/MAFbx (Muscle atrophy F-box) and Trim63 (MuRF1-Muscle ring finger protein 1) (Figure 13). They belong to the ubiquitin-proteasome system (UPS) and were found to be upregulated in these different atrophic models [188]. Indeed, in combination with the autophagic–lysosomal pathway, the ubiquitin-proteasome pathway forms the two major pathways involved in protein breakdown [189].

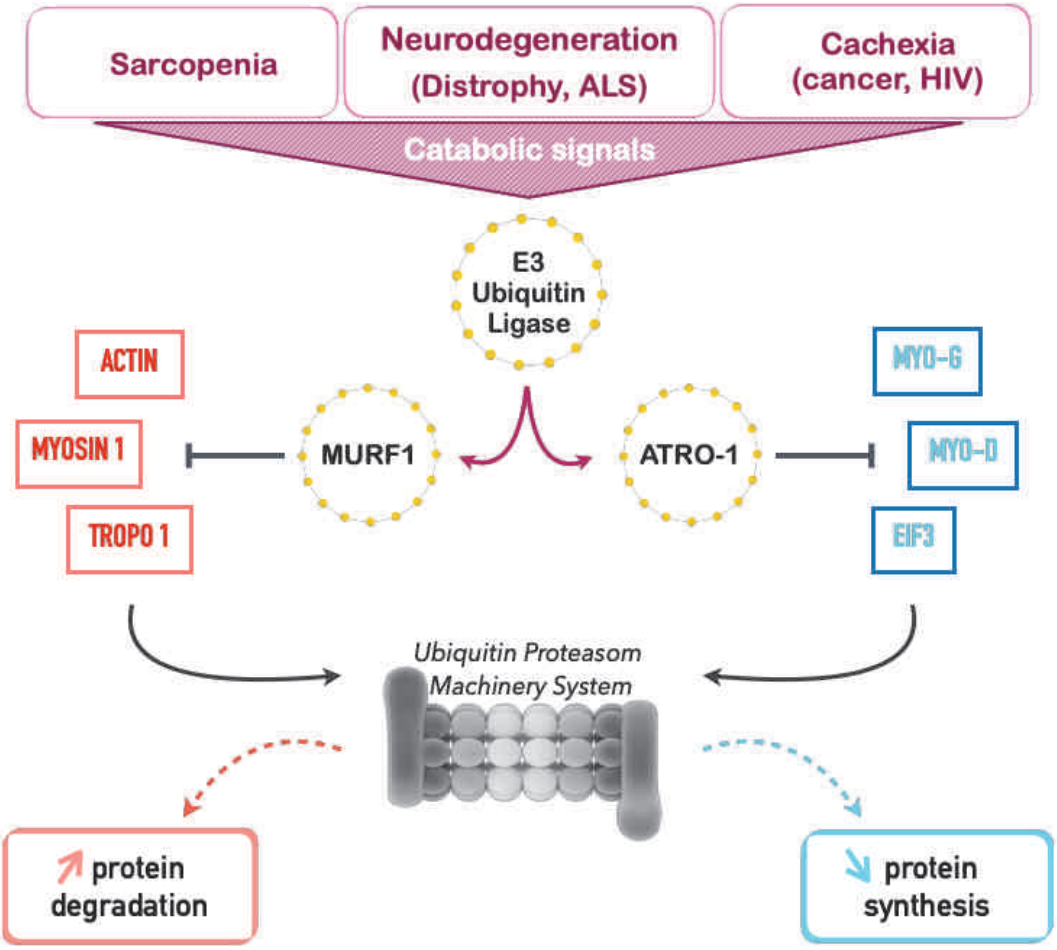

Fig.13 Catabolic signals driving to muscle waste through MuRF1 and Atrogin-1 regulation.
Inspired from Foletta et al., 2011

1. *The ubiquitin-proteasome pathway*

The ubiquitin-proteasome pathway belongs to the proteolysis systems and is responsible of the major part of intracellular protein breakdown in all body cells. In particular, within the UPS, the 26S proteasome system is the primary involved in muscle protein degradation during atrophy [190]. The proteasome function is to recognize proteins owning ubiquitin groups to degrade them into small peptides through a multi-catalytic protease complex. To ubiquitinate proteins, three major enzymatic components; E1, E2 and E3; are required to link ubiquitin chains onto targeted protein (Figure 14) [187][189].

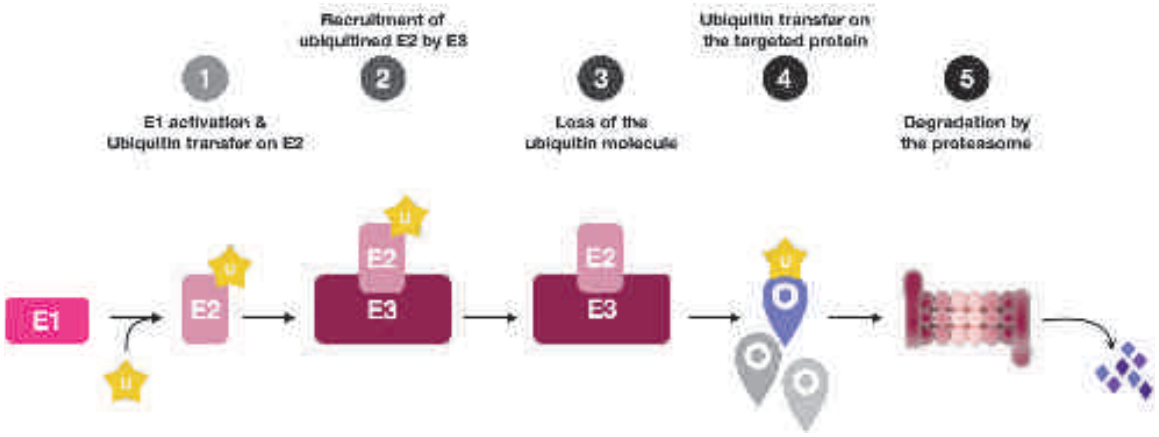

Fig.14 Schematic representation of the ubiquitination steps.

In a primary phase, E1 which is a ubiquitin-activating enzyme activates and transfers ubiquitin molecules to its partner: E2, the ubiquitin-conjugating enzyme, which in turn, carries and prepares ubiquitin for conjugation. The last and rate-limiting step of the ubiquitylation process is controlled by the ubiquitin ligase E3, whose role is to recruit E2 ubiquitin loaded enzymes, then to recognize and interact with the specific substrate. The E3 ubiquitin ligase then catalyzes the transfer of the ubiquitin molecule from the E2 enzyme to the targeted protein, which one is at the end, recognized and degraded by the proteasome machinery system [187][189].

2. *Trim63 (Murf1) and Atrogin-1 E3 ubiquitin ligase*

Among all existing E3 ubiquitin ligases, **Trim63** and **Atrogin-1** are known to be muscle-specific, more precisely, upregulated in muscle atrophy. The E3 enzymes are composed of a RING (really interesting new gene) finger domain, characteristic of small proteins. They can act as monomers, dimers or multi-subunit complexes, which carry the ubiquitin ligase property when assembled together [192].

Trim63 (Murf1) belongs to the TRIM superfamily of multidomain (RING domain, zinc-finger B-box domain, and leucine-rich coiled-coil domain) [191][192][195] ubiquitin E3 ligases and can exist as a monomer or dimer like its two other homologues, Murf2 and Murf3. They are all present in muscle cells but the Trim63 is the only family member shown to be related to muscle atrophy, while the others play a role in muscle development [196].

Concerning Atrogin-1 (Fbxo32), it is the cullin-RING E3 ligase (CRL) superfamily, also containing a RING finger protein that permits it to associate with a cullin protein (CUL1) and recruit the ubiquitin-charged E2s. Unlike Trim63, Atrogin-1 is in a multisubunit complex, consisting of a SKP1 (S-Phase Kinase Associated Protein 1) and a F-box protein that serve as the substrate adaptor element [197].

It was shown that mutated mice models lacking Atrogin-1 and/or Trim63 are resistant to denervation, fasting and dexamethasone-induced muscle atrophy [196][197]. Other experiments demonstrated an upregulation of their expression in a wide range of atrophy-inducing conditions and diseases (immobilization, hindlimb suspension, denervation, ageing, sepsis, fasting, cancer, HIV, chronic obstructive pulmonary disease, diabetes and due to the use of glucocorticoids) [186][198][198][199][200][203].

Altogether, these results pointed out the relevance of the atrogenes as muscle-atrophy key effectors. However, Trim63 and Atrogin-1 are both described as transcriptionally upregulated under most atrophy-inducing conditions, highlighting that they do not always act in the same way. Indeed, each one of them has specific targets and is differently regulated in distinct types of atrophy with distinct roles.

Trim63, for example, is associated with myofibrillar protein degradation through ubiquitination of muscle structural proteins, like troponin I [204], myosin heavy chains [158][203] and actin [206].

Conversely, Atrogin-1 is associated to growth-related processes because it promotes degradation of MyoD [207], which in response to exercise and muscle injury promotes satellite cells activation and proliferation, contributing to myoblast differentiation and muscle regeneration [159][206]. To summarize, Atrogin-1 controls protein synthesis while Trim63 regulates protein degradation molecular pathways.

Directly in relation to my PhD project, it was described that doxorubicin stimulates the ubiquitin proteasome pathway, in particular upregulates the E3 ubiquitin ligase Atrogin-1 upregulation in cardiac myocytes and skeletal muscles [2][43][207].

This important proteolytic pathway is positively or negatively regulated by multiple signaling pathways, such as the IGF-Akt-mTOR-FoxO cascade (Figure 16) [210].

3. *Regulation by the IGF-PI3K-Akt-FoxO signaling*

Atrogenes are notably regulated by the FoxO genes' family, itself controlled by the IGF-1/PI3K/Akt pathway. The FoxO (or class O-type forkhead) transcription factor family contains four genes, FoxO1, FoxO3, FoxO4 and FoxO6, which are expressed in skeletal muscle [211]. During muscle waste, FoxO genes are usually upregulated. Indeed, it was demonstrated using *in vitro* and *in vivo* models that they promote muscle atrophy by direct binding onto the *Trim63* and *Atrogin-1* promoters [209][210]. In addition, skeletal muscle mass of transgenic mice overexpressing FoxO1 was decreased [214]. Also, under stress condition, FoxO3 gene is activated in myofiber, leading to *Trim63* and *Atrogin-1* mRNA upregulation [215].

As mentioned before, Akt regulates FoxO transcription factors in a negative manner through the IGF-1/PI3K/Akt pathway [216]. Interestingly, type 2 diabetic mice, where PI3K and Akt activities are reduced, showed an increased FoxO phosphorylation concomitantly to Atrogin-1 and Trim63 upregulation [217], suggesting that the Akt pathway promotes muscle growth and simultaneously blocks protein degradation [218].

4. *Regulation by the Jak/Stat pathway*

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is a family of four tyrosine kinases associated with cytokine receptors. In response to cancer cells, different molecules such as cytokines, hormones, interleukines bind on specific type I cytokine receptors, thus activating the JAK/Stat pathway signalization [219].

When activated, this pathway plays a role in cell proliferation, differentiation, cell migration and apoptosis. It was notably showed that Jak-induced phosphorylation of Stat3 induces proteolysis with caspase-3 and ubiquitin-proteasome activation in two cancer-associated cachexia models [220]. Indeed, this study demonstrated that the component structure by caspase-3 serve as substrate for the ubiquitin-proteasome system. In addition, as described in the same study, Jak/Stat pathway also regulates muscle loss by activating CAAT/enhancer-binding protein delta (C/EBPd). Interestingly, the ubiquitin-proteasome components MuRF-1 and Atrogin-1 are stimulated via C/EBPd, which when knocked-down in mice, increase muscle mass and grip strength by decreasing myostatin and Atrogin-1 expression.

5. *Regulation by inflammatory cytokines and NF-κB signaling*

Muscle atrophy is directly associated with released inflammatory cytokines, notably TNF-α described as a potent actor during muscle wasting, previously known under the name "cachectin" [221]. TNF-α mainly acts through the NF-κB pathway that contains dedicated transcription factors responding in muscles to pro-inflammatory signals like TNF-α [222]. NF-κB activation is noticed in muscle atrophy-related ageing and cancer cachexia [220][221] demonstrated by *in vivo* analysis showing that mice lacking NF-κB subunits were resistant to muscle atrophy [225]. The role of NFKB in muscle atrophy remains partly controversial, as studies showed that it may activate the *trim63* promoter (Figure 16) [226], while other demonstrated that it could only stimulate *Atrogin-1* expression, but not *Trim63* [227]. Furthermore, an additional *in vitro* study suggests that TNF-a enhances muscle atrophy by inhibiting myocyte differentiation through a NF-κB activation [228].

6. *Regulation by glucocorticoids*

As mentioned above, glucocorticoids participate to muscle wasting. Indeed, glucocorticoid levels were found to be elevated in many pathological conditions, such as, sepsis, metabolic acidosis or insulinopenia and is associated with muscle loss as during cachexia [203][226]. Through their receptors, glucocorticoids activate *Atrogin-1* and *Trim63* promoters leading to an increase of their expression both *in vitro* and *in vivo* [213].

7. *Regulation by the Hippo pathway*

Hippo signaling pathway is described as the key regulator of organ development such as growth and size in mammals [8][227]. The interest of this pathway has begun to be considered in recent years more precisely for its involvement in cancer aggressiveness because of its involvement in cell proliferation, survival and metastasis associated with a poor prognosis, but also mostly for its mechanotransduction property. Mechanotransduction is defined as mechanical stimuli-to-biological activities conversion and is assumed by the Hippo pathway components [231].

Hippo core contains the "Yes-associated protein" YAP and its paralog TAZ (Tafazzin) ubiquitously expressed, notably in skeletal muscle, both acting as transcriptional co-activators of specific transcription factors: TEAD1-4 (TEA domain family members 1-4) [8][229]. Specific kinases such as MST1/2 (Mammalian Sterile 20-like kinase 1/2) and LATS1/2 (Large Tumor Suppressor 1/2) also belong to the Hippo family whose role is to negatively regulate YAP activity through cytoplasm sequestration after phosphorylation [10][90][230][231]. When active, YAP/TAZ interact with TEAD1-4 allowing the expression of specific target genes, such as *Ctgf* (connective tissue growth factor) and *Cyr61 (*cellular communication network factor 1) (Figure 15) [8]. However, YAP can also interact with other transcription factors that are not part of the canonical Hippo core. For instance, YAP can interact with p73 after DNA damage to induce the expression of *Puma* (p53 upregulated modulator of apoptosis).

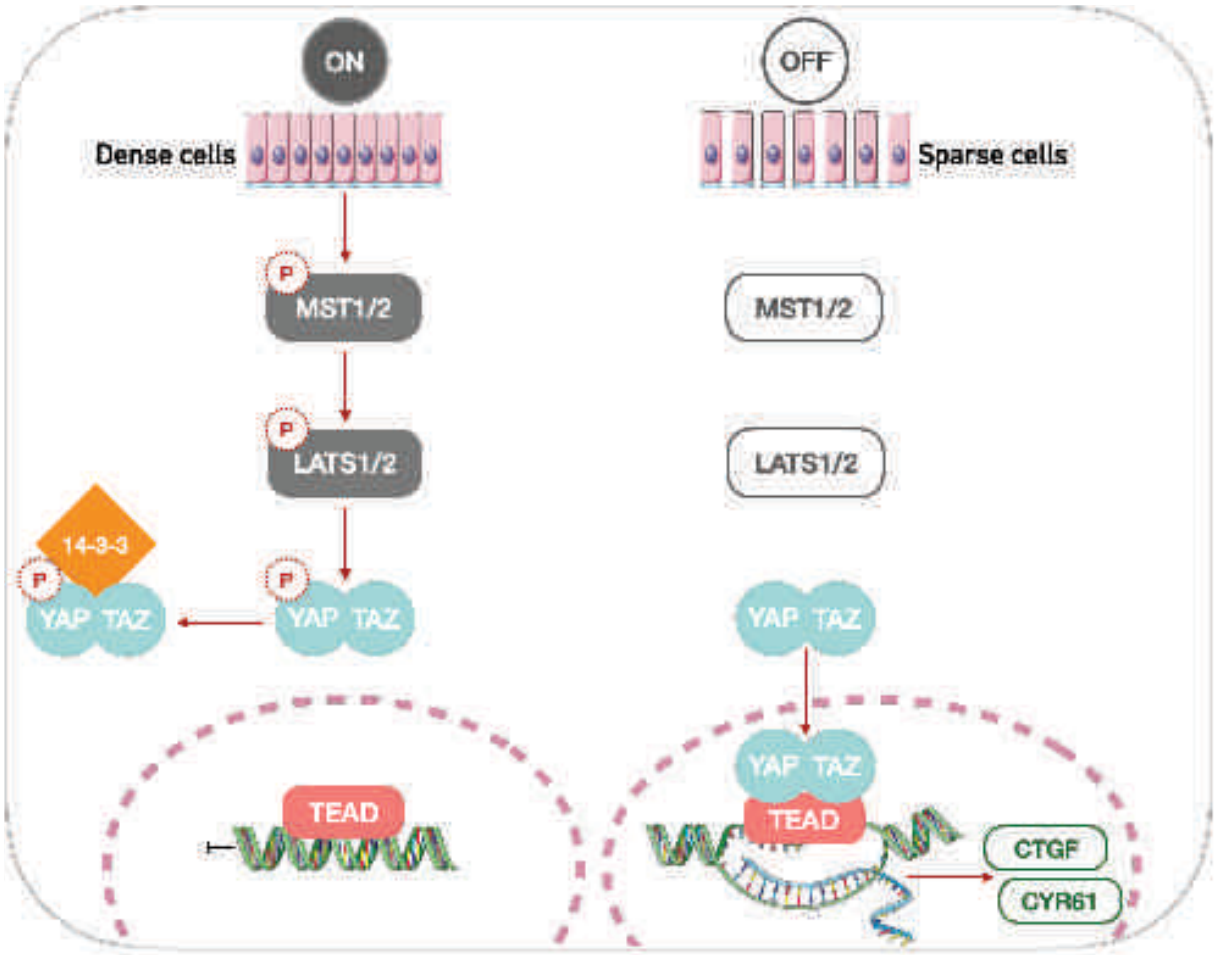

Fig.15 Schematic representation of the Hippo signaling pathway activation or inactivation according cell density.
Adapted from https://mechanobio.info

The Hippo pathway involvement has been particularly described to regulate muscle development, homeostasis and plasticity by acting on the transcription of the associated genes. For example, during heart size growth where it was found that cardiomyocytes of developing mice possess specific Hippo pathway' promoters [8].

It has also been demonstrated that hippo pathway components are positively implicated in skeletal muscle cells proliferation through a TEAD-dependent regulation of protein synthesis [230]. Indeed, YAP is expressed both in slow and fast muscle fibers of skeletal muscle [235], where *in vitro* and *in vivo* studies of murine myoblasts have demonstrated that YAP promoted the proliferation of Pax7 and MyoD positive muscle progenitor cells. Maturation of satellite cells also depends of the Hippo pathway because YAP knockdown induced a decrease of their proliferation [236]. However, it seemed that this loss of function had no impact on the

differentiation process, consistent with data reporting that YAP inactivation is required for myogenic differentiation. This is explained by activation of MST1 kinase expression during myoblast differentiation that consequently inhibit YAP activity [237]. The same expression profile has been noticed for TAZ, described to promote proliferation of muscle cells, but unlike YAP seemed to also induce myoblast differentiation by interaction with TEAD4 [238]. Indeed, muscle growth is affected in response to TAZ knockdown while only regenerative properties are impaired after specific YAP knockdown in satellite cells.

By the fact that YAP contributes to muscle mass development, it also means that aberrant expression leads to muscle pathology such as skeletal dystrophies [230]. Indeed, denervation-related atrophy is potentialized after YAP knockout in skeletal muscle cells [239]. Inversely, it has been reported that constitutive YAP expression through phosphorylation blocking is sufficient to induce hypertrophic phenotype through the transcription of myofibrillar genes by TEAD1-4 [7][8]. In addition, YAP expression is increased in response to mechanical overload such as stretch, strength training, hypertrophy compensation or exercise [240]. Also, YAP expression was linked to doxorubicin-induced senescence in muscle cells through its accumulation into nucleus. Interestingly, YAP inhibition drives cells to apoptosis instead of senescence due to a higher sensibility to doxorubicin administration [241]. It is explained by the high expression of the pro-survival protein survivin in senescent cells, suggesting that YAP protects senescent cells from death through an anti-apoptotic mechanism, and subsequently, YAP deletion leads to the opposite effect.

The role of the Hippo pathway in muscle atrophy was confirmed by Judson and colleagues, who highlighted in 2013 the link between the hippo pathway and the ubiquitin proteasome system (Figure 16). Unexpectedly, they observed that a constitutive activated YAP mouse model developed symptoms of atrophy such as necrotic fibers and muscle degeneration [8], consistent with elevated MuRF-1 and Atrogin-1 expressions. This suggests that YAP constitutive activation in adult tissue induces an atrophic phenotype instead of provoking hypertrophy. However, these unexpected results were discussed by another study demonstrating that YAP overexpression is sufficient to generate tissue hypertrophy consequently consistent with the downregulation of MuRF-1 expression and its pro-atrophic activity [7]. Moreover, an upregulation of MST1 after denervation clearly represses YAP, in

correlation with an increase of atrogenes expression mediated by Foxo3a phosphorylation [242]. Taken together, these discrepancies between different studies highlight the necessity to better understand the precise involvement of the Hippo pathway during skeletal muscle atrophy process.

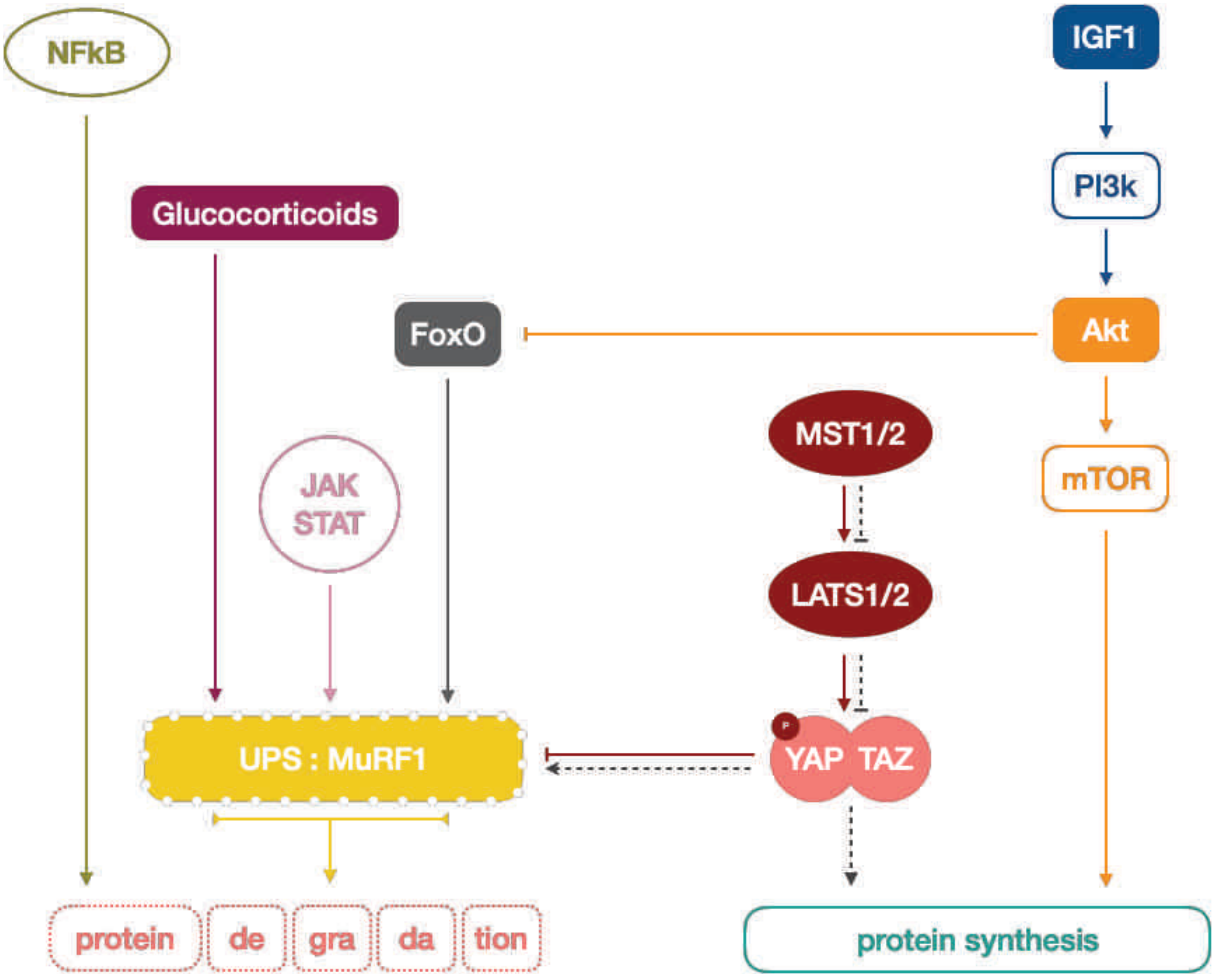

Fig.16 Major pathways involved in the balance of protein synthesis and degradation.
Inspired from Sandri 2008 and Schiaffino et al., 2013.

Taken together, the ability of the different signaling pathways and transcription factors to cooperate in the modulation of Trim63 and Atrogin-1 may explain the variable expression patterns observed depending on the source of the muscle atrophy.

G. Therapeutic opportunities

Cancer-associated cachexia is a multi-factorial syndrome, suggesting that it requires a multidirectional treatment keeping in mind both the cancer type and the cachexia stage (Figure 17).

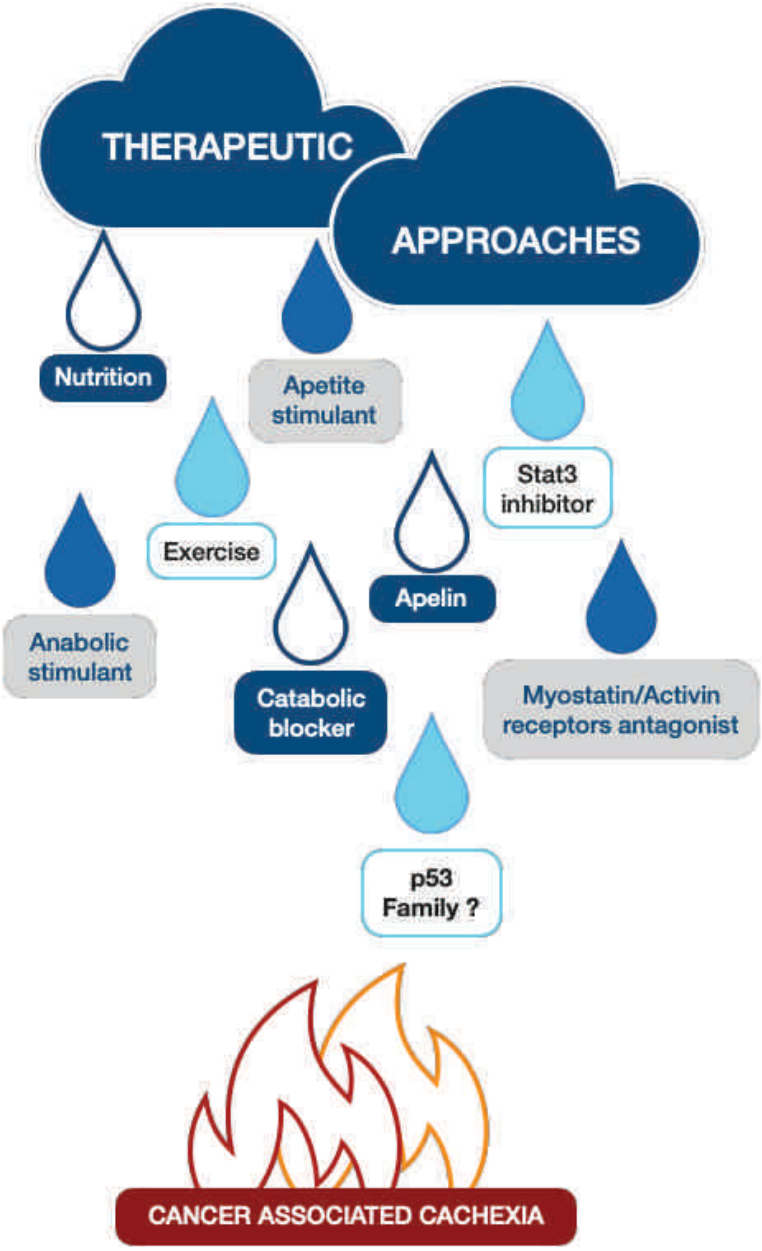

Fig.17 Illustration of therapeutic approaches developed to counteract cancer-associated cachexia.

Unfortunately, it was proved that cancer-associated cachexia could not be totally restored by only a classical nutritional support. This is why exercise, when possible, with nutritional supplements are used. Indeed, first approaches were investigating supplements such as sterile liquids, semi-solids or powders in order to provide micro and macronutrients [158][240]. Patients are also accompanied by nutritional healthcare professional to improve the quantity and quality of their food intake. Drugs that may help to stimulate appetite are being testes, such as anamorelin, which is an analog of ghrelin, showed positive results with food intake increase and anti-inflammatory effects [241][242]. However, in the case where calorie deficit is too important (more than 1200 kcal/day) active nutritional management is used through enteral and/or parenteral nutrition. In addition, appetite stimulants such as orexigenic drugs have been developed to restore appetite in cachectic patients [160].

More recently, alternatives were developed based on the better understanding of the molecular mechanisms triggering cancer related cachexia and were or are subjected to clinical trials. For example, studies using cytokine pathway inhibitors such as Progestogens (megestrol acetate and medroxyprogesterone), catabolic pathways blockers such as ecosapentaenoic acid [246] or stimulating anabolic pathways (oxandrolane) in skeletal muscle. For instance, it was shown that myostatin and TGFB (Transforming growth factor beta) family members blocking was able to reduce cancer-associated cachexia symptoms [159]. Similarly, SMARs molecules for selective androgen receptor modulators, derived from testosterone for its anabolic properties, showed promising results in phase III clinical trials by increasing lean body mass in cancerous patients [247]. Furthermore, it was shown that a small-molecule Stat3 inhibitor could have therapeutic properties due to its cancer-cachexia preventing effect. Indeed, Stat3 inhibition was successfully blocking cancer-mediated muscle wasting [248]. A recent study also demonstrated that the endogenous peptide generated by muscle contraction named Apelin where importantly decreased in muscle atrophy. Indeed, mice lacking apelin had dramatic alteration in muscle functioning, however, a specific peptide that restored apelin gives promising results through the enhancement of muscle-related pathways in myofibers and regenerative capacities [249].

The myostatin/activin intrinsic pathway was also investigated through a designed ligand that target activin receptor: sACVR2B-Fc. Indeed, serum level of myostatin/activin have been reported to be increased in cachectic patients [250]. This molecule has shown decreased doxorubicin-related cachectic effects by decreasing MuRF1 expression, without interfering with anticancer drug [251]. It was confirmed by Fisher and colleagues [252] through pre-clinical and clinical studies that clearly confirmed the potential effect of soluble receptor antagonists of myostatin/activin, not only preventing muscle wasting but also prolonging overall survival. In this sense, other myostatin specific antibody indicated promising results in clinical trials [250].

Unfortunately, one of the most predictable therapy which involved an inhibition of synthesis or release of cytokines (eicosapentaenoic acid-EPA) and proteasome inhibitor (bortezomib), showed; despite their positive effects in animal models; surprisingly disappointing results in human clinical trials [250][251]. Indeed, corticosteroids are frequently used and indeed show improvement in appetite, however, long-term exposure leads to inverse side effects, such as protein breakdown and insulin resistance [255]. More recently, considering inter-organ metabolism such as the crosstalk between bone and skeletal muscle could be an option for novel therapeutic drug design. Indeed, it was shown that bone-derived factors play an important role in skeletal muscle cachexia during cancer.
In this respect, gut microbiota which emerges as an essential entity involved in unsuspected functions could also represent an investigation field [159].

However, despites all these interesting leads for treatment, there at the moment no therapeutic approved drug to treat or prevent cancer cachexia in clinic. Clearly, cancer associated cachexia remains a field that needs to be explored, as despite numerous efforts it is still a non-curable syndrome. That is why it is necessary to further profoundly investigate the molecular pathways in order to gain a better and comprehensive understanding of the underlying mechanisms. An extremely well described family, which in relationship to muscle wasting still almost unexplored, is the p53 family and its up- and downstream effectors. Besides a considerable amount of studies showing its involvement in the most indispensable molecular pathways (development and cellular and tissular homeostasis), increasing evidence point out the p53 family as an essential regulator or modulator of development and muscle atrophy.

V. P53 family and muscle

Multiple in vitro studies have evidence that the p53 family plays a role in muscle development by controlling muscle cell differentiation, proliferation and survival. Surprisingly, deletion of the p53 family members have different reported effects. Indeed, mice deleted for total p53, p63 or p73, as well as selective TA or ΔN isoform displayed no obvious defects during muscle development [253][254]. However, these results were discussed by Porrello demonstrating that p53 null mice do not display any muscle impairments whereas *in vitro* myoblast failed to induce Rb when p53 is deleted [258]. It suggests a non-essential role of p53 in muscle development or compensatory mechanisms by p63 and/or p73 [259]. In addition, p63 and p73 null mice display developmental defects as described before, but do not seem to have severe muscle alteration [260]. However, it was also questioned by Cefalu in 2015 showing that TAp63 knock out cells displayed alteration in myotubes fusion [261].

In addition, p53 family members importance in muscle development is clearly demonstrated by the study of Huttinger-Kirchhof in 2006 showing that overexpression in the muscle of a dominant negative isoform ΔNp73 strongly alter skeletal muscle development [262]. Furthermore, the p53 family has proved its importance during cardiac development where p63 is the most implicated member but also in skeletal muscle physiology revealing a new role of the p53 family [263].

Hence, the p53 family has been linked to the development and pathologies of different types of muscles that are further detailed bellow.

A. Cardiac muscle

Despite its role in skin morphogenesis and epithelial stem cell maintenance, p63 was also described to be essential during cardiogenesis, notably in heart myogenesis, differentiation [255][261] and more precisely during heart development [265], where its pro-apoptotic isoform TAp63 is a major actor. TAp63 is expressed during early phases of endodermal cells development and modulates the expression of endodermal factors [263]. p63 also plays a crucial role in cell fate by regulating the proliferation and survival of cardiac progenitor cells [262][263]. It was show that mice lacking p63 have serious cardiac embryonic

development defects after histological analysis. Indeed, p63 knocked down mice displayed thickness problems within ventricular walls, ventricles dilatation, septation and trabeculation abnormalities and also reduced myofibers content [263].

In addition, to understand the underlying mechanisms of p63 in the muscles, further experiments were carried out using $p63^{-/-}$ embryonic stem cells (ESCs). In accordance with the *in vivo* results, absence of p63 resulted in downregulation of the early cardiac progenitor markers such as Tbx5 (Tbox 5), Nkx2.5 (NK2 Homeobox 5) and isl1 (Insulin Gene Enhancer Protein), as well as mature cardiomyocyte markers such as TroponinT2, α-Actinin and Mlc2v (Myosin regulatory light chain 2). Concomitantly, the ESCs were unable to differentiate into beating cardiomyocytes [263].

B. Skeletal muscle

Importantly, within skeletal muscles, the balance between TA and ΔN-isoform expression influence on developmental and differentiation processes [115]. However, the exact role of the p53 family in embryonic skeletal muscle context is still a little unclear.

1. Differentiation

Physiologically, skeletal muscle differentiation involves the commitment of muscle embryonic progenitors, which under Pax3 and Pax7 influence express the myogenic regulatory factors: Myf5 and MyoD. Satellite cells stay quiescent or are differentiated into myoblast and start to proliferate after further expression of Myf5, MyoD, MyoG/myogenin, and Mrf4 [267].

Differentiation process end occurs when proliferating myoblasts enter in an irreversible cell-cycle step. Tamir and colleagues [268] were in the first scientists to show that p53 is expressed during myoblast differentiation and is necessary for induction of new muscle differentiation-specific genes, such as myosin heavy chain (*myHC*) or muscle creatine kinase (*Mck*) allowing the fusion of myoblasts into myofibers.

Later, it has also been demonstrated that p53 contributes to skeletal myocytes differentiation through expression of its target genes *p21* and *p57* expressed that prevent cell-

cycle re-entry [259], but also through its cooperation with Rb (retinoblastoma tumor protein) by interacting together [258].

Rb is essential during embryogenesis cell death, proliferation and differentiation of skeletal muscle, more precisely required for the last step of myogenesis [259]. It has been shown that when p53 expression is deleted, Rb activity is impaired leading to an arrest of cell proliferation. Interestingly, it appears that the other p53 family members also are involved in muscle differentiation through ΔNp73α that inactivates differentiation, and since TAp63γ and TAp73β were reported to counteract p53 deletion by transactivating Rb.

In this sense, the role of p73α has been described to delay myogenic differentiation by leading to a decreased MyoD transcriptional activity, highlighted by a repression of its target genes, such as *p21*, myHC and MyoG [269].

About p63, its pro-apoptotic isoform TAp63, was described to be involved in myocytes fusion, since its knockdown showed an important reduction of fused myotubes *in vitro* [261].

Surprisingly, despite all previous reported findings, some studies questioned the role of p53 during muscle development and stipulated that p53 is not essential for muscle development, myogenic potential and regeneration [267][268][272]. Indeed, p53 null mice showed no drastic impairments in skeletal muscle development and $p53^{-/-}$ myoblasts were able to differentiate *in vitro* into myofibers. Furthermore, Cam and colleagues showed that myogenesis inhibition by TNF-α was dependent of a p53 signalization and promoted activation of the PW1 mediated pathway to block the differentiation of myogenic cells. In comparison, when a p53 specific-described inhibitor; pifithrin; is used, the differentiation is restored in a TNF-α context highlighting another role of p53 as an essential actor in the myogenesis blocking through TNF mediation [259].

Findings can be summarized as: p53 positively regulates RB by inducing its transcription, whereas ΔNp73α inactivates it through phosphorylation and impairs cell differentiation. Surprisingly, it was shown that p53-mutated C2C12 myoblasts formation was severely impaired due to a missing induction of RB [258]. They also showed that p63 and p73, more precisely TAp63γ and TAp73β, are able to compensate p53 deletion and interfere with Rb by transactivating it through its hypophosphorylation [259]. This fact was already demonstrated by Fontemaggi and colleagues in 2001, showing that, in addition to p53, p73 is implicated

during muscle differentiation [264]. However, against all odds, p53 is not considered as an essential actor during muscle development and regeneration as described before, because of p53 null mice showed no drastic impairments in skeletal muscle development [267][268][272].

2. *Regeneration*

Similarly to differentiation, the role of the p53 family in muscle regeneration is little studied. However, Yun and co-workers [273] showed that p53 expression was observed during myogenic regeneration in salamander limb, as well as in regenerating axolotl limbs, strict p53 regulation is required for muscle differentiation. Indeed, pifithrin use for inhibiting p53 or expression of ΔNp73 (dominant negative in this context) prevents myotube formation *in vitro* that result in impaired limbs regeneration. The authors hypothesize that ΔNp73 regulates this process, as its expression pattern is inverse to that of p53 *in vivo*. However, other molecular analyses indicate that the proliferation of myoblasts and the fusion of myotubes in regenerating adult $p53^{-/-}$ skeletal muscle of C57Bl/6J mice are normal [272], indicating that p53 might also not be essential in mice or during this specific muscle injury recovery.

Taken together, those results indicate that the members of the p53 family cooperate in a complex network to control myogenic differentiation and regeneration. Unfortunately, neither of the groups seemed to have pursued their work on these questions and the p53 family since. Thus, a straightforward mechanism of the involvement of the p53 family in these myogenic processes cannot be proposed.
More than in differentiation and regeneration, the p53 family, more precisely p53 and p63, also play a role during muscle metabolism.

3. *Metabolism*

p53 has been described to be involved in the regulation of mitochondrial biogenesis of skeletal muscle [274]. The metabolic responses mediated by the p53 family are associated with their ability to promote cell survival, modulating responses that compromise the cell energy production and consequently cell fate. For instance, low rates of nutrients or energy

directly affect p53 stability. For example, in response to metabolic stress like nutrient deprivation signals or O_2 availability, p53 is activated and phosphorylated by AMPK and directly interact with the LKB1 (Liver kinase B1) pathway leading to a decrease in cell growth mediated by *p21* expression [272][273]. Indeed, glucose limitation drives to a reversible cell cycle checkpoint [274][275], that promote cell survival [279].

During the metabolic changes occurring within the muscles, p53 reacts to energy production variation. Indeed, ATP and ADP (Adenosine diphosphate) rations can modulate the ability of p53 to bind to DNA, ADP in a positive and ATP (Adenosine triphosphate) negative manner [280]. Additionally, p53 negatively regulates the expression of glucose transporters in myotubes [281], modulates mitochondrial functions and promotes its biogenesis as an adaptation to exercise in skeletal muscle. These effects can be observed in knockout mice for p53, which presented reduced exercise performance due to impairment on oxygen consumption [282]. Furthermore, p53 regulates the expression of Sirt1 (Sirtuin 1), a major actor of muscle development, metabolism and aging [283].

Other p53 family members can also play a role in response to starvation in muscles. In this case, TAp63 appears to be involved in muscle homeostasis due to its regulatory function on Sirt1 leading to ATP production via the tricarboxylic acid (TCA) cycle in order to ensures cells energy supply and prolong survival [284]. P63 actually seems to be a critical regulator of lipid and glucose metabolism. Indeed, Su and colleagues [284] observed metabolic disorders and lipid deposits in several tissues of TAp63 null mice. They also showed that TAp63 regulates the Sirt1/AMPK/LKB1 pathway, key metabolic regulators, which modulate metabolic adaptation in skeletal muscles by balancing the catabolic and anabolic activity. Indeed, Sirt1 is increased during proliferation and myogenesis, promoting satellite cells proliferation [285], however, in response to nutrient deprivation, Sirt1 has a p53-dependent negative effect on myogenesis.

Additionally, ablation of p63 also affected mitochondria biogenesis, altering cardiac function and resulting in impaired excitation-contraction coupling [263].

C. P53 family and muscle atrophy

As described before, the p53 family owns major roles during muscle homeostasis. However, the p53 family members are also described during pathological context such as muscle atrophy where they modulate muscle wasting in different situations. Indeed, muscles can adapt according to the demands of the body in order to optimize its function of sustaining and promoting movement and the p53 family members have been linked to the adaptive processes of atrophy/hypertrophy.

1. Cancer non-associated muscle atrophy

Fox and colleagues in 2014 showed that p53 was overexpressed in skeletal muscles during immobilization [286]. More precisely, they demonstrated that the augmented p53 level appears as an early event of muscle immobilization, associated with decreased size of muscle fibers, and that p53 null mice were more resistant to immobilization-induced atrophy, showing the capacity of p53 to induce muscle atrophy [286]. This protective effect was potentialized by the concomitant deletion of ATF4 (Activating transcription factor 4) expression, a transcription factor also described to be upregulated after limb immobilization.

Their results were validated by Stocks and colleagues [274] showing that p53 knock-out mice were protected against atrophy, whereas in non-mutated mice increased p53 expression contributes to muscle wasting through an induction of *p21* gene.

In this sense, it was shown that specific apoptotic-response through p53 targets genes expression happens during muscle atrophy. For example, Bax protein (Bcl-2–associated X protein) which owns pro-apoptotic properties was increased [284][285][286] while the pro-survival protein Bcl-2 (B-cell lymphoma 2) was decreased in chronic heart failure or plexus injury-related atrophy, indicating an apoptosis mechanism [289].

Indeed, Dupont-Versteegden and colleagues summarized the work of numerous studies about it [290]. It was in particular proved that caspase-3 dependent pathways have an important role during muscle wasting. In this respect, caspase-3 participates in *in vitro* myotubes apoptosis [291] where its activity or activated protein level was increased in muscle atrophy models such as during heart failure, limb immobilization or in response to burns [289][289][290]. Caspases activation is the initial proteases of myofibrillar protein breakdown

[291][292]. Next to caspase-3 involvement, it was highlighted that caspase-9 and -7 expressions were upregulated in muscle fibers in response to human muscle denervation [296]. Interestingly, their expressions showed segmental localization within myofibers suggesting that all fibers do not enter in apoptosis at the same time. To continue, it was considered that caspase-3 induction, in addition to m-calpain expression (muscle-specific).

Interestingly, we underlined the p53 family relation with muscle atrophy showing that p63 is upregulated in amyotrophic lateral sclerosis and able to directly modulate Trim63 (Murf1) expression [6]. Amyotrophic lateral sclerosis is a neuromuscular disease, characterized by the loss of motor neurons and concomitant atrophy of skeletal muscles, leading to paralysis and ultimately death [297]. Recently, Grabowiecki and colleagues have shown that the p53 family, and more particularly p63, can have an important role during muscular atrophy [6]. Indeed, they have also shown that p53, TAp63 and TAp73 mRNA (messenger ribonucleic acid) are induced during muscular atrophy in a mouse model (SOD1-G86R) of ALS that reproduces the symptoms. P53 family target gene expression correlated with the severity of atrophy in ALS patients and disease progression in the mouse model. According to this work and similarly to myoblast differentiation, p63 seems to have the most important function between the p53 family members in the context of ALS.

In addition, the group have identified a new muscle-specific function of p63 in the context of muscular atrophy. They identified putative binding sites for the p53 family in the promoter region of the ubiquitine ligase Trim63 (Murf1), an enzyme that triggers muscle protein degradation. While p53 and TAp73 had less effect, when overexpressed in C2C12 myoblasts, TAp63 binds to the promoter and strongly activates the transcription of Trim63. On the contrary, knockdown of TAp63 resulted in a lower expression of Trim63 in a basal state and following cellular stress induced by the mitochondrial uncoupling drug FCCP (Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone), highlighting the physiological importance of p63 in the context of muscular atrophy [6].

2. *Cancer-associated muscle atrophy: cachexia*

An important part of muscle atrophy is directly linked to cancer and various studies highlighted that the p53 pathway is also active in skeletal muscle during cachexia. The p53 involvement during muscle atrophy lies in the fact that it is usually mutated or chronically activated during cancers leading the hypothesis that p53 could be at the origin of cancer-related cachexia.

It was shown that p53 is required during cachexia where its hyperactivation promotes muscle atrophy *in vivo* [3][267]. Indeed, p53 plays a role in response to anticancer drug since it was demonstrated that its expression in myocytes was related to doxorubicin treatment [209][298][299][300]. As mentioned before, doxorubicin induces CAC through different mechanisms notably ROS production [301] or increase of the TNF-α level [302] and it appears that p73 is required through its pro-apoptotic role after pro-inflammatory cytokines release such as TNF- in vascular smooth muscle cells [303]. Indeed, *in vitro*, as well as in regenerating muscles *in vivo*, TNF-α inhibits myogenesis and differentiation, downregulates the myogenic factors MyoD and MyoG [304], and upregulates Atrogin-1 expression, leading to muscular atrophy [305]. In this sense, it also seemed that p53 was necessary to TNF-α mediated inhibition of myogenesis because of p53 null mice becoming less susceptible to develop muscle atrophy.

Interestingly, a new role of the p53 family in cancer-associated cachexia was pointed out by Schwarzkopf and colleagues in 2006 in their study by evidencing the link between the p53 family and the ubiquitin proteasome pathway [270]. Indeed, p53 is known to interact with the ubiquitin proteasome pathway since a long time because of Mdm2 (mouse double minute homolog 2) which is the primary E3 ubiquitin ligase protein that negatively modulate its expression by ubiquitination [306]. In their study they demonstrated the ability of the p53 family to interact with other E3 ubiquitin-ligases, such as atrogenes. They demonstrated that C26 tumor-induced cachexia was attenuated in p53-deficient mice since they observed that weight loss, muscular atrophy and Atrogin-1 expression were markedly reduced, compared to p53 wild-type mice.

These results highlight an important role of p53 in the regulation of muscular atrophy. The activation of a p53 response in muscle cachexia results in an opposite outcome than the mechanisms observed in muscle development. The p53 family activity seems necessary for correct differentiation and the prevention of aberrant cell proliferation, therefore exhibiting a beneficial function in muscles.

PROBLEMATIC & THESIS OBJECTIVES

The subject of my thesis was to investigate the role of the **p53 family** in molecular mechanisms of the **side effects** caused by **anticancer drugs on healthy tissues**. To do so, I focused on two systems that were previously established as causing health issues for patients:

1) the toxicity induced by **cisplatin** on the **enteric nervous system**,
2) the toxicity caused by **doxorubicin** on **skeletal muscle**.

To be as closer as possible to the clinical context, I made the choice to use as much as possible ***in vivo* models**, which included myenteric nervous system *ex vivo* cultures (Figure 18) or *in vivo* tissues, and gastrocnemius tissues of mice models. In addition, I used pertinent *in vitro* models to answer for mechanistic questions when necessary.

In complement to the investigation of the p53 family, I performed **transcriptomic (RNA seq)** experiments associated with **bioinformatics analyses** in these two systems in order to identify unexpected deregulated pathways that might include therapeutic targets or provide information on the molecular networks that might be connected to the p53 family. The use of *in vivo* experimental models allowed me to address the problematics of my thesis with a closer possibility to consider potential therapeutic solutions.

My project was divided into two parts:

A. Characterize the role of the p53 family in the impact of cisplatin on enteric nervous system homeostasis. This part included 4 specific objectives:

1. Determine if the p53 family is expressed within the ENS and which members.

2. Investigate the cell subtypes where p53 family members are expressed and how their expression participates to neuronal loss.

3. Identify precisely the chronologic events of cisplatin toxicity (cell death, proliferation, quiescence).

4. Performed a transcriptomic approach to analyze the pathways that are deregulated.

B. Characterize the expression of the p53 family members in skeletal muscle during doxorubicin-induced cachexia.

1. Identify which p53 family member is the most involved in cancer-associated cachexia.

2. How the p53 family members expression modulate pro-atrophic factors such Trim63.

3. Determine if other signaling pathways would participate into that regulation by using a transcriptomic approach.

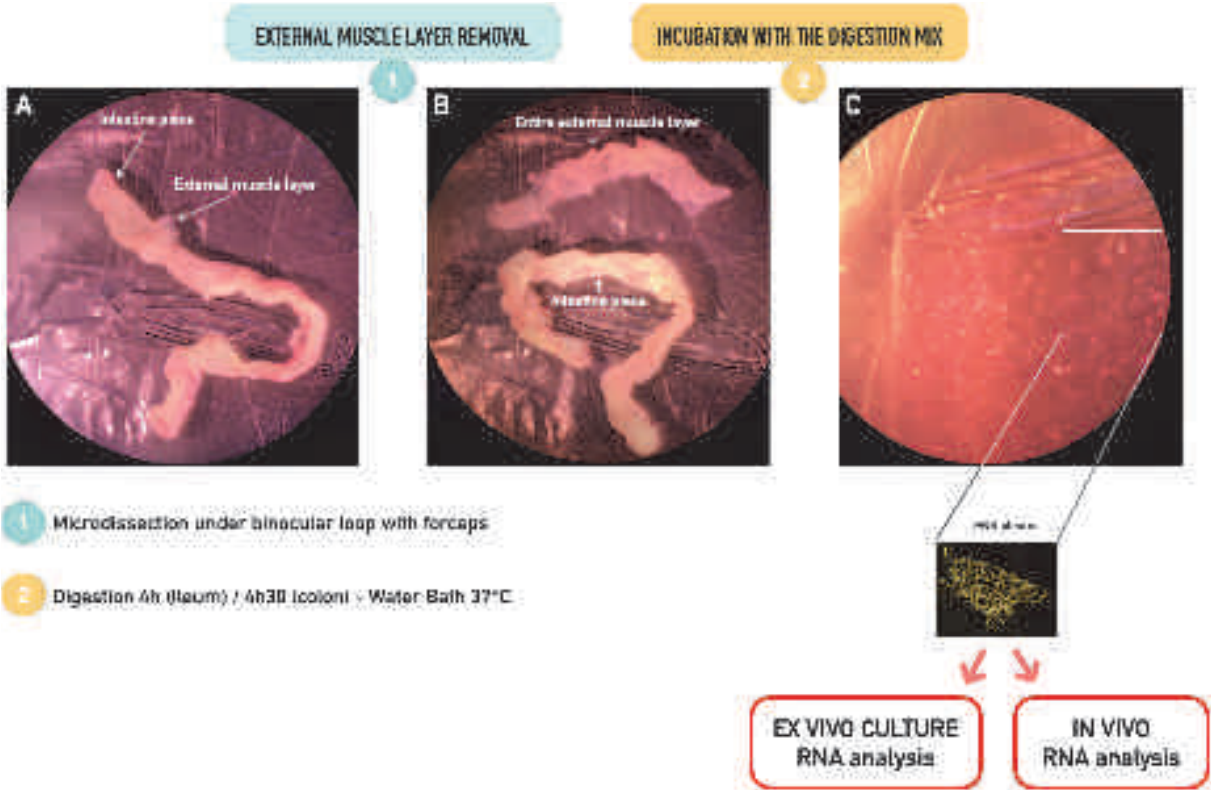

Fig.18 Myenteric plexus dissection and isolation.

A. Removal of mouse gastrointestinal tract from the lower part of the stomach until the rectum and separation of the external muscle layer that comprises ENS (1).

B. Entire removal of external muscle layer and sectioning into 0.5cm pieces for digestion (2).

C. Isolated myenteric plexus after fat and muscle cell destruction and fat

Central nervous system comprises brain and spinal cord nerves. Peripheral nervous system is divided into somatic and autonomic nervous systems itself giving sympathetic and parasympathetic nervous systems but also enteric nervous system.

RESULTS

My PhD work contributed in two projects that will be summarized in two publications about to be submitted.

The first publication is the unique characterization of the p53 family in the enteric nervous system, and its role in the response to toxic stress induced by cisplatin. This work provides the first evidence that p73 pay participate in the neuronal apoptosis in the enteric nervous system. Our results also suggest activation of enteric neuron regeneration and neurogenesis processes, following the cytotoxic impact of cisplatin.

The second highlights the modulation of Trim63 (Murf1) by the p53 family in muscle atrophy induced by doxorubicin. We characterized the role of p63 in the physical and functional modulation of Trim63, both in mouse myoblast cells and mice models. This study also investigated the potential other molecular pathways involved in doxorubicin-induced cachexia.

Enteric neurons develop a dynamic and coordinated pro-apoptotic and regenerative transcription program in response to cisplatin toxicity: role of the p53 family

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Running title: Role of p53 family in the ENS

Keywords: p53, p63, p73, apoptosis, enteric nervous system, cisplatin

Highlights:

- The cisplatin-based chemotherapy induces apoptosis in enteric nervous system
- Transcriptomic analysis of ENS treated with cisplatin indicates highlights activation of p53-related target genes
- ENS expresses p53, p63 and p73
- Apoptosis induced by cisplatin correlates with increase p53 and p73 expression as well their target genes
- Inhibition of p53 and p73 activity decreases cisplatin induced p53/p73 target genes.

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The authors declare no potential conflicts of interest

Abstract

The inability of the current therapies to be perfectly selective towards cancer cells leads to toxic effects on healthy tissues that hinder patient's quality of life and force eventually the clinicians to stop the anticancer treatment. It has been proposed that long lasting chemotherapy-induced gastrointestinal (GI) dysfunction is caused by neuronal loss in the enteric nervous system (ENS), but the exact mechanism has not been identified yet. We performed a transcriptomic analysis on ENS myenteric plexus of mice treated with cisplatin that revealed dynamic and coordinated alterations in pathways that account for ENS plasticity. For instance, apoptotic pathways involving the p53 family (p53, p63, p73) are transiently induced 1 day after treatment, while processes involved in neuronal plasticity are rather induced later on. In contrast, inflammation pathways are induced rapidly and maintained up to 7 days after treatment. *In vivo* and *ex vivo* analyses confirmed that cisplatin induces apoptosis in enteric neurons characterized by cleaved caspase 3, AIF relocalization and induction of pro-apoptotic genes (ex. *puma*), which correlated with expression of p53, p73 and p63. *Ex vivo* and *in vivo* loss of function experiments established further the implication of the p53 family during apoptosis in the ENS. Following cell death, neuronal plasticity processes were established *in vivo*, characterized by cell proliferation and labelling of neuronal/glial precursors. Altogether, these results established a cascade of events induced by chemotherapies in the ENS causing neuronal loss and regeneration that may contribute to digestive impairment.

Introduction

The management of cancer remains a difficult challenge due to the extreme adaptability of the cancer cells to stress and their intimate interconnection with healthy tissues within the organism that favor their survival and impact negatively on patient's physiology. For instance, clonal selection leads quickly to resistance against most targeted therapies. Hence, basic cytotoxic chemotherapies (i.e. oxaliplatin, cisplatin) that present less selectivity toward cancer cells remain the most frequent approaches to treat cancer despites their toxicity on healthy tissues. Although used on daily-basis to help patients, the efficiency of supportive care is unsatisfactory, inducing itself severe side effects (i.e. arrhythmia, heart failure), and therefore chemotherapies' toxicities often decrease patient's quality of life and lead eventually to dose limitations and/or total cessation of anticancer treatment, hindering patient's survival [1-5]. This is typically the case for cisplatin that is used to treat multiple cancers, including head and neck, testicular and lung cancers.

One of the most common side effects of anticancer chemotherapies is the alteration of function of gastrointestinal (GI) tract, characterized by diarrhea, nausea and constipation [6]. Furthermore, in contrary to what is frequently assumed, these side effects are increased by combining targeted therapies with chemotherapies [7]. If some of these alterations (i.e. diarrhea) coincide perfectly with the cure, chemotherapy leads also to long-term disfunctions up to 10 years after treatment (e.g. constipation) [8]. The etiology of these pathophysiological processes is still poorly known. Obviously, the well-known negative impact of the various chemotherapies on the highly proliferative epithelia of the digestive tract may account for the acute side effects of the drugs [9, 10], but likely not for the long term disfunctions. One alternate hypothesis that emerged proposes that GI side effects may be mediated by degradation of the enteric nervous system (ENS) integrity. Indeed, another important side effects of anticancer chemotherapies is neurotoxicity on the peripheric and central nervous system [11 12 13-16].

The enteric nervous system (ENS) is the most complex division of the peripheral nervous system specific of the gastrointestinal tract (GIT), controlling both secretion/absorption and motility [17]. It comprises neurons and enteric glial cells encompassed into ganglionic structures organized into two plexuses, the myenteric (motility control) and the submucosal (absorption control) plexus. ENS contains around 400 to 600 millions of neurons, classified in 15 different subtypes according their morphology, expressed neurotransmitters, and electrophysiological properties. Enteric glial cells provide support to neurons but also regulate synaptic transmission and maintenance of the epithelio-intestinal barrier. This strong and dynamic system is affected by several pathologies specific of the GI tract, such as inflammatory and congenital bowel diseases, but also neurodegenerative

pathologies, such as Parkinson. In this later case, neuronal loss and enteric glial cells are suspected to be involved in the pathology initiation [18-20]. In the adulthood, ENS displays a permanent plasticity characterized by a balance between neuronal apoptosis and regeneration [21, 22].

In addition to these pathologies, ENS is impacted negatively by chemotherapies, which contributes significantly to the gastrointestinal dysfunctions [23]. For instance, Vera et al. [24] and Wafai et al. [25] have shown by immuno-histological counting in mouse and rat that a repeated exposure of cisplatin or oxaliplatin reduces the neuronal population and the ganglion size in the ENS, particularly 14 days after oxaliplatin administration, causing enteric neuronal loss with colonic motility reduction. The impact on the neuronal population is selective, as HUC/D-positive neurons have their number reduced, in contrast to NOS-positive neurons that increase in number. In contrast, 5-Fluorouracyl (5-FU) induces neuronal loss of both PGP9.5 and NOS-positive neurons after 7 days of chronic treatment [26]. Surprisingly, a pilot study performed in patients showed an increase in number of HUC/D- and NOS-positive neurons associated with abnormal neuronal excitability after anticancer treatment [27]. This discrepancy is likely caused by the heterogeneity of group of the treated patients encompassing patient of different age and sex and with different types of treatment, highlighting the limits and difficulties to perform such studies in human.

In addition to motility dysfunction, it has been hypothesized that chemotherapy-induced (e.g. 5-fluoruracyl, Irinotecan) ENS neuronal loss may cause alimentary mucositis, which occurs in 40% case of standard dose and 100% in high dose chemotherapy [28-30]. Alimentary mucositis is linked with mucin expression and secretion, physiologically involved in the protection of the gastrointestinal tract from stress, bacteria as well as penetration or digestion of mucosa. Hence, it has been proposed that ENS neuronal loss may alter the production and secretion of mucin by Goblet cells of the intestinal epithelium through changes in secretion of the neurotransmitter VIP (vasointestinal peptide) [31].

Neurons are not the only cell type affected by chemotherapies. A recent study demonstrated that enteric glial cells (EGC) are also impacted by platinum treatment [32]. For instance, immunostaining with specific glial makers showed a decrease of the proportion of GFAP (glial fibrillary acidic protein positive EGCs and an augmentation in number of the S100beta-positive EGC, another glial marker specific of cell damages and apoptosis, after a longer-term exposure (7 and 14 days).

If the negative impact of various chemotherapies on ENS neurons and glial cells is now relatively well established, the molecular mechanism and signaling pathways involved are almost completely unknown. A pioneer study recently investigated the role of PARP1 and PARP2 in the neuronal loss [33, 34]. It showed an elevated expression of PARP1/2 in the ENS in response to oxaliplatin, which

correlated with release of cytochrome C, suggesting mitochondrial defect. The use of the PARP inhibitor BGP15 avoided in part nChat- and nNOS-positive neuron loss and restored some of gastrointestinal functions. Despite this interesting information, whether PARP expression, mitochondrial disfunction or even apoptosis takes place in the neurons upon oxaliplatin treatment was not determined, neither the pathways that may be involved.

In order to decipher the molecular mechanisms involved in neuronal loss in the ENS upon chemotherapy we used a transcriptomic approach to identify the deregulated genes in myenteric plexus isolated from mice treated with an acute dose of cisplatin and extracted 1 day or 7 days after treatment. Following bioinformatics analyses of deregulated pathways, immunohistochemical and functional validation where performed to establish chronology and importance of selected molecular events that may affect ENS function

Materials and methods

Animal models

TAP63$^{CRE/+}$ mice were generated by the Clinique de la Souris (Strasbourg) by introducing the CRE recombinase cDNA after the TAp63 promoter. The ROSA26-tomato mice that contains the cDNA for the fluorescence protein Tomato after a LoxP flanked STOP cassette. By crossing these two mouse lines TAp63$^{CRE/+}$:ROSA26 mice were generated in house. Wild type C57BL/6, TAp63$^{CRE/CRE}$ or TAp63$^{CRE/+}$:ROSA26 C57BL/6 mice of 10 weeks were weighed and intraperitoneally injected with 10 mg/kg cisplatin in 200 µl 0.009% saline. Mice were weighed on a regular basis. Treated mice were sacrificed either 1, 7 and 21 days after treatment. Small intestines and colons were taken out for immunofluorescent staining or RTqPCR. All animal experiments were approved by the Regional and National ethical committees.

Colon embedding and sectioning for immunofluorescent studies

Intestinal segments were dissected from the mice, placed in phosphate-buffered saline (PBS) to wash and intestinal contents were removed by rinsing the gut with PBS using a syringe. Then the segment was cut open longitudinally and rolled up by using watchmakers' forceps to push the roll. They were directly placed in a 12-well plate filled with 4% paraformaldehyde (PFA) and a paper roll to ensure the rolled-up position of the intestinal segment. PFA-fixated tissues were washed 3 times with PBS, dehydrated, and embedded in paraffin. The paraffin-embedded specimens were sectioned using a microtome (Leica RM2145), with a thickness of 6 µm. The slices were transferred to a Superfrost® Plus

microscopic slide (Thermo Scientific) with water and left on a warm plate to let the specimens stretch. After aspiration and evaporation of the water, the slides were placed in an incubator overnight at 37°C. If staining was not performed directly, slides were stored in the dark at room temperature.

Immunofluorescent staining

IHC detection and analysis were performed on 6 µm deparaffinized ileum and colon sections. For Antigen retrieval, the slides were incubated in a 0.01 M sodium citrate solution (pH 6) at 100°C for 10 min in a microwave, followed by a 2h cool-down period.

The slides were washed twice with H_2O and PBS and permeabilization was achieved using PBS – Triton X100 0.1% for 3 to 5 minutes. Thereafter, the slides were blocked using PBS-Triton 0.1% with 5% normal goat serum (NGS) during one to 2h at room temperature. Primary antibodies were diluted in blocking solution and slides incubated overnight at 4°C. P73 protein was detected by rabbit monoclonal antibody (1: 500 diluted (EP436Y); Abcam, UK); Synaptobrevin was detected with a mouse monoclonal antibody (1: 400 (104211); Synaptic System, Germany), HUC/D with a monoclonal antibody (1: 300 (16A11); ThermoFisher Scientific, USA); Cleaved Caspase 3 with a rabbit polyclonal antibody (1: 200 (9661); Cell Signaling, Netherlands); AIF was detected with a monoclonal antibody (1: 100 (Sc 5586); Santa Cruz Biotechnologie, USA) ; Ki67 was detected with a rabbit monoclonal antibody (1: 500 (RM-9106S); ThermoFisher Scientific, USA). The slides were rinsed with PBS-Triton 0.1% 4 times, 3-5 min. Each IHC was controlled negatively by processing sections in the absence of primary antibody.

For fluorescent detection Alexa Fluor Secondary antibodies (ThermoFisher Scientific, USA) were diluted in PBS-Triton 0.1% + 5% NGS and after two hours at room temperature, the slides were washed 4 times in PBS-Triton 0.1%. Nuclei were labeled with a DAPI solution (1/20,000) for 5 min. Slides were then rinsed in distilled H_2O and mounted using Calbiochem® FluorSave™ Reagent (Merck Millipore, Germany).

For DAB staining, sections were incubated with biotinylated goat antibody (Sigma, France) diluted 1:200 for 1 h at room temperature. After four PBST washes (for 3 min each), endogenous peroxidase activity was blocked by a 10-min incubation in a 0,5% hydrogen peroxide solution in methanol. The slides were washed two times 5 min in PBS and incubated in Avidine streptavidin-peroxidase complex (ABC kit, Vector Laboratories, France) for 1 to 2 h at room temperature. After two PBST washes of 5 min, bound peroxidase was identified using DAB (1/10, Santa cruz, USA). Nuclear counterstaining was performed with a Harris hematoxylin coloration. Pictures of ganglia were taken with a Zeiss Axio Imager M2-Apotome2 fluorescence microscope.

Dissection, digestion and culture of mouse myenteric plexuses for ex vivo treatment

C57BL/6 mice of 10 weeks were sacrificed using CO_2, and the peritoneal cavity was opened. The segment of intestine closest to the rectum was identified, and a cut was made. By gently pulling and removing mesentery and fat at the same time, the gut was removed from the cavity. The caecum provided the distinction between the small intestine and the colon. Right under the stomach, another cut was made to completely remove the tract from the mouse. The tract was placed in phosphate-buffered saline (PBS) to wash and remove fecal content from the intestines. Intestinal contents were removed by rinsing the gut with PBS using a syringe. Segments of approximately two to three centimeters at a time were placed in a Petri dish filled with dissection medium consisting of MEM glucose medium with streptomycin (50 ml medium with 500 µl streptomycin). Under a microscope with extra light sources, the outer muscular layer with the plexus was identified, and with watchmaker forceps the layer was pulled off the intestine very carefully. The procedure was performed for the entire colon and small intestine of each mouse. The segments were cut into small pieces and placed in digestion medium consisting of HBSS, streptomycin, DNAse and Liberase (highly purified collagenase I and II, Sigma Aldrich). Liberase was only added at the end to ensure its optimal enzymatic activity. Colon segments were digested for at least 4.5 hours, and small intestine segments for at least 4 hours in Eppendorf tubes in a 37˚C water bath. Approximately every hour the tubes were inverted to ensure effective digestion. Under the microscope, digestion efficacy was confirmed (white-colored tissues indicated insufficient digestion of the muscle layer), and the presence of plexuses was visually appraised. The parts were washed and gently pipetted up and down, if this did not disrupt the plexus networks too much. The segments were transferred to an Eppendorf tube with a high plexus/fluid ratio by trying to only aspirate the plexus particles. After sedimentation of the plexuses to the bottom and a short spin in an Eppendorf centrifuge, the supernatant was very carefully aspirated. The plexuses were brought into a 12-well plate with 500 µl of proliferation medium consisting of DMEM High Glucose medium, BSA, B27, streptomycin and β-mercaptoethanol. The cells were placed in a 37˚C incubator with 5% CO_2, and could be treated and/or kept in culture.

RNA extraction

RNA was extracted from cells using Trizol-mediated cell lysis. After extraction and RNAs precipitation, supernatants were removed and the RNA pellet was washed with 75% EtOH, centrifuged at 9000g for 5 minutes at 4˚C and again 75% EtOH was added. Then, the RNA was re-suspended in nuclease-free H_2O. After at least two hours in the -20˚C freezer, the RNA was quantified using NanoDrop

Spectrophotometer (Thermo Scientific). The A_{260}/A_{280} ratio and the A_{260}/A_{230} ratio were also documented, and should both be close to 2.0 for pure RNA samples.

RTqPCR

Owing to the very small RNA yield from myenteric plexuses, a special kit (Superscript™ III, Invitrogen) was used to optimize the reverse transcription of the ENS material. For every tube with RNA, first strand buffer, DTT, RNaseOUT™ and Superscript™ III enzyme were added according to the manufacturers protocol. The samples were placed into a thermic cycler to initiate the reverse transcription. The 20 µl of cDNA yielded thereafter was not diluted prior to further use. The Master mix for the RT-qPCR contained SybR Green reagent, primer mix (20 µl of forward primer, 20 µl of reverse primer and 60 µl of H_2O) and H_2O. For each well, 16 µl of Master mix was pipetted, and 4µl of cDNA. The plate was centrifuged for 1 minute at 1200 rpm at 4˚C, and the plate was run in a 7500 Real Time PCR System (Applied Biosystems) machine and the 7500 software (V2.3). Analysis of the quantity was performed using the comparative ΔΔCt method in Microsoft Excel. The housekeeping gene TATA box binding protein (TBP) expression was used as the reference value.

RNA sequencing

Sequencing was performed by the Sequencing platform of the IGBMC (Strasbourg) and results were first analyzed using AltAnalysis software [28]. Deregulated gene were identified based on two-fold change expression and t-test p-value <0.05. Deregulated genes were then analyzed for over-representation in selected biological processes in several resources: Gene Ontology, MPhenoOntology, Disease Ontology, GOSlim, PathwayCommons, KEGG, Transcription Factor Targets, miRNA Targets, Domains, BioMarkers, RVista Transcription Sites, DrugBank, BioGrid.

Statistics

Means were compared using the independent student t –test, and with one-way analysis of variance (ANOVA) with Tukey's post hoc test. Differences were considered significant when $p<0.05$ (indicated with *). P-values of <0.01 and <0.001 were illustrated with ** and *** respectively to indicate the significance levels as analyzed by GraphPad Prism. Error bars represent the SD (standard deviation).

Results

Myenteric ganglia develop a dynamic, time-dependent and coordinated transcriptome in response neuronal loss induced by cisplatin

To investigate the molecular mechanisms governing chemotherapies toxicity on the ENS, we treated Black 6 mice with cisplatin (10 mg/Kg) intraperitoneally and analyzed tissues 1 day or 7 days after injection. First, we performed immunohistofluorescence on tissues from the digestive system to label neurons using the selective neuronal maker HUC/D (Figure 1A). Quantification of neurons per ganglia indicated that cisplatin treatment decreased neuronal numbers by about 20% after 7 days of treatment and had no significant impact after 1 day (Figure 1B).

Then, to identify the deregulated genes associated with neuronal loss, we purified myenteric networks from mice non-treated or treated for 1 day or 7 days and performed RNA seq analysis (Figure 1C). Deregulated genes with fold change > 2 and *adjp* value <0.05 where selected and pathways analysis were performed using multiple databases (ex. DAVID, STRING, Reactome, TRAP). 625 and 1436 genes were deregulated after 1 and 7 days of treatment compared to non-treated mice, respectively (Figure 1D). 156 genes were estimated significantly differently deregulated between 1 days and 7 days of treatment. Pathways and mechanisms identified with z score > 2 in one of the conditions and with *adjp* value <0.05 were considered as deregulated.

Genes transiently deregulated after 1 day of treatment segregated within pathways related to control of cell death and survival, such as apoptosis and DNA damage response. In addition, several of these genes were identified as direct targets (ex. *bax*, *puma*, *mdm2, sesn2*) of transcription factors of the p53 transcription factor family (p53, p63 and p73) (Figure 1E) [35]. These 3 genes encode for multiple isoforms with function that can be opposite. For instance, TAp63 and TAp73 isoforms are often considered to favor cell death or cell cycle arrest via the regulation of various target genes, such as *bax*, *puma*, *cdkn1a*, similarly to p53. These genes are involved in response to different types of cellular stresses [36]. Concomitantly, pro-survival pathways such as AKT/PI3K and PDGF signaling were induced, suggesting the activation of compensatory mechanisms to protect cells from apoptosis within the myenteric ganglia.

After 1 day of treatment, signaling pathways associated with inflammation and inflammasome (ex. IL3, NOD like-receptor) were induced and mostly remained elevated after 7 days of treatment (Figure 1F). In addition, the expression of several interleukins (*cxcl3*, *cxcl2*, *ccl6*, *ccl9*) is induced at 7 days (Figure S1A). Concomitantly, genes that are direct targets of transcription factors (i.e. IRF4, Fox3P) involved in inflammation response followed the same profile, indicating that the activity of these transcription factors is induced in the myenteric ganglia after cisplatin treatment. To note,

mechanisms related to IFNγ seemed to be induced only transiently. Possible involvement of inflammation processes is further supported by the observation of cells with elevated COX2 expression and increased recruitment of muscularis macrophages in the vicinity of the enteric ganglias (Figure S1B and C).

In contrast, genes strongly induced after 7 days of treatment segregated within pathways and processes associated with neuronal plasticity (ex. long term potential) and identity (ex. frontal cortex) and stem cells activity (ex. fetal brain, induced pluripotent stem cells) (Figure 1G and H). Similarly, genes considered as target genes of transcription factors important for neuronal identify (ex. REST, CREB1) seem preferentially activated at 7 day of treatment [37, 38]. These results indicated that in response to cisplatin, cells within the myenteric ganglia develop a dynamic, time-dependent, and coordinated response starting with activation of pathways controlling cell death/survival and inflammation that is followed by processes related to neuronal plasticity.

Cisplatin induces apoptosis of ENS neurons

To validated the results of the transcriptomic analysis, we performed a novel experiment and analyzed pro-apoptotic genes and target genes of the p53 family by RT-qPCR on RNA extracted from purified myenteric ganglia of mice treated with cisplatin. Two pro-apoptotic and target genes of the p53 family (*noxa*, *puma*) were transiently induced by cisplatin after 1 day of treatment (Figure 2A). Two other known target genes of the p53 family, *mdm2* and *cdkn1a*, followed a similar profile.

To further established if cisplatin induced neuronal cell death, we analyzed by immunohistofluorescence the activation of markers of apoptosis in the ENS ganglia. First, we quantified the cells with cleaved caspase 3 (Casp3*). Surprisingly, we detected in the ganglia of the ENS of non-treated mice a weak basal level of cleaved caspase 3 (Figure 2B and C). However, the intensity of the labeling and the number of cells labeled were significantly increased after 1 day of treatment, reaching about 50% of the cells. These results suggested that the neuronal loss observed (Figure 1B) might be due to neuronal apoptosis. To confirm that, co-labeling was performed using the specific neuronal marker HUC/D and the pro-apoptotic marker AIF. Cisplatin induced the localization of pro-apoptotic maker AIF in the nucleus of neurons labelled by HUC/D (Figure 2D). In addition, the effect of cisplatin correlated with the appearance of altered nuclei characterized by condensed or fragmented chromatin (Figure 2E). The AIF labeling significantly decreased after 7 day of treatment. These results confirmed that cisplatin induced neuronal apoptosis in ENS, which may account for the neuronal loss observed. An alternative mechanism for cell elimination is senescence and it was previously shown that cisplatin induces senescence in the GI epithelial crypt [39]. Indeed, we

detected senescence in the crypts after 1 days of treatment with cisplatin (Figure 2F and G), which lasted up to 21 days post-treatment (data not shown), using beta-galactosidase assays and expression of the senescence marker $p16^{INK4A}$. However, no signal was observed in the enteric ganglia, suggesting that apoptosis is the main process for elimination of enteric neurons.

To assess whether cisplatin can directly induce cell death in ganglia independently of the influence of the surrounding tissues (intestinal epithelium, smooth muscles), we isolated the myenteric plexus, cultured them and treated them *ex vivo* for 24 hours with two concentration of cisplatin (C1, 5µM; C2, 20µM). As observed *in vivo*, pro-apoptotic genes (*bax*, *noxa*, *puma*) and target genes of the p53 family (*mdm2*, *cdkn1a*) were induced after 24 hours of treatment with cisplatin (Figure 3A). Inversely, the anti-apoptotic gene *Bcl2* was repressed. The elevated expression of pro-apoptotic genes correlated with increased fragmentation/condensation of nuclei (Figure 3B) and cleaved caspase 3 labeling (Figure 3C).

Activation of apoptosis in isolated myenteric ganglia is dependent upon proteins of the p53 family

The expression of p53 family members in the ENS has never been investigated. As several target genes of the p53 family were induced by cisplatin in the myenteric ganglia, we decided to analyze the expression profile of p53, p63 and p73 under these conditions. First, we extracted RNA from purified and isolated myenteric ganglia that were non-treated or treated with cisplatin and performed RT-qPCR to measure the expression of p53 and the TAp73 and TAp63 isoformes, which are susceptible to positively regulate p53 family target genes, such as *bax*, *puma*, *mdm2*. P53, TAp73 and TAp63 isoforms were expressed in the ENS under basal (non-treated) condition (Figure 3E). TAp63 and TAp73 were induced by 24 hours treatment of the ENS ganglia with cisplatin. As expected, p53 mRNA were not induced, but immuno-cytofluorescence studies indicated a nuclear relocalization of p53 after cisplatin treatment (Figure 3D).

To assess the potential role of the p53 family member in the response to cisplatin in isolated myenteric ganglia, we used pifithrin a molecule described as a p53 inhibitor but that can also inhibit the activity of other p53 family member [40]. Isolated myenteric plexus was pretreated with pifithrin for 1 hours, before treatment with cisplatin for 24 hours. As observed above the expression of the pro-apoptotic gene Noxa was induced by cisplatin. This induction was completely abolished with pifithrin pretreatment (Figure 3F).

Cisplatin induces p73 expression in ENS neurons in vivo

To further establish whether p53 family members were indeed induced by cisplatin in ENS, we performed immunocytofluorescence studies on the gut of mice non-treated or treated for 1 day or 7 days with cisplatin. We detected a basal level of expression of p53 in the ENS plexus (Figure 4A). Surprisingly, cisplatin tended to diminish the protein level of p53 in the ENS plexus after 1 day of treatment (Figure 4A and B). At the mRNA level, cisplatin did not modify significantly the expression of p53 in myenteric plexus isolated from mice non-treated or treated (Figure 4B). In contrast, cisplatin induced the number of cells labelled for p73 and the intensity of the labelling after 1 day of treatment (Figure 4C and D). Labelling for p73 decreased significantly at 7 days of treatment. suggesting a transient induction of p73 in the ENS upon cisplatin. At the mRNA level, TAp73 expression was only induced at 7 days of treatment. This suggested that in vivo the induction of TAp73 mRNA is slower compared to the isolated plexus and that the elevated protein level is due to stabilization of the protein [41].

To established whether the p73 protein was expressed in the ENS neurons, co-labelling with two neuronal markers, synaptobrevin and HUC/D, were performed on the gut of mice non-treated or treated with cisplatin [42]. P73 protein localized in the nucleus of cells that were positive for two neuronal markers, synaptobrevin and HUC/D (Figure 5A, B and C). In particular, the cytoplasmic labelling of HUC/D allowed to visualize well the nuclear localization of p73 protein in ENS neurons (Figure 5C).

Loss of TAp63 expression favors sustained expression of proapoptotic genes in ENS

We also attempted to visualize p63 expression in the ENS. Despite the use of multiple antibodies, we were never able to see p63 labelling (personal data). This might be explained by the lower mRNA expression level of TAp63 detected in myenteric plexus compared to p53 or TAp73 (Figure 6A). Hence, we used a genetic approach to assess the existence and the role of the expression of TAp63 in ENS. Mice with CRE recombinase knockin into the promoter of TAp63 ($TAP63^{CRE/+}$) were breaded with ROSA26-Tomato mice that contains the cDNA for the fluorescence protein Tomato flanked by LoxP sequences (Figure 6B). The generated compound mice ($TAp63^{CRE/+}$:Rosa26-Tomato) expressed the fluorescent Tomato proteins in cells that have or have had an active TAp63 promoter. Co-labelling for Tomato and neuronal markers expression were performed on gut tissues of mice non-treated or treated with cisplatin. Expression of Tomato proteins was present in cells expressing neuronal markers (Figure 6C and D). However, cisplatin treatment did no modify the expression level of the Tomato protein. This can be explained by the fact that if ENS neurons express TAp63 at basal

condition and therefore CRE dependent recombination has already happened, it is not possible to detect an increase recombination visualized by Tomato labelling.

To analyze the possible role of TAp63 in ENS, we inter-crossed TAp63$^{CRE/+}$ mice to generate TAp63$^{CRE/CRE}$ mice that do not express TAp63 protein. TAp63$^{CRE/+}$ and TAp63$^{CRE/CRE}$ mice were treated with cisplatin for 1 and 7 days and compared to non-treated mice. Analyses of the tissues by immunostaining did not reveal any significant differences in term of general ENS structure or number of neurons in any conditions (data not shown). However, analysis of the expression of selected genes revealed that loss of p63 elevated TAp73 mRNA level after 1 days of treatment with cisplatin (Figure 6E). In addition, the expression of two target genes of the p53 family, Noxa and Cdkn1a, were maintained elevated in absence of TAp63 expression. These results indicate that TAp63 is expressed in ENS neurons and participates in the regulation of genes involved the regulation of cell survival and death.

ENS undergoes cellular plasticity and regeneration processes after apoptosis

The transcriptomic analysis revealed that at 7 days after treatment with cisplatin, cellular plasticity processes related to stem cell activation, neuronal identity and activity were induced, suggesting that following the neuronal apoptosis regeneration within the ENS was taking place (Figure 1). For instance, STRING pathway analysis highlight "neurogenesis" as Biological process enriched at 7 days of treatment that include 107 genes over 1650 (*fdr* = 8.45^{-14}) (Figure 7A). Several genes related to neurogenesis are amongst the genes the most deregulated (i.e. *kcnq3*, *unc80*, *ncam2, ntrk3*). As before, we validated the expression changes of some of these genes, such as *rtnk3, unc80 and kcnq3* (Figure 7B). To further characterize the existence of possible plasticity and remodeling events, we treated mice with cisplatin for 7 and 21 days and analyzed gut tissues. We first characterized the expression of P73 and observed that it was significantly reduced after 21 days of treatment with cisplatin (Figure 7C). Then, we analyzed cell proliferation and presence of stem cells in the ENS. For instance, immunolabeling for SOX10, a marker for ENS stem cells, was performed. We observed an increased in cells expressing SOX10 7 days after treatment, which diminished after 21 day (Figure 7D). Labelling for Ki67, a marker of cell proliferation, identified cells proliferating in the vicinity of the ganglia and along the plexus tract (Figure 7E). The exact identity of these cells remains to be established, but the proliferative activity and increase in SOX10 positive cells confirm the existence of plasticity processes following the apoptosis induced by cisplatin in ENS.

Discussion

Cytotoxic chemotherapies remain the standard treatment for most types of cancer despite induction of severe acute and long-term side effects, for which supportive care are not completely satisfactory. Hence, the development of innovative therapeutic approaches requires a better understanding of the inherent molecular mechanisms. Long-term gastrointestinal dysfunctions induced by chemotherapies have been linked to alterations of enteric nervous systems integrity. More specifically, loss of specific neuronal populations has been described in response to cisplatin, oxaliplatin, 5-FU or irinotecan in murine models. However, the molecular mechanisms underlying the neuronal loss in response to these chemotherapies have not yet been properly explored. In this study, we demonstrate that cisplatin induces a dynamic coordinated transcription program starting with a pro-apoptotic step involving proteins of the p53 family and induction of inflammation response, followed by the activation of neuronal plasticity/differentiation processes.

Cisplatin induced apoptosis in enteric neurons

We established that cisplatin induces apoptosis in enteric neurons that is characterized by expression of pro-apoptotic genes (i.e. *bax*, *puma*, *noxa*) cleavage of caspase 3, relocalization of AIF and condensation/fragmentation of nuclei that have been observed *ex vivo* and *in vivo*. In particular, AIF relocalization have been observed in enteric neurons expressing the HUC/D neuronal marker. This apoptosis can explain the neuronal loss observed in our study after cisplatin treatment, and also the neuronal losses previously described following treatment with cisplatin, oxaliplatin, 5-FU or irinotecan [23 24 25 26 43]. In mice treated with acute dose of cisplatin, this neuronal cell death is observed already 1 days after treatment, decreases at 7 days, and was undetectable 21 days after treatment, showing a transient and dynamic induction of a cell death program. The apoptosis is also induced in *ex vivo* cultures of myenteric plexus treated with cisplatin, suggesting that the pro-apoptotic transcriptional program is due to a direct impact of cisplatin on the myenteric plexus rather than an indirect action via the surrounding tissues (i.e. smooth muscle, epithelium). Because AIF is part of a caspase 3-independent apoptotic program that includes cleavage of PARP1 [44], our results suggest that at least two cell death programs are involved in enteric neurons loss, one caspase-dependent and another caspase-independent. Furthermore, the activation of AIF in myenteric neurons, which we observed, can explain the protective activity of the PARP inhibitor BGP15 on the GI tract function altered by oxaliplatin that has been previously described [33, 34]. The exact type of neurons suffering from apoptosis remains to be established. For instance, a general loss of neurons associated with a selective increase of nNOS-positive neurons have been described by Wafai et al. [25], while McQuade et al. [26] described a loss of both nNOS-positive and PGP9.5-positive neurons.

Role of the p53 family in enteric neuronal apoptosis

We showed that cisplatin induces the expression of p53 and TAp73 in enteric neurons, which correlated with the induction of several pro-apoptotic (*bax*, *noxa*, *puma*) and other targets genes (*mdm2*, *ptgs2*, *cdkn1a*) of the transcription factors of the p53 family. This induction peaks already 1 day after the treatment and is likely to play a role in apoptosis as the inhibitor of p53 and TAp73 (pifithrin) blocks cisplatin-induced expression of the pro-apoptotic genes. The exact contribution of each member of the p53 family in enteric neuronal apoptosis remains to establish, especially since we also observed in enteric neurons an expression of TAp63, whose silencing maintained elevated mRNA levels of selected pro-apoptotic genes 7 days after treatment. This observation suggests that a complex interplay may exist between the p53 family members to fine tune the promoter activity of their target genes. It is to note that the silencing of TAp63 did not lead to significant changes in ENS structure, in control condition or upon stresses, as we could evaluate so fare. Change in expression and the role of p53 and TAp73 in enteric neuron apoptosis is coherent with what has been described previously in the central nervous system by us and others [14, 15, 45-47]. However, additional functions for p73 and p63 isoforms have been observed in the central nervous system that include stem cell maintenance, differentiation and senescence [48, 49]. Therefore, we cannot exclude that p53 family members participates into such activities in the enteric neurons.

ENS plasticity in response to chemotherapy

Upon cisplatin challenge and following enteric neuronal apoptosis, we observed an induction of gene signatures associated with neuronal identity, differentiation, plasticity and stem cell activity. Some of these signatures start simultaneously with the apoptotic signature, but they are further increased 7 days after the treatment. For instance, induction of *ngfr*, *ret*, *gfap* that are markers of neuronal/glial precursors are elevated 7 days after the treatment. In addition, several receptors (i.e. *ret*, *gfra1*, *gfra3*) for GDNF, an important trophic factor for enteric neuronal differentiation are also induced, as well as expression of receptors for neurotransmitters (e.g. glutamatergic), such as *grik1*, 2 and 3. These plasticity transcriptional programs are associated with elevated expression of SOX10-positive cells within the enteric ganglia, an indicator of glial precursor cells, and proliferative cells in their vicinity. These plasticity events involving a succession of proliferation and differentiation of neuronal and glial precursors are coherent with the recent findings of Kultarni et al. describing a dynamic balance between neuronal apoptosis and neurogenesis in the ENS in adults [22]. The master genes controlling these events remain to be identified. Some of the signature suggest that the transcription factors CREB1 and REST might be involved. For instance, CREB1 has been previously

associated with neuronal survival and plasticity [50] [51] [52, 53]. Furthermore, it has been shown that CREB is involved in neuronal survival and neurogenesis via the 5HT receptor activity [54]. REST is also an essential factor to restrict gene expression in neuron and has been shown to silence neuronal pro-apoptotic genes [38]. Furthermore, as indicated above, proteins of the p53 family might also play a role in these processes. The exact contribution of these various transcription factors in the plasticity processes within the ENS in response to chemotherapies remains to be established.

Chemotherapies induce inflammation in the ENS

The gene signatures in the ENS generated after cisplatin treatment also suggest that a persistent inflammation takes place up to 7-day post-treatment. However, there seems to exist specific kinetics of activation as IL-2/3/5 signaling remains elevated while IFN-a/b signaling seems to be induced only transiently at 1-day post-treatment. These processes correlate with activation of specific transcription factors involved in the inflammatory mechanisms, such as IRF4 or FOXp3 [55] [56], and increased expression of several cytokines and chemokines (i.e. *cxcl3*, *cxcl2*, *ccl6*, *ccl9*). The possible activation of inflammation processes is further confirmed by the strong induction of *ptgs2* (Cox2) expression. Cox2 is a key protein in prostaglandin-dependent inflammation, in particular in the gastrointestinal tract where its deregulation has been associated with pathologies [57]. Although additional studies are required to understand the exact nature of this inflammation, it appears now to be a general feature in the ENS in response to chemotherapies as it was also observed in response to 5-FU[26]. It remains also still to understand the contribution of the inflammation processes in the neuronal cell death or plasticity processes following chemotherapies, and in particular which types of immune cells might participate. Interestingly, we observed that muscularis macrophages are more present close to the ganglia in response to cisplatin. Several hypotheses can be proposed. For instance, muscularis macrophages have been described to be recruited in the vicinity of the enteric ganglia driven by a neuron-mediated-beta2-adrenergic signaling that is partly dependent on the expression of *arg1* [58]. *Arg1* is one of the genes that is strongly induced 7 days after treatment. In addition, some of the chemokines (*ccl6*, *ccl9*) expressed in response to cisplatin are preferentially expressed by macrophages. Interestingly, muscularis macrophages participate in the function and protection of the ENS, including to cleanup apoptotic debris [59]. Hence, these cells could help regenerating enteric ganglia. However, inflammation via the secretion of cytokines/chemokines might also have deleterious effects. For instance, some chemokines/cytokines have been shown to induce neuronal cell death, such as Cxcl2 [60, 61].

This study identified signaling pathways and molecular mechanisms that may account for the neuronal loss and regenerative processes upon chemotherapy. The protein of the p53 family seems to play a role in it. The succession of neuronal cell death and regenerative processes with the ENS may account for some of the long-term side effect induced by the chemotherapy. In addition, we also showed the induction of significant inflammatory mechanisms that may have complex impact on the pathophysiology of the gastrointestinal tract. We cannot either exclude that the secretion of cytokines or of chemokines by the ENS may impact the on the aggressiveness of the cancer cells in the case of colon or gastric cancers that are in the vicinity of the ENS [62]. The next challenge is to use these informations to develop therapeutic approaches protective of the ENS upon chemotherapy.

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Reference

1. Von Hoff DD, Schilsky R, Reichert CM, et al. Toxic effects of cis-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 1979;63:1527-31.
2. Mazevet M, Moulin M, Llach-Martinez A, et al. Complications of chemotherapy, a basic science update. Presse Med 2013;42:e352-61.
3. Di Fiore F, Van Cutsem E. Acute and long-term gastrointestinal consequences of chemotherapy. Best Pract Res Clin Gastroenterol 2009;23:113-24.
4. Stein A, Voigt W, Jordan K. Chemotherapy-induced diarrhea: pathophysiology, frequency and guideline-based management. Ther Adv Med Oncol 2010;2:51-63.
5. Feyer P, Jordan K. Update and new trends in antiemetic therapy: the continuing need for novel therapies. Ann Oncol 2011;22:30-8.
6. Gibson RJ, Keefe DM. Cancer chemotherapy-induced diarrhoea and constipation: mechanisms of damage and prevention strategies. Support Care Cancer 2006;14:890-900.
7. Wong H, Yau T. Targeted therapy in the management of advanced gastric cancer: are we making progress in the era of personalized medicine? Oncologist 2012;17:346-58.
8. Denlinger CS, Barsevick AM. The challenges of colorectal cancer survivorship. J Natl Compr Canc Netw 2009;7:883-93; quiz 894.

9. Keefe DM, Brealey J, Goland GJ, et al. Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. Gut 2000;47:632-7.
10. Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004;4:277-84.
11. Ly KI, Arrillaga-Romany IC. Neurologic Complications of Systemic Anticancer Therapy. Neurol Clin 2018;36:627-651.
12. Zajaczkowska R, Kocot-Kepska M, Leppert W, et al. Mechanisms of Chemotherapy-Induced Peripheral Neuropathy. Int J Mol Sci 2019;20.
13. Meng X, Leyva ML, Jenny M, et al. A Ruthenium-Containing Organometallic Compound Reduces Tumor Growth through Induction of the Endoplasmic Reticulum Stress Gene CHOP. Cancer Res 2009;1;69(13):5458-66.
14. Benosman S, Gross I, Clarke N, et al. Multiple neurotoxic stresses converge on MDMX proteolysis to cause neuronal apoptosis. Cell Death Differ 2007.
15. Benosman S, Meng X, Von Grabowiecki Y, et al. Complex regulation of p73 isoforms after alteration of amyloid precursor polypeptide (APP) function and DNA damage in neurons. J Biol Chem 2011;286:43013-25.
16. Bajic JE, Johnston IN, Howarth GS, et al. From the Bottom-Up: Chemotherapy and Gut-Brain Axis Dysregulation. Front Behav Neurosci 2018;12:104.
17. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 2012;9:286-94.
18. Clairembault T, Leclair-Visonneau L, Neunlist M, et al. Enteric glial cells: new players in Parkinson's disease? Mov Disord 2015;30:494-8.
19. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. World J Gastroenterol 2015;21:10609-20.
20. Chalazonitis A, Rao M. Enteric nervous system manifestations of neurodegenerative disease. Brain Res 2018;1693:207-213.
21. Kulkarni S, Ganz J, Bayrer J, et al. Advances in Enteric Neurobiology: The "Brain" in the Gut in Health and Disease. J Neurosci 2018;38:9346-9354.
22. Kulkarni S, Micci MA, Leser J, et al. Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. Proc Natl Acad Sci U S A 2017;114:E3709-E3718.
23. Escalante J, McQuade RM, Stojanovska V, et al. Impact of chemotherapy on gastrointestinal functions and the enteric nervous system. Maturitas 2017;105:23-29.
24. Vera G, Castillo M, Cabezos PA, et al. Enteric neuropathy evoked by repeated cisplatin in the rat. Neurogastroenterol Motil 2011;23:370-8, e162-3.

25. Wafai L, Taher M, Jovanovska V, et al. Effects of oxaliplatin on mouse myenteric neurons and colonic motility. Front Neurosci 2013;7:30.
26. McQuade RM, Stojanovska V, Donald E, et al. Gastrointestinal dysfunction and enteric neurotoxicity following treatment with anticancer chemotherapeutic agent 5-fluorouracil. Neurogastroenterol Motil 2016;28:1861-1875.
27. Carbone SE, Jovanovska V, Brookes SJ, et al. Electrophysiological and morphological changes in colonic myenteric neurons from chemotherapy-treated patients: a pilot study. Neurogastroenterol Motil 2016;28:975-84.
28. Thorpe D, Stringer A, Butler R. Chemotherapy-induced mucositis: the role of mucin secretion and regulation, and the enteric nervous system. Neurotoxicology 2013;38:101-5.
29. Saegusa Y, Ichikawa T, Iwai T, et al. Changes in the mucus barrier of the rat during 5-fluorouracil-induced gastrointestinal mucositis. Scand J Gastroenterol 2008;43:59-65.
30. Stringer AM, Gibson RJ, Logan RM, et al. Irinotecan-induced mucositis is associated with changes in intestinal mucins. Cancer Chemother Pharmacol 2009;64:123-32.
31. Thorpe D, Butler R, Sultani M, et al. Irinotecan-Induced Mucositis Is Associated with Goblet Cell Dysregulation and Neural Cell Damage in a Tumour Bearing DA Rat Model. Pathol Oncol Res 2019.
32. Robinson AM, Stojanovska V, Rahman AA, et al. Effects of Oxaliplatin Treatment on the Enteric Glial Cells and Neurons in the Mouse Ileum. J Histochem Cytochem 2016;64:530-45.
33. McQuade RM, Stojanovska V, Bornstein JC, et al. PARP inhibition in platinum-based chemotherapy: Chemopotentiation and neuroprotection. Pharmacol Res 2018;137:104-113.
34. McQuade RM, Stojanovska V, Stavely R, et al. Oxaliplatin-induced enteric neuronal loss and intestinal dysfunction is prevented by co-treatment with BGP-15. Br J Pharmacol 2018;175:656-677.
35. Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ 2006;13:962-72.
36. Pflaum J, Schlosser S, Muller M. p53 Family and Cellular Stress Responses in Cancer. Front Oncol 2014;4:285.
37. Belgacem YH, Borodinsky LN. CREB at the Crossroads of Activity-Dependent Regulation of Nervous System Development and Function. Adv Exp Med Biol 2017;1015:19-39.
38. Zhao Y, Zhu M, Yu Y, et al. Brain REST/NRSF Is Not Only a Silent Repressor but Also an Active Protector. Mol Neurobiol 2017;54:541-550.
39. Frey N, Venturelli S, Zender L, et al. Cellular senescence in gastrointestinal diseases: from pathogenesis to therapeutics. Nat Rev Gastroenterol Hepatol 2018;15:81-95.

40. Codelia VA, Cisterna M, Alvarez AR, et al. p73 participates in male germ cells apoptosis induced by etoposide. Mol Hum Reprod 2010;16:734-42.
41. Ozaki T, Hosoda M, Miyazaki K, et al. Functional implication of p73 protein stability in neuronal cell survival and death. Cancer Lett 2005;228:29-35.
42. Phillips RJ, Hargrave SL, Rhodes BS, et al. Quantification of neurons in the myenteric plexus: an evaluation of putative pan-neuronal markers. J Neurosci Methods 2004;133:99-107.
43. McQuade RM, Stojanovska V, Donald EL, et al. Irinotecan-Induced Gastrointestinal Dysfunction Is Associated with Enteric Neuropathy, but Increased Numbers of Cholinergic Myenteric Neurons. Front Physiol 2017;8:391.
44. Nikoletopoulou V, Markaki M, Palikaras K, et al. Crosstalk between apoptosis, necrosis and autophagy. Biochim Biophys Acta 2013;1833:3448-3459.
45. Jacobs WB, Kaplan DR, Miller FD. The p53 family in nervous system development and disease. J Neurochem 2006;97:1571-84.
46. Jacobs WB, Walsh GS, Miller FD. Neuronal survival and p73/p63/p53: a family affair. Neuroscientist 2004;10:443-55.
47. von Grabowiecki Y, Abreu P, Blanchard O, et al. Transcriptional activator TAp63 is upregulated in muscular atrophy during ALS and induces the pro-atrophic ubiquitin ligase Trim63. Elife 2016;5.
48. Talos F, Abraham A, Vaseva AV, et al. p73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis. Cell Death Differ 2010;17:1816-29.
49. Niklison-Chirou MV, Killick R, Knight RA, et al. How Does p73 Cause Neuronal Defects? Mol Neurobiol 2016;53:4509-20.
50. Herold S, Jagasia R, Merz K, et al. CREB signalling regulates early survival, neuronal gene expression and morphological development in adult subventricular zone neurogenesis. Mol Cell Neurosci 2011;46:79-88.
51. Bustos FJ, Jury N, Martinez P, et al. NMDA receptor subunit composition controls dendritogenesis of hippocampal neurons through CAMKII, CREB-P, and H3K27ac. J Cell Physiol 2017;232:3677-3692.
52. Gaiddon C, Larmet Y, Trinh E, et al. Brain-derived neurotrophic factor exerts opposing effects on beta2- adrenergic receptor according to depolarization status of cerebellar neurons. J Neurochem 1999;73:1467-76.
53. Gaiddon C, Loeffler JP, Larmet Y. Brain-derived neurotrophic factor stimulates AP-1 and cyclic AMP-responsive element dependent transcriptional activity in central nervous system neurons. J Neurochem 1996;66:2279-86.

54. Liu MT, Kuan YH, Wang J, et al. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. J Neurosci 2009;29:9683-99.
55. Biswas PS, Bhagat G, Pernis AB. IRF4 and its regulators: evolving insights into the pathogenesis of inflammatory arthritis? Immunol Rev 2010;233:79-96.
56. O'Connor RA, Anderton SM. Inflammation-associated genes: risks and benefits to Foxp3+ regulatory T-cell function. Immunology 2015;146:194-205.
57. Meriwether D, Sulaiman D, Volpe C, et al. Apolipoprotein A-I mimetics mitigate intestinal inflammation in COX2-dependent inflammatory bowel disease model. J Clin Invest 2019;130.
58. Gabanyi I, Muller PA, Feighery L, et al. Neuro-immune Interactions Drive Tissue Programming in Intestinal Macrophages. Cell 2016;164:378-91.
59. De Schepper S, Stakenborg N, Matteoli G, et al. Muscularis macrophages: Key players in intestinal homeostasis and disease. Cell Immunol 2018;330:142-150.
60. De Paola M, Buanne P, Biordi L, et al. Chemokine MIP-2/CXCL2, acting on CXCR2, induces motor neuron death in primary cultures. Neuroimmunomodulation 2007;14:310-6.
61. Brandolini L, d'Angelo M, Antonosante A, et al. Chemokine Signaling in Chemotherapy-Induced Neuropathic Pain. Int J Mol Sci 2019;20.
62. Ruiz de Porras V, Bystrup S, Martinez-Cardus A, et al. Curcumin mediates oxaliplatin-acquired resistance reversion in colorectal cancer cell lines through modulation of CXC-Chemokine/NF-kappaB signalling pathway. Sci Rep 2016;6:24675.

Figure Legends

Figure 1. *ENS enteric plexuses develop a dynamic, coordinated and time-dependent transcriptomic program in response to the cytotoxicity induced by cisplatin*. **A.** Labelling of ENS neurons for HUC/D (red) expression by immunohistofluorescence in colon tissues of non-treated mice. DAPI was used to label nuclei (Bleu). Scale = 200 µm. **B.** Counting of HUC/D immunoreactive (IR) neurons in colon of non-treated or cisplatin-treated (10mg/Kg, single dose) mice, 1 day (D1) or 7 days (7D) after treatment. Graph represent % of neurons per myenteric ganglia. * indicate $p<0.05$ (n=4). **C.** Schematic representation of the protocol followed to analyze the transcriptome of myenteric plexuses of mice treated with cisplatin (10mg/Kg, single dose). **D.** Venn diagram illustrating the repartition of the deregulated genes between the different condition (NT, non-treated; D1, 1 days after treatment; D7, 7 days after treatment). Genes with fold change > 2 and *adjp* < 0.05 were selected as deregulated. **E-H.** Deregulated pathways associated with the positively deregulated genes. Graphs display z score for indicated deregulated pathways with *adjp* < 0.05. Deregulated pathways were identified using STRING, Reactome, DAVID and Altanalysis software.

Figure 2. *Cisplatin induces apoptosis in ENS neurons*. **A.** Cisplatin induces expression of pro-apoptotic genes (*noxa*, *puma*) and target genes (*mdm2*, *cdkn1a*) of the p53 family in myenteric cells. Myenteric plexuses of mice non-treated (NT) or treated for 1 day (D1) or 7 days (D7) with cisplatin were isolated and mRNA were analyzed by qPCR. Graphs represent means with SD relative to the control. **, *** indicates $p < 0.01$ or 0.001, respectively (n=3). *Tbp* was used a house-keeping gene. **B.** Cisplatin induces caspase 3 cleavage in ENS plexuses. Immunoreactive cells for cleaved caspase 3 (Casp 3*) were counted in ENS plexuses of non-treated (NT, left picture) mice or mice treated for 1 day (D1, right picture) with cisplatin. Cleaved caspase 3 was revealed by immunohistofluorescence. Graphs represent means with SD relative to the control. *** indicates $p < 0.001$, respectively (n=5). Scale = 100 µm. **C.** Cisplatin induces caspase 3 cleavage in ENS plexuses. Cleaved caspase 3 (Casp 3*) was revealed by DAB immunohistochemistry and is visualized as brown deposits. Scale = 100 µm. **D.** Cisplatin induces AIF relocalization in ENS neurons. Gut tissues of non-treated (NT) mice and mice treated for 1 day with cisplatin were labelled for the pro-apoptotic effector AIF (green) and the neuronal marker HUC/D (red). Nuclei were labelled with Dapi (blue). Scale = 100 µm. **E.** Cisplatin induces nuclei fragmentation/condensation in ENS neurons. Gut tissues of non-treated (NT) mice and mice treated for 1 day with cisplatin were labelled for the neuronal marker HUC/D (red) and nuclei were labelled with Dapi (blue). **F.** Cisplatin induces senescence in GI crypts but not in enteric neurons. Senescence was detected in cells using the Senescence beta-galactosidase staining kit (Cell

Signaling Technology, USA). Senescence is visualized as bleu deposits. **G.** The senescence marker $p16^{INK4}$ was revealed by DAB immunohistochemistry and is visualized as brown deposits. Scale = 100 µm.

Figure 3. *Cisplatin induces apoptosis and expression of the p53 family members in ex vivo culture of myenteric plexuses*. **A.** Cisplatin induces the expression of pro-apoptotic and other target genes of the p53 family in ex vivo culture of myenteric neurons. Myenteric plexuses were isolated from mice, placed in ex vivo culture, and then treated for 24 h with cisplatin (C1, 5µM; C2 10µM). RNAs were extracted and RT-qPCR were performed on mRNA for the indicated genes. *Tbp* was used as housekeeping gene. Graphs represent means with SD relative to the control. **, *** indicates p > 0.01 or 0.001, respectively (n=3). **B.** Cisplatin induced nuclei condensation in ex vivo cultured myenteric plexuses. Nuclei of *ex vivo* cultured and non-treated (NT, left image) and cisplatin-treated (C2, right image) myenteric plexuses were labelled with Dapi and condensed nuclei were counted. Graphs represent means with SD relative in % of condensed nuclei compared to all nuclei. **, *** indicates p > 0.01 or 0.001, respectively (n=3). **C., D.** Cisplatin induces caspase 3 cleavage and nuclear relocalization in myenteric cells. *Ex vivo* cultured and cisplatin-treated (C2) or non-treated (NT) myenteric plexuses were labelled by immunocytofluorescence for cleaved caspase 3 (C) and p53 (D). **E.** Cisplatin induce the expression of TAp63 and TAp73 in myenteric cells. RNAs from non-treated (NT) or cisplatin-treated (C1, 5µM; C2 10µM; 24 h) myenteric plexuses were extracted and RT-qPCR were performed on mRNA for the indicated genes. *Tbp* was used as housekeeping gene. Graphs represent means with SD relative to the control. *** indicates p < 0.001, respectively (n=3). **F.** An inhibitor of p53 and TAp73 reduces cisplatin-induced pro-apoptotic gene expression. RNAs from non-treated (NT) or cisplatin-treated (C1, 5µM; C2 10µM; 24 h) myenteric plexuses, in absence of in presence of pifithrin, were extracted and by RT-qPCR were performed on *Noxa* mRNA. *Tbp* was used as housekeeping gene. Graphs represent means with SD relative to the control. *** indicates p < 0.001, respectively (n=3).

Figure 4. *Cisplatin induced p73 protein in ENS plexuses*. **A.** Gut tissues of non-treated (NT) mice and mice treated for 1 day (D1) with cisplatin were labelled for p53 (green). **B.** Immunoreactive cells for p53 were counted in ENS plexuses of non-treated (NT) mice or mice treated for 1 day (D1) or 7 days (D7) with cisplatin. Graphs represent means with SD relative to the control. RNAs of myenteric plexuses isolated from non-treated or cisplatin-treated mice were extracted and by RT-qPCR were performed on *p53* mRNA (n=3). *Tbp* was used as housekeeping gene. Graphs represent means with

SD relative to the control. **C.** Gut tissues of non-treated (NT) mice and mice treated for 1 day (D1) with cisplatin were labelled for p73 (green). **D.** Immunoreactive cells for p73 were counted in ENS plexuses of non-treated (NT) mice or mice treated for 1 day (D1) or 7 days (D7) with cisplatin. Graphs represent means with SD relative to the control. *** indicates $p < 0.001$, respectively (n=5). RNAs of myenteric plexuses isolated from non-treated or cisplatin-treated mice were extracted and by RT-qPCR were performed on *TAp73* mRNA (n=3). *Tbp* was used as housekeeping gene. Graphs represent means with SD relative to the control. *** indicates $p < 0.001$, respectively (n=3).

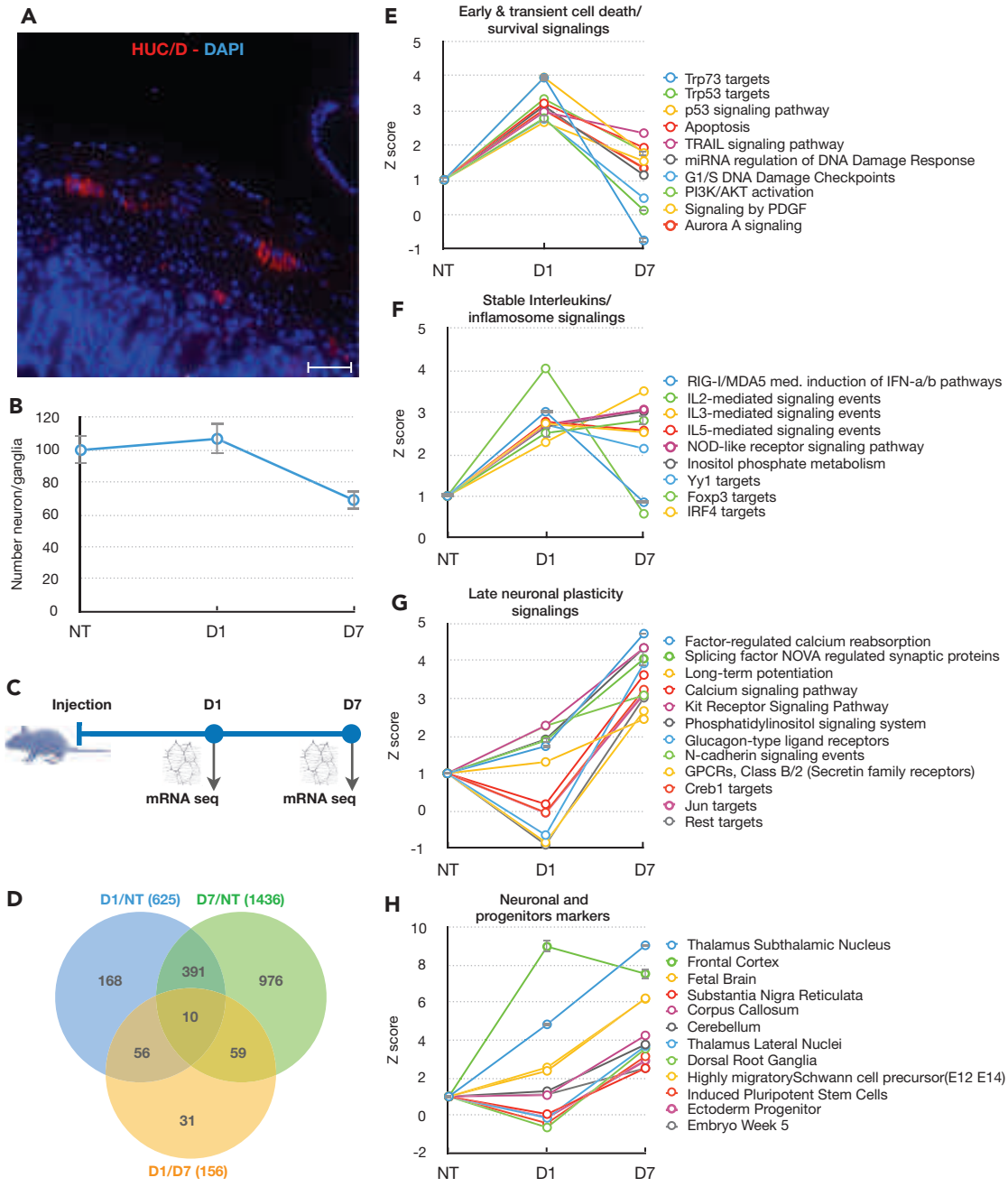
Figure 5. *P73 expression localized in ENS neurons*. A. Gut tissues of mice treated for 1 day (D1) with cisplatin were labelled for p73 (green), synaptobrevin (red) and Dapi (blue). B. Gut tissues of mice treated for 1 day (D1) with cisplatin were labelled for p73 (green), HUC/D (red) and Dapi (blue).

Figure 6. *TAp63 participates in the regulation of pro-apoptotic genes' expression in enteric neurons.* **A.** Relative expression level of p53, TAp63 and TAp73 in myenteric plexuses. RNAs of myenteric plexuses isolated from non-treated mice were extracted and by RT-qPCR were performed on *p53*, *TAp63* and *TAp73* mRNA (n=3). *Tbp* was used as housekeeping gene. Graphs represent means with SD relative to the Tbp expression. *** indicates $p > 0.001$, respectively (n=3). **B.** Schematic representation of the genetic approach used to assess the expression of TAp63 in the ENS. **C. D.** Gut tissues of mice treated for 1 day with cisplatin were labelled for Tomato (green), synaptobrevin (red) and Dapi (blue). Scale = 100 µm (C) or 20 µm (D). **E.** Genetic inactivation of TAp63 prolonged activation of the pro-apoptotic gene Noxa. Myenteric plexuses of wild-type and $TAp63^{CRE/CRE}$ (KO) mice, non-treated (NT) or treated for 1 day (D1) or 7 days (D7) with cisplatin, were isolated and mRNA were analyzed by qPCR for the indicated genes. Graphs represent means with SD relative to the control. **, *** indicates $p < 0.01$ or 0.001, respectively (n=3). *Tbp* was used a house-keeping gene.

Figure 7. *Cisplatin induces late cellular plasticity in the ENS*. **A.** Induction of neurogenesis related genes at 7 post treatment. Graph represent expression level of 107 genes from the RNA seq indicated as related to neurogenesis in STRING GO Biological process terms. Expression level are represented as fold change versus *adp* values for each gene. **B.** Validation by RT-qPCR of change in mRNA level of *nrtk3* in myenteric plexus of mice non-treated or treated with cisplatin (10 mg/Kg) for 1 day (1D) or 7 days (7D). Graphs represent means with SD relative to the control. **, indicates $p > 0.01$ (n=4). **C.** p73 expression in enteric neurons decreases 21 days after cisplatin treatment.

Immunoreactive cells for p73 and p53 were counted in ENS plexuses of non-treated (NT) mice or mice treated for 7 days (D7) or 21 days (D21) with cisplatin. Graphs represent means with SD relative to the control. ** indicates $p > 0.01$, respectively (n=3). **D.** Expression of the neuronal/glial cell precursor SOX10 is induced by cisplatin after 7 days of treatment. Immunoreactive cells for SOX10 were counted in ENS plexuses of non-treated (NT) mice or mice treated for 7 days (D7) or 21 days (D21) with cisplatin. Graphs represent means with SD relative to the control. ** indicates $p > 0.01$, respectively (n=3). **E.** Cisplatin induced cell proliferation in the ENS 7 days after treatment. Immunoreactive cells for the proliferative marker KI67 (green) and the neuronal marker HUC/D (red) were counted in ENS plexuses of non-treated (NT) mice or mice treated for 7 days (D7) or 21 days (D21) with cisplatin. Graphs represent means with SD relative to the control.

Figure 1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B
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100
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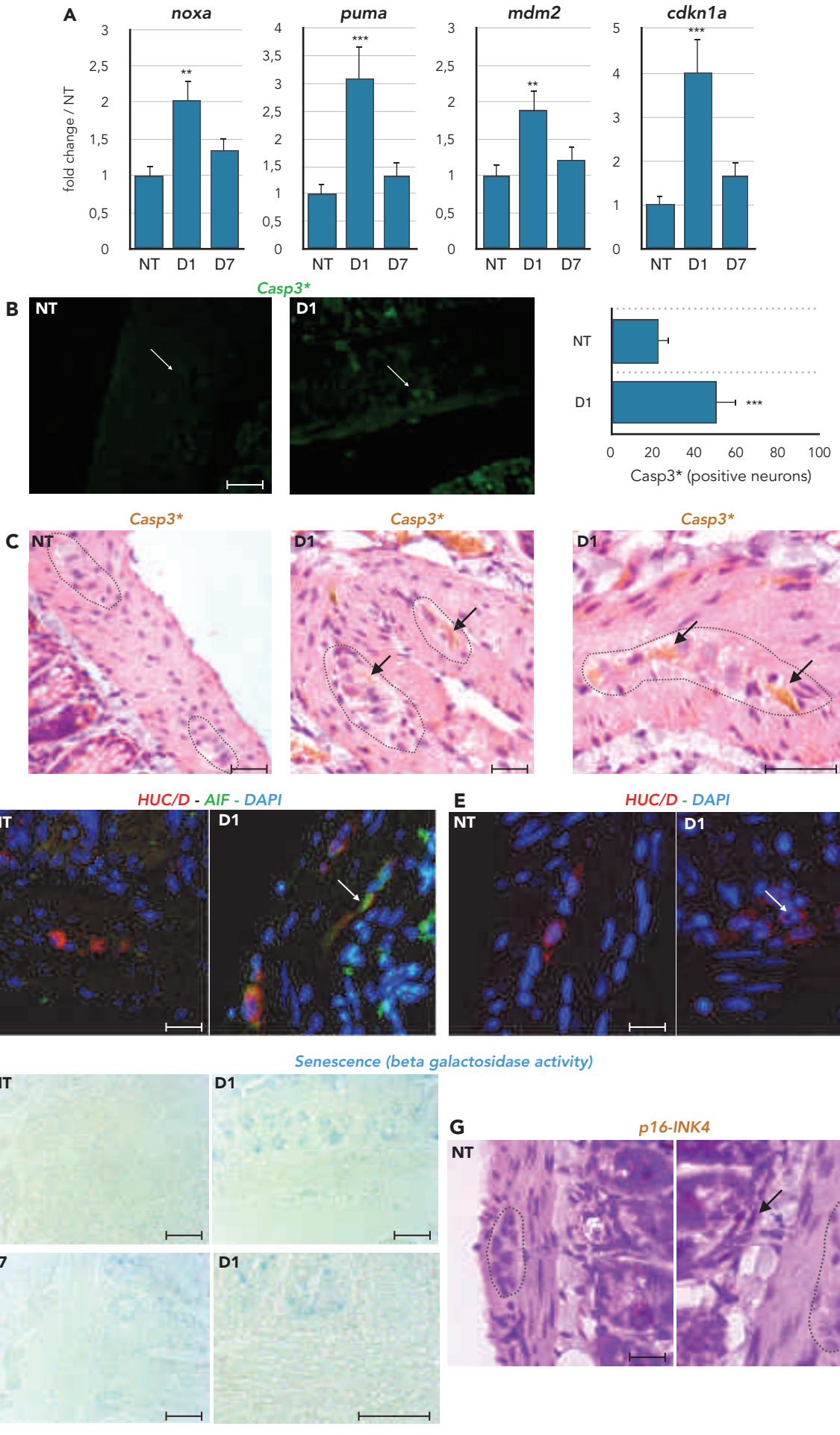
60
40
20
0
NT
D1
D7
C
Injection
D1
D7
mRNA seq
mRNA seq
D
D1/NT (625)
D7/NT (1436)
168
391
976
10
56
59
31
D1/D7 (156)
E
Early & transient cell death/ survival signalings
Z score
Trp73 targets
Trp53 targets
p53 signaling pathway
Apoptosis
TRAIL signaling pathway
miRNA regulation of DNA Damage Response
G1/S DNA Damage Checkpoints
PI3K/AKT activation
Signaling by PDGF
Aurora A signaling
F
Stable Interleukins/ inflamosome signalings
RIG-I/MDA5 med. induction of IFN-a/b pathways
IL2-mediated signaling events
IL3-mediated signaling events
IL5-mediated signaling events
NOD-like receptor signaling pathway
Inositol phosphate metabolism
Yy1 targets
Foxp3 targets
IRF4 targets
G
Late neuronal plasticity signalings
Factor-regulated calcium reabsorption
Splicing factor NOVA regulated synaptic proteins
Long-term potentiation
Calcium signaling pathway
Kit Receptor Signaling Pathway
Phosphatidylinositol signaling system
Glucagon-type ligand receptors
N-cadherin signaling events
GPCRs, Class B/2 (Secretin family receptors)
Creb1 targets
Jun targets
Rest targets
H
Neuronal and progenitors markers
Thalamus Subthalamic Nucleus
Frontal Cortex
Fetal Brain
Substantia Nigra Reticulata
Corpus Callosum
Cerebellum
Thalamus Lateral Nuclei
Dorsal Root Ganglia
Highly migratorySchwann cell precursor(E12 E14)
Induced Pluripotent Stem Cells
Ectoderm Progenitor
Embryo Week 5

Figure 2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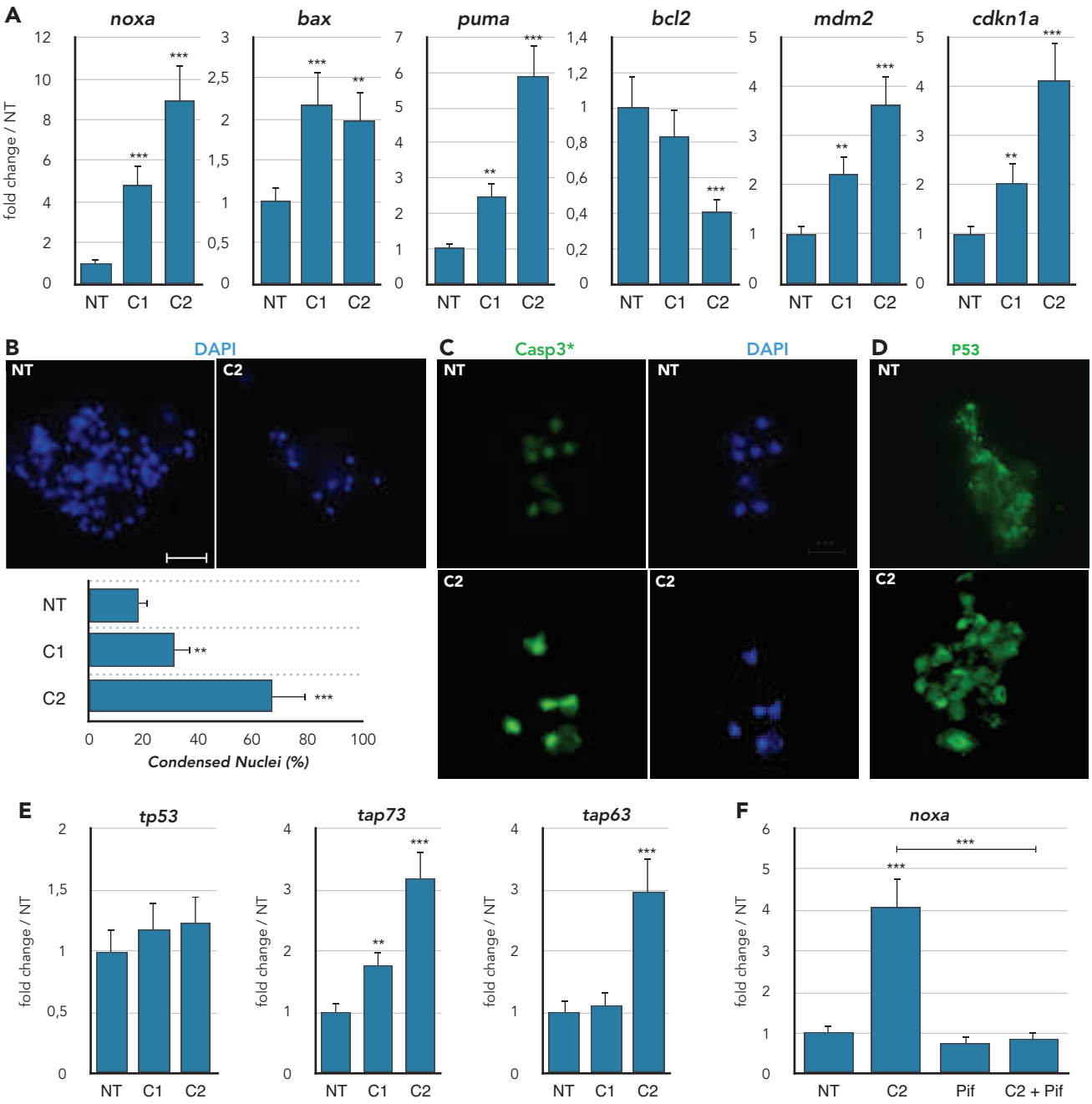
noxa
puma
mdm2
cdkn1a
fold change / NT
NT
D1
D7
B
Casp3*
NT
D1
Casp3* (positive neurons)
C
Casp3*
D
HUC/D - AIF - DAPI
E
HUC/D - DAPI
F
Senescence (beta galactosidase activity)
D7
G
p16-INK4

Figure 3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noxa
bax
puma
bcl2
mdm2
cdkn1a
fold change / NT
NT
C1
C2
B
DAPI
NT
C2
Condensed Nuclei (%)
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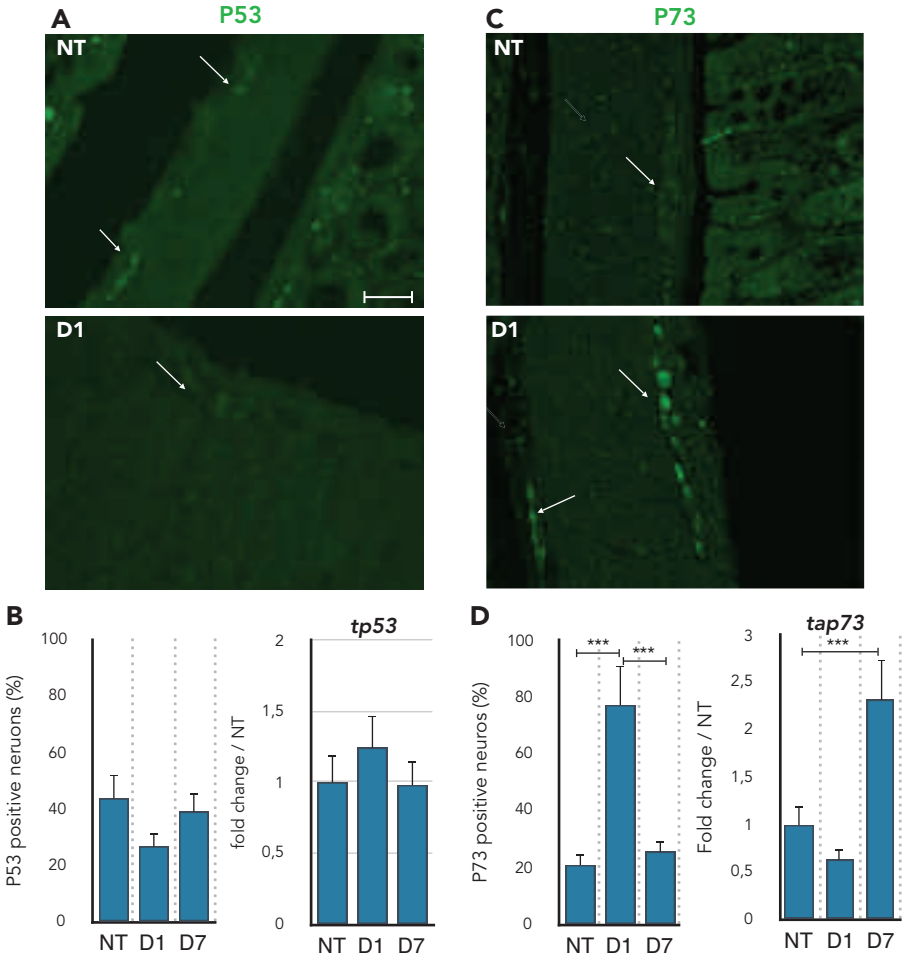
tap73
tap63
F
noxa
Pif
C2 + Pif

Figure 4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P53
NT
D1
C
P73
NT
D1
B
P53 positive neruons (%)
100
80
60
40
20
0
NT D1 D7
fold change / NT
tp53
2
1,5
1
0,5
0
NT D1 D7
D
P73 positive neuros (%)
100
80
60
40
20
0
***
***
NT D1 D7
Fold change / NT
tap73
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2,5
2
1,5
1
0,5
0
***
NT D1 D7

Figure 5

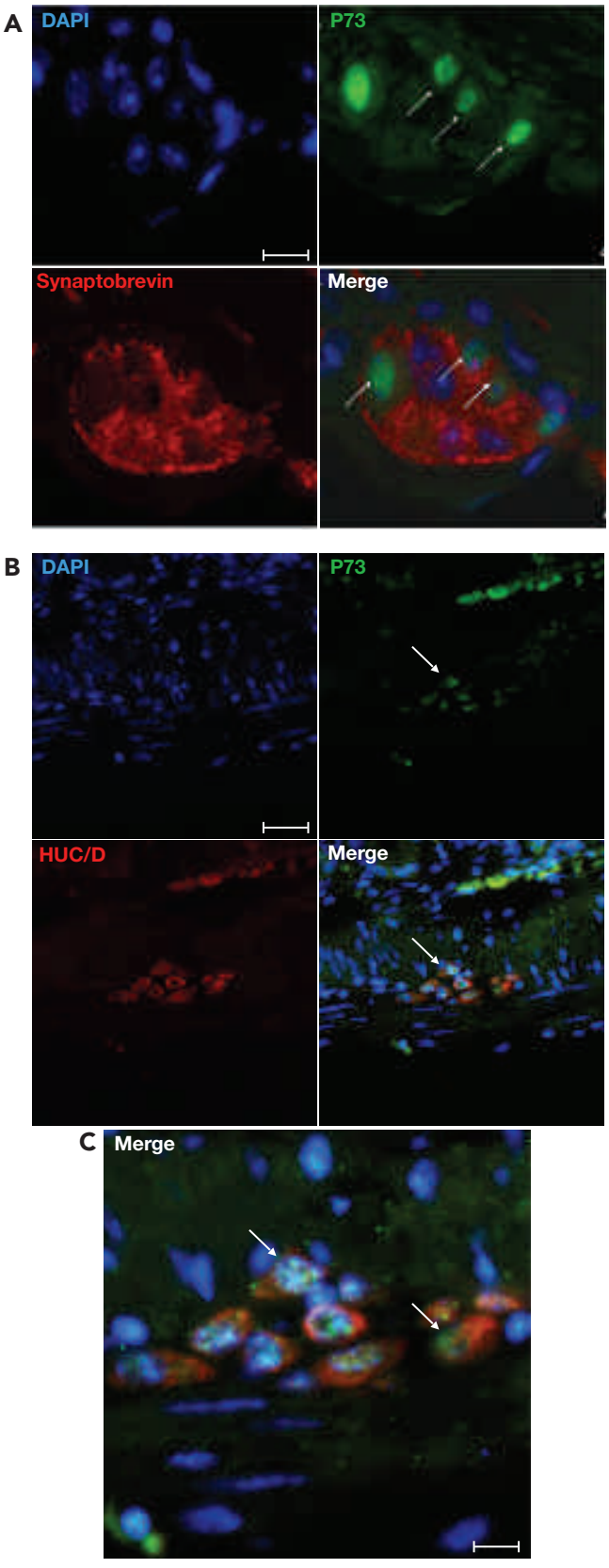
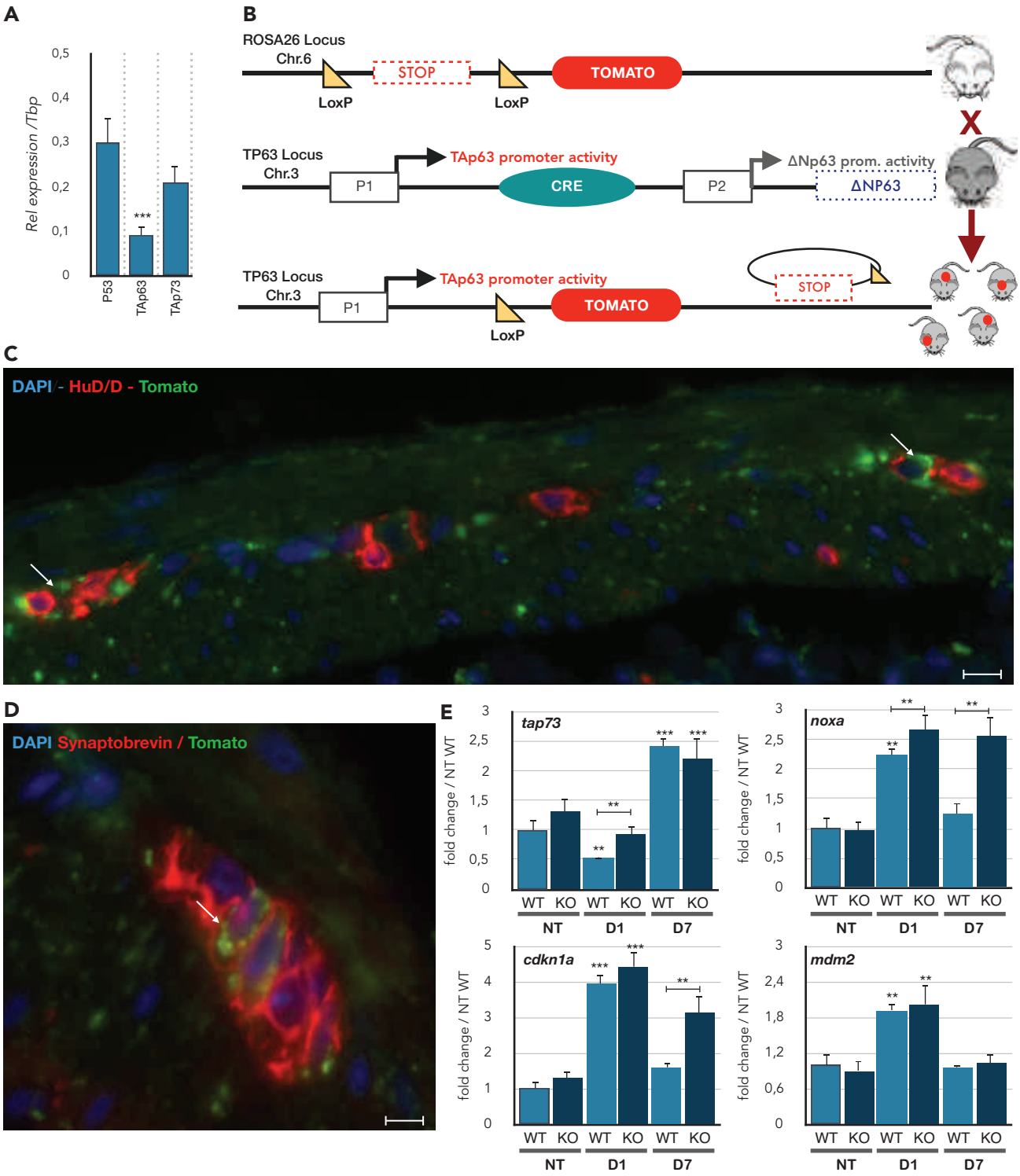


Figure 6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Rel expression /Tbp
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0,4
0,3
0,2
0,1
0
***
P53
TAp63
TAp73
B
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Chr.6
STOP
TOMATO
LoxP
LoxP
X
TP63 Locus
Chr.3
TAp63 promoter activity
P1
CRE
P2
ΔNp63 prom. activity
ΔNP63
TP63 Locus
Chr.3
TAp63 promoter activity
P1
TOMATO
LoxP
STOP
C
DAPI - HuD/D - Tomato
D
DAPI Synaptobrevin / Tomato
E
tap73
noxa
cdkn1a
mdm2
fold change / NT WT
WT KO WT KO WT KO
NT D1 D7

Figure 7

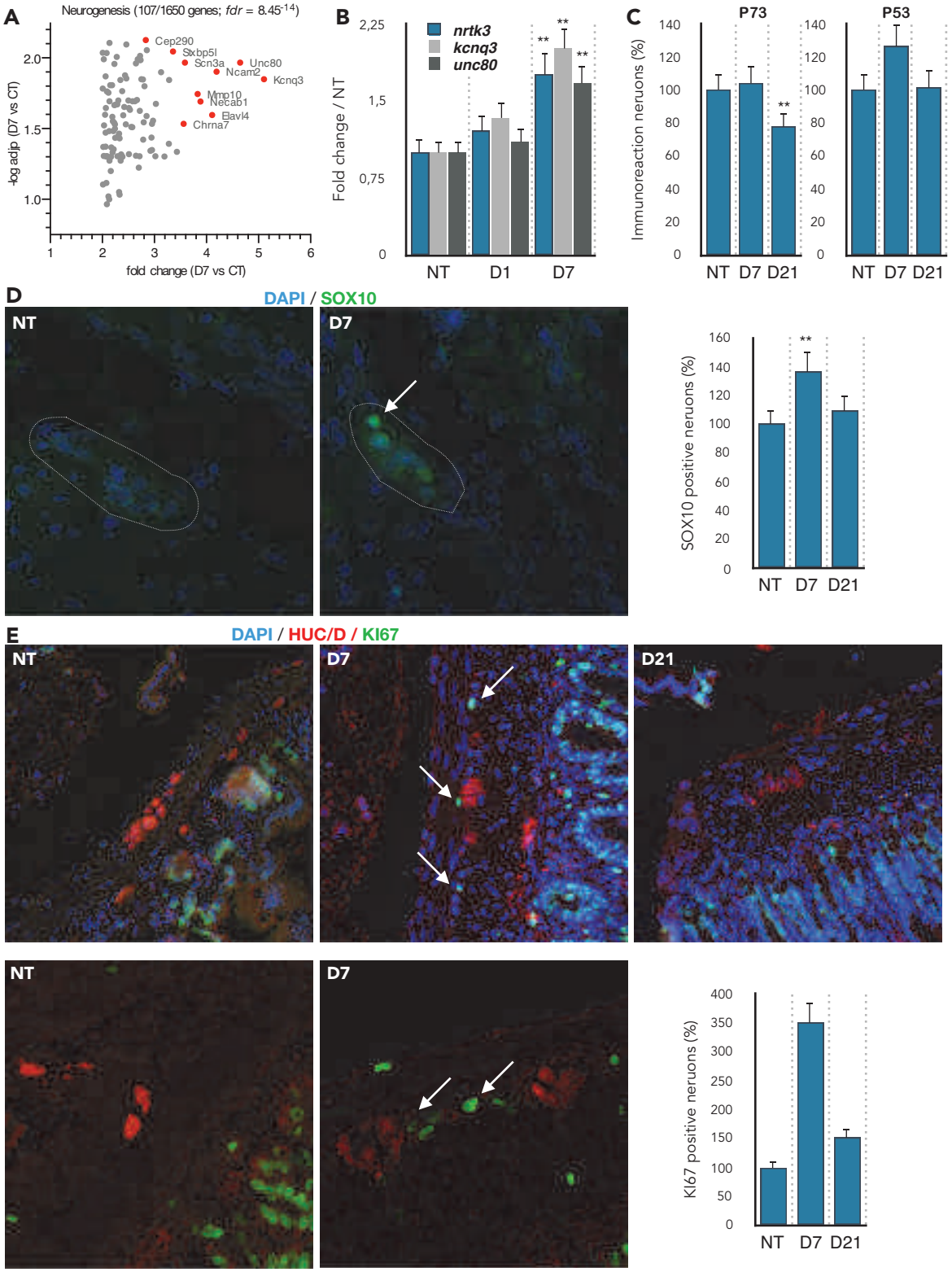
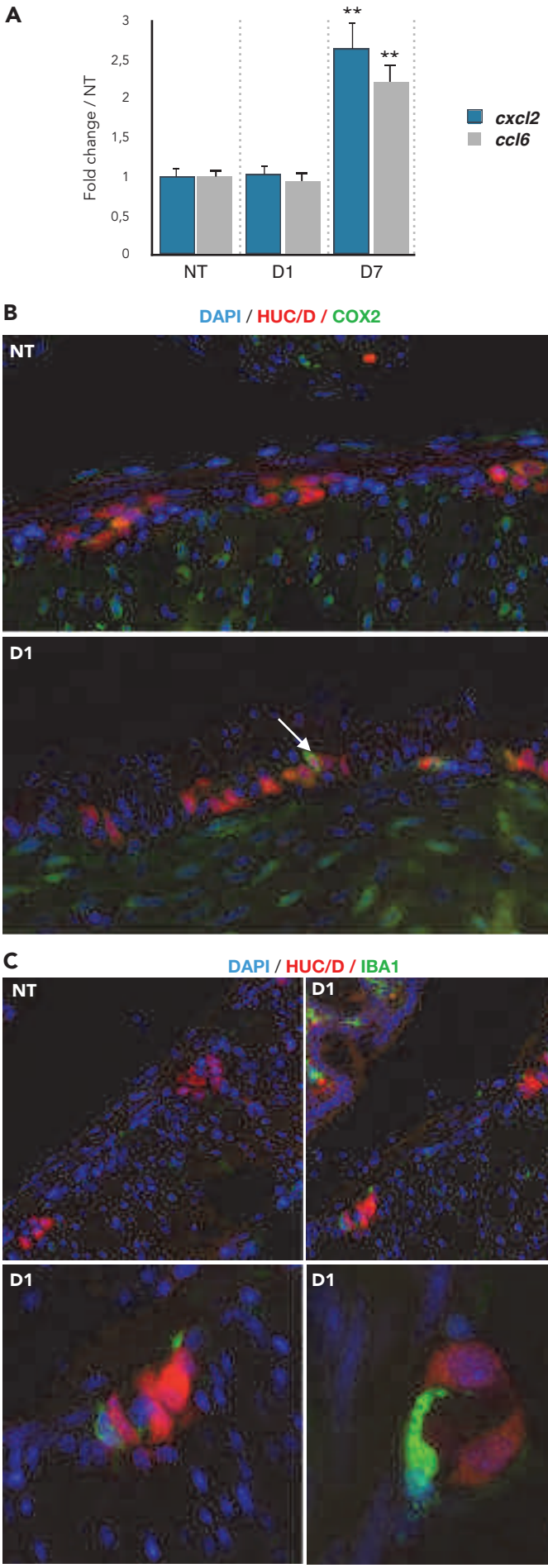

A
Neurogenesis (107/1650 genes; fdr = 8.45-14)
Cep290
Stxbp5l
Scn3a
Ncam2
Unc80
Kcnq3
Mmp10
Necab1
Elavl4
Chrna7
-log adjp (D7 vs CT)
fold change (D7 vs CT)
B
nrtk3
kcnq3
unc80
Fold change / NT
NT D1 D7
C
P73
P53
Immunoreaction neruons (%)
NT D7 D21
D
DAPI / SOX10
NT
D7
SOX10 positive neruons (%)
NT D7 D21
E
DAPI / HUC/D / KI67
NT
D7
D21
KI67 positive neruons (%)
NT D7 D21

Figure S1



A. Cisplatin induces expression of genes encoding cytokines (*cxcl2*, *ccl6*) in myenteric cells. Myenteric plexuses of mice non-treated (NT) or treated for 1 day (D1) or 7 days (D7) with cisplatin were isolated and mRNA were analyzed by qPCR. Graphs represent means with SD relative to the control.** indicates p > 0.01, respectively (n=3). *Tbp* was used a house-keeping gene.

B. Cisplatin induces Cox2 in ENS neurons. Gut tissues of non-treated (NT) mice and mice treated for 1 day with cisplatin were labelled for COX2 (green) and the neuronal marker HUC/D (red). Nuclei were labelled with Dapi (blue).

C. Cisplatin induces Iba1 in ENS neurons. Gut tissues of non-treated (NT) mice and mice treated for 1 day with cisplatin were labelled for Iba1 (green) and the neuronal marker HUC/D (red). Nuclei were labelled with Dapi (blue).

Role of the p63 in the regulation of Trim63 in chemotherapy-induced cachexia.

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Running title: Role of TAp63 in the chemotherapy-induced muscle atrophy

Keywords: p53, p63, muscle atrophy, chemotherapy, trim63

Highlights:

• The doxorubicin-based chemotherapy induces TAp63 in skeletal muscles

• Transcriptomic analysis of skeletal muscles treated with doxorubicin highlights activation of p53-related target genes

• Skeletal muscle cells express TAp63

• TAp63 and p53 control the expression of trim63

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Abstract

Muscle atrophy is a severe syndrome associated with certain types of cancers and chemotherapies that worsened the prognosis for patients. We performed a transcriptomic analysis on skeletal muscle of mice treated with the chemotherapeutic drug doxorubicin to identify the deregulated pathways that may play a role in muscle atrophy. Beside increase in muscle atrophy mechanisms, such as *trim63*, we observed an activation of cell death (i.e. *puma*) and DNA damage (i.e. *gadd45b*) mechanisms, and of p53 and TAp63 target genes (i.e. *peg3*) or regulators (i.e. *aen*), as well as a putative YAP inhibitor, *arrdc2*. We first validated the deregulated genes and characterized the expression of p53 and TAp63 in muscle treated with doxorubicin. We then showed that p53 as well as TAp63 are involved in the regulation of the expression of the pro-atrophic gene *trim63* via a direct interaction on its promoter. Finally, we showed that the YAP transcription factor participates in the regulation of *trim63* expression and is regulated by doxorubicin. Altogether, our results characterized novel molecular mechanisms induced by the chemotherapeutic drug doxorubicin in skeletal muscle that may account for atrophy observed in cancer patients.

Introduction

Muscle atrophy can occur as part of many diseases and is often an indicator of bad prognosis, impairing quality of life and increasing mortality [1, 2]. In addition, several conditions like aging, starvation, disuse and immobilization can lead to muscle loss and, depending on the cause, different molecular pathways can be involved in the muscle atrophy process. However, the imbalance between protein synthesis and degradation mediated by the proteasome pathway and modulated by the muscle specific ubiquitin ligases Trim63 (Murf1) and Atrogin-1 (MAFbx) are a common feature among the diverse causes of muscle loss [3]. These muscle specific ubiquitin ligases - Trim63 and Atrogin-1 - are described to be consistently upregulated in a range of skeletal muscle atrophy models.

Cancer cachexia is a multifactorial syndrome characterized by pronounced muscle atrophy, systemic inflammation and metabolic alterations [4-6]. Importantly, cachexia affects between 50 to 80% of cancer patients and is responsible for about 20% of their death [7]. Increasing evidences point out the impact of the tumors and/or the therapies on muscle function and integrity in cancer patients. Studies suggest that the tumor itself plays an active role in muscle wasting due to its ability to secrete pro-inflammatory cytokines and the large requirement of nutrients to maintain cell growth, creating a tumor-host interaction that aggravates the condition of the patient [4]. At the same time, cancer chemotherapy presents side effects that could contribute to cachexia, like anorexia, nausea and diarrhea. But more than that, it has been shown that chemotherapeutic agents have a direct negative effect on protein metabolism leading to cachexia [8] [9]. In this context, the anticancer drug doxorubicin is well recognized for having muscle catabolic effects [10]. Some works suggest that its atrophic effect would be due to production of ROS [11], TNF-α [12] or ceramides [13]. In addition, it was shown that doxorubicin is able to induce p53 accumulation in cardiac myocytes, which can contribute to muscle wasting [14].

However, despites the identification of these different mechanisms involved in muscle atrophy, no efficient therapy has been yet developed to restore muscle mass in cancer patients. Hence, we used a transcriptomic approach on muscle of mice treated with doxorubicin to identify novel mechanisms that might account for skeletal muscle atrophy and that could represent therapeutic targets. Among the deregulated pathways, this approach pointed to an activation of p53 and its homologue TAp63.

Despite the classical roles of the p53 family of transcription factors (p53, p63 and p73) in tumorigenesis, cell cycle, apoptosis regulation, epithelial and neuronal development, numerous elements in the literature suggest that the p53 family modulates myogenesis and differentiation [15]

[16]. For example, p63 is particularly important during heart development [17], while p73 modulates myoblast differentiation [18]. In addition, p53 and p63 play a role during muscle regeneration and metabolism by controlling Sirt1, TIGAR and AMPK expression [19] [20] [21]. Within the p53 family, distinct promoters generate two classes of isoforms generally recognized for having antagonist function, containing (TA) or not (ΔN) the N-terminal transactivation domain [22]. Importantly, within skeletal muscles, the balance between TA and ΔN-isoform expression influence on developmental and differentiation processes [23]. Interestingly, p53 has been shown to be induced during limb immobilization [24]. In addition, we underlined the p53 family relation with muscle atrophy showing that p63 is upregulated in amyotrophic lateral sclerosis (ALS) and able to directly modulate *Trim63* (Murf1) expression [25].

Hence, in this work, we investigate whether the p53 and TAp63 can modulate muscle wasting in a cancer-associated cachexia model induced by doxorubicin and if it could be a common regulatory mechanism of muscle atrophy associated to diseases.

Materials and Methods

Cell culture

C2C12 cells were obtained from ATCC (ATCC CRL-1772) and grown in DMEM (Dulbecco's modified Eagle's medium; Life Technology, Carlsbad, CA) with 10% fetal bovine serum (Life Technology) at 37°C in a humidified atmosphere and 5% CO2. Mycoplasma contamination has been tested negatively using PlasmoTest (Invivogene, San Diego, CA). Cells and animals were treated with doxorubicin (Pfizer 50mg).

Quantitative PCR

TRIzol (Invitrogen, Carlsbad, CA) was used to extract RNA. One µg of RNA was used for reverse transcription (iScript cDNA kit, Bio-Rad, France) and qPCR was carried out (iQ SYBR Green, Bio-Rad). Expression levels were normalized TBP as previously described [26].

Western blotting

Cells or tissue were lysed with LB (125 mM Tris-HCl pH 6.7, NaCl 150 mM, NP40 0.5%, 10% glycerol). Proteins were denatured and deposited directly (35 µg of proteins) onto a SDS-PAGE gel. Western

blotting was performed using antibodies raised against p53 (mouse monoclonal anti-p53, 1C12, Cell Signaling Technology, France), p63 (mouse anti-p63, 4A4, Santa Cruz Biotechnology; p63, Abcam, France), p73 (rabbit monoclonal anti-p73, EP436Y, Epitomics Abcam Company, France), TAp63 (rabbit polyclonal anti-Tap63, 618901, Biolegend), Trim63 (rabbit anti-Trim63, D01, Abnova; Taipei, Taiwan), Yap (mouse monoclonal anti-Yap, sc-101199, Santa Cruz) and phospo-Yap (rabbit monoclonal anti-pYap, 13008, Cell Signaling). Secondary antibodies (anti-rabbit, anti-mouse: Sigma, France) were incubated at 1:1000. Loading was controlled with actin (mouse anti-beta-actin, Sigma, 1:10000) (Antoine *et al.,* 1996).

Transfection assays

Cells were transfected by JetPrim (Polyplus, Strasbourg, France) as previously described [27]. SiRNA transfection was performed using 10 nM of siRNA against p63-pan (Trp63 Stealth RNAi MSS212111, Invitrogen), 50 nM of siRNA against p53 (Trp53 s75474, Ambion) and 30 nM of siRNA against Yap (Yap s76160, Ambion), using RNAiMAX or Dharmafect as described by the protocol provider (Life Technology and Dharmacon™ respectively).

Trp53 sequence: GCU UCG AGA UGU UCC GGG Att

Trp63-pan sequence: CCG AGG UUG UGA AAC GAU GCC CUA A

Yap sequence: GGA UGA AAU GGA UAC AGG Att

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using the standard protocol from the Magna ChIP G kit (Millipore). C2C12 lysates were sonicated 12 times at 10% power (Bioruptor™ UCD-200, Diagenode). For each 1 million cells, 1 µg of antibody was used. P53 and p73 were immunoprecipitated with a mouse antibody raised against total p53 (mouse monoclonal anti-p53, 1C12, Cell Signaling Technology, France) and p73 (mouse monoclonal anti-p73, E-3, Santa Cruz Biotechnology; Abcam, France). Mouse-anti-RAB11A was used as negative control (Santa Cruz Biotechnology).

RNA sequencing

Sequencing was performed by the Sequencing platform of the IGBMC (Strasbourg) and results were first analyzed using AltAnalysis software [28]. Deregulated gene were identified based on two-fold

change expression and t-test p-value <0.05. Deregulated genes were then analyzed for over-representation in selected biological processes in several resources: Gene Ontology, MPhenoOntology, Disease Ontology, GOSlim, PathwayCommons, KEGG, Transcription Factor Targets, miRNA Targets, Domains, BioMarkers, RVista Transcription Sites, DrugBank, BioGrid.

Wild-type mice and doxorubicin treatment

In total forty wild-type (WT) male mice C57BL/6 background 5 months old were randomly assigned to four groups of 10 mice each. The animals received a single intraperitoneal injection of doxorubicin (Pfizer 50mg) diluted at 18mg/kg in 0.9% NaCl, during 1, 3 or 5 days. Vehicle-treated littermates received the same formulation without doxorubicin. In vivo experiments were repeated twice with a number of animals recommended to optimize statistical analyses according to the regional and national animal ethics committee. All animal manipulations were performed under appropriate supervision and observing protocols validated by the regional and national animal ethics committee.

TAp63$^{+/Cre}$ROSA26^{Tomato} reporter mouse line

TAP63$^{CRE/+}$ mice were generated by the Clinique de la Souris (Strasbourg) by introducing the CRE recombinase cDNA after the TAp63 promoter. The ROSA26-tomato mice that contains the cDNA for the fluorescence protein Tomato after a LoxP flanked STOP cassette. By crossing these two mouse lines TAp63$^{CRE/+}$:ROSA26 mice were generated in house. Wild type C57BL/6, TAp63$^{CRE/CRE}$ or TAp63$^{CRE/+}$:ROSA26C57BL/6 mice of 10 weeks were weighed and intraperitoneally injected with 18 mg/kg doxorubicin in 200 µl 0.009% saline. Mice were weighed on a regular basis. Treated mice were sacrificed either 1, 3 and 5 days after treatment. Gastrocnemius muscles were taken out for immunofluorescent staining or RTqPCR. All animal experiments were approved by the Regional and National ethical committees.

Statistical Analyses

Statistical analyses were performed using a one-way analysis of variance test followed by a Tuckey post-test to allow a comparison between all the conditions. In the graphs, an asterisk indicates a statistically significant difference. Tests confirmed a statistically significant difference between control and treated mice. Statistical analyses were performed using Prism (GraphPad Software, San Diego, CA).

Results

RNA sequencing of doxorubicin-treated skeletal muscles shows multiple deregulated pathways

To identify novel molecular mechanisms involved in chemotherapy-induced muscle atrophy, we treated mice with doxorubicin for 3 days and analyzed gastrocnemius skeletal muscle by RNA sequencing (Figure 1A). Under these conditions, muscle mass diminished by about 20% (Figure 1B) as previously observed [29]. Sequencing results were analyzed by Altanalysis software to first established genes deregulated by 2-fold with an *adjp* value < 0.05. Then, pathways analyses were performed using multiple databases (ex. DAVID, STRING, Reactome, TRAP). Deregulated processes or pathways were selected when the false discovery risk was bellow 0.05 or the z score above 2.

As expected, muscle atrophy related pathways were identified as induced, which included the Murf-1/Trim63 ubiquitin ligase (Figure 1C). In addition, pathways related to cell death, autophagy, cell growth, and DNA damage, were also activated. Activation of these pathways correlated with induction of target genes for Tp53 and TAp63 transcription factors (Figure 1D). Target genes for other transcription factors, such as Nfkb and Fox3p, were also induced. In particular, target genes for MyoD1 were induced, indicating that muscle plasticity mechanisms leading to precursor proliferation are activated upon doxorubicin treatment [30].

Inversely, several mechanisms were strongly downregulated, such as cell adhesion and extracellular matrix components, skeletal muscle development and pro-survival mechanisms (AKT-Pi3K and PDGF signaling) (Figure 1E). Similarly, target genes for several transcription factors, such as SP1, NFAT3, and Myogenin were also downregulated, suggesting that the activity of these transcription factors were downregulated in muscle upon doxorubicin treatment. Interestingly, these transcription factors are also part of the molecular mechanisms of muscle cell differentiation processes [30, 31].

TAp63 is induced during atrophy of skeletal muscle

The pathway analyses identified the upregulation in skeletal muscles upon doxorubicin treatment of several target genes and regulators of Tp53 and TAp63, such as *gadd45*, *nqo1*, *aen*, *eda2r*, *ddit4* and *peg3* (Figure 2A). This observation suggested that Tp53 and TAp63 were involved in the process leading to muscle atrophy induced by doxorubicin. This was previously shown for p53 but never for TAp63 [14]. However, we previously showed that TAp63 expression was induced during skeletal

muscle atrophy in amyotrophic lateral sclerosis (ALS) [25]. Therefore, we decided to investigate further the role of TAp63 in the response on muscle to doxorubicin.

Mice were treated with doxorubicin and gastrocnemius muscles were analyzed after 1, 3 and 5 days of treatment. Under these conditions muscle mass decreased of about 25% after 3 days and stayed diminished at 5 days (Figure 2B). As expected, expression of the two major pro-atrophic ubiquitin ligases, *trim63* and *atrogin1*, were strongly upregulated at 3 days of treatment, but small increases were also seen at 1 day and 5 days of treatment (Figure 2C and D). Interestingly, increased protein expression of p53 was also seen at 5 days of treatment while increased mRNA level of TAp63 was already observed at 3 days of treatment (Figure 2E and F).

We also attempted to visualize TAp63 expression in the muscles. Despite the use of multiple antibodies, we were never able to see TAp63 labelling (personal data). Hence, we used a genetic approach to assess the existence and the role of the expression of TAp63 in skeletal muscles. Mice with CRE recombinase knock in within the promoter of TAp63 ($TAP63^{CRE/+}$) were breaded with $ROSA26^{Tomato}$ mice that contains the cDNA for the fluorescence protein Tomato flanked by LoxP sequences (Figure 3A). The generated compound mice ($TAp63^{CRE/+}$:$Rosa26^{Tomato}$) expressed the fluorescent Tomato proteins in cells that have or have had an active TAp63 promoter. Expression of Tomato proteins was present in myotubes of the compound mice ($TAp63^{CRE/+}$:$Rosa26^{Tomato}$) but not in the $Rosa26^{Tomato}$ mice (Figure 3B). Tomato expression was also observed in nuclei. Interestingly the number of tomato-positive nuclei was increased upon doxorubicin treatment (Figure 3B and C). These results suggested that TAp63 was expressed in muscle cells and that this expression is activated by doxorubicin in some nuclei.

To analyze the possible role of TAp63 in muscles, we inter-crossed $TAp63^{CRE/+}$ mice to generate $TAp63^{CRE/CRE}$ mice that do not express TAp63 protein. $TAp63^{CRE/+}$ and $TAp63^{CRE/CRE}$ mice were treated with doxorubicin for 3 days and compared to non-treated mice. No significant changes in morphology was observed. However, analysis of *trim63* expression showed that doxorubicin induced more strongly *trim63* in TAp63 KO mice (Figure 3D). This result was surprising as we previously reported that TAp63 was capable of inducing *trim63* expression [25]. Hence, we analyzed under the same condition other genes that might be impacted by either doxorubicin treatment or TAp63 expression. Interestingly, under the same conditions atrogin-1 induction by doxorubicin is not changed. In contrast, *sirt1* that have previously shown to be regulated by TAp63 [21], is induced in WT mice but not in TAp63 KO mice (Supplementary figure 1). These results indicate that TAp63 is expressed in muscles and participate in the regulation of specific genes involved in muscle atrophy.

TAp63 is induced by doxorubicin in myoblastic cells

To clarify the role of TAp63 in the response of skeletal muscle to doxorubicin, we used the murine myoblastic cell line C2C12. In these cells, doxorubicin treatment induced also the mRNA levels of several p53/TAp63 target genes (i.e. *nqo1*, *noxa*) (Figure 4A) that were upregulated in the RNA sequencing experiment, indicating that the change in mRNA level was happening in muscle cells of the gastrocnemius muscle and not in some other cell types that might be present in this muscle. We then analyzed the expression level of p53 and TAp63 in C2C12 cells treated with two concentration of doxorubicin (0.4 and 0.6 µM) over different time. This time course experiment revealed that doxorubicin strongly induced the mRNA level of TAp63 at 18h and maintained at 24h of treatment (Figure 4B). P63 proteins were also induced although with less intensity (Figure 4C). p53 protein level was also induced by doxorubicin, in a time and dose dependent manner. The induction of p53 protein expression and nuclear localization was confirmed by immunohistochemistry (Supplementary Figure 2).

Interestingly, the doxorubicin-induced expression of p53 and TAp63 correlated with *trim63* (Figure 4D) and *atrogin-1* upregulation (Figure 4E), similarly to what was observed *in vivo* (Figure 2C and F). These results showed that doxorubicin induces the expression of p53 and TAp63 and atrogenes in myoblast cells in a similar time and dose dependent manner, suggesting that they might act in the same pathway.

Functional interaction between p53/TAp63 and Trim63

Based on the correlation existing between the expression of the different p53 family members and atrogenes, we aimed at investigating whether the different members of the p53 family could regulate the expression of *trim63* and *atrogin-1*. To do so, we performed gain of function experiments in murine myoblastic cells (C2C12), treated or not treated with 0.6 µM of doxorubicin for 24 hours, and subsequently analyzed for the expression of *trim63* and *atrogin-1* mRNA by RT-qPCR. The results showed that overexpression of TAp63 strongly induces the expression of *trim63*, which was further potentiated by the treatment with doxorubicin (Figure 5A). Interestingly, ΔNp63, normally described for having a dominant negative effect, also stimulated Trim63 expression. Overexpression of p53 also induced *trim63* expression, but with less intensity than TAp63. Importantly, overexpression of

the different p53 family members did not affect *atrogin-1* expression (data not shown). These results confirmed our previous data suggesting that the p53 family regulates *trim63* expression [25].

To investigate if the expression of *trim63* is not only induced but also depends on the expression of the p53 and TAp63, we transfected C2C12 cells with p53 or p63 specific siRNA, treated cells or not with 0.6 µM of doxorubicin for 24 hours and analyzed again for the expression of *trim63* by RT-qPCR (Figure 5B-E). The efficacy of the p53 and p63 siRNAs was confirmed by RT-qPCR (Figure 5B and C) and Western blot (Supplementary Figure 3A and B). Importantly, transfection of siRNA against p53 or p63 strongly decreased Trim63 expression in the presence of doxorubicin (Figure 5D and E) suggesting that doxorubicin induced expression of *trim63* in part depends on p53 and p63 in C2C12. Hence, it is possible that in TAp63 KO mice p53 compensate for the lack of TAp63 in regulating *trim63*.

Physical interaction between the p53/TAp63 and Trim63.

Our results support the hypothesis that p53 family members may participate in the regulation of *trim63* expression during muscle atrophy induced by doxorubicin. Indeed, we have previously shown that TAp63 can bind and transactivate *trim63* promoter [25]. In order to evaluate if doxorubicin might influence the p53 family binding to the *trim63* promoter we performed Chromatin immunoprecipitation (ChIP) assays covering two possible p53 binding sites in the *trim63* promoter in presence of doxorubicin (Figure 6A). These binding sites were identified using both the "Eukaryotic promoter database" and the "p53FamTAG" bioinformatic tools. One of the sites is located at 660/-690 bp and the second at 2015/2045 bp. The ChIP assay demonstrated that both p53 and TAp63 bind to the *trim63* promoter (Figure 6B). However, it showed also that although doxorubicin strongly induced *trim63* expression, it does not seem to increase the binding of p53 or p63 to the *trim63* promoter.

Role of YAP in the expression of trim63

One of the most induced genes in the RNA seq experiment was arrdc2 (fold change = 7.2) (Figure 7A). Two family members of *arrdc2*, *arrdc1* and *arrdc3*, are negative regulators of YAP [32] [33]. YAP is an important cofactor that belongs to the Hippo pathway, which insure the conversion of mechanical stimuli into biological activity [34]. YAP participates in multiple processes through the

interaction with several transcription factors, such as TEAD1-4 and p73, which leads to the expression of different types of target genes, such as *ctgf*, *cyr61*, *igfbp5*, *puma*, or *ankrd1,* depending which transcription factor YAP is associated with. In addition, p53 regulates the Hippo pathway by interfering with upstream regulators (Lats1/2) [35]. TAp63 has also been shown to induce the Hippo pathway [36]. Interestingly, YAP has been shown to inhibit *trim63* expression [37]. Besides that, the Hippo pathway seems to play an important role in controlling muscle plasticity [38]. Hence, we decided to analyze the impact of doxorubicin on the activity of YAP in the skeletal muscle and if YAP participated in the activation of trim63 expression.

Interestingly, analysis of the RNA sequencing data showed that several genes that are coregulated by YAP, such as *cyr61*, *puma* of *ankrd1* are upregulated. However, other YAP co-regulated genes are downregulated, such as *axl* or *igfbp5* (Figure 7A). The deregulation of two of the most canonical targets genes of the Hippo pathway, *cyr61* and *ctgf*, were validated by RT-qPCR on an independent experiment (Figure 7B). Similarly, upregulation of *cyr61* and *puma*, other YAP target genes, was also observed in C2C12 cells treated with doxorubicin, while *ctgf* and *igfbp5* were downregulated (Figure 7C). These results further suggested that YAP might be regulated upon doxorubicin treatment.

To analyze YAP regulation, we performed immunofluorescence on C2C12 cells treated with doxorubicin using a specific YAP phospho-antibody that recognizes YAP phosphorylated at s127. This phosphorylation sequesters YAP in the cytoplasm into an inactive form. C2C12 cells at confluency and in control condition showed a cytoplasmic and nuclear localization of YAP (Figure 7D). Treatment with doxorubicin induced a nuclear relocalization of YAP without a significant decrease of YAP phosphorylation. These results suggested that doxorubicin induced YAP capacity to induce gene expression by its nuclear localization.

To investigate the role of YAP in *trim63* expression, we used loss of function experiment using the YAP pharmacological inhibitor verteporfin and a siRNA towards YAP. Treatment of C2C12 with verteporfin blocked the induction of *trim63* expression by doxorubicin without affected the basal expression of *trim63* (Figure 7E). Under the same conditions, verteporfin diminished the expression of two YAP target genes, *cyr61* and *ctgf*, both in basal and doxorubicin-treated conditions. This result suggested that YAP might be involved in the induction of *trim63* expression by doxorubicin but not in its basal expression. Similarly, YAP siRNA diminished the expression of *cyr61* in doxorubicin-treated cells (Figure 7F). However, the effect of YAP siRNA on *trim63* was less marked compared to verteporfin. This might be explained by the involvement of the YAP paralogue, TAZ. To gain further understanding on how inhibition of the YAP and Hippo pathway might be impacting on *trim63*

expression, we analyzed the expression of the TAp63 and one of its target gene, *aen*. YAP siRNA diminished their mRNA level after doxorubicin treatment, suggesting that YAP might control in part the expression of *trim63* through TAp63.

Discussion

Despites numerous studies and the identification of several molecular mechanisms involved in muscle atrophy, including when it is caused by anticancer treatment, no efficient cure exists yet to restore muscle mass and strength. This lack of therapeutic solution is significantly impairing the quality of life and survival of cancer patients, and highlight the necessity to improve our understanding of the signaling pathways that causes muscle atrophy. To do so, we performed, to our knowledge, the first transcriptomic analysis on skeletal muscles of mice treated with an anticancer drug. This allowed us to identified several pathways and processes that were deregulated, either induced or repressed, upon treatment of mice with doxorubicin. Among them, target genes and regulator of TP53 and TAp63 were identified as significantly over-represented. Based on this observation and our previous findings that TAp63 was induced in muscle atrophy during ALS and regulated trim63, we further analyzed the role of TAP63 in the response of muscle to doxorubicin.

Doxorubicin deregulates multiple pathways in skeletal muscle

The transcriptomic analysis identified multiple pathways that were deregulated in the muscle of doxorubicin treated mice. Some of them were expected, such as the activation of the *trim63*/*atrogin-1* muscle protein degradation program [39] or the induction of apoptosis [40] and autophagy [41], that were previously described in this context. The negative regulation pro-survival pathway, such as AKT and MAPK pathways are consistent with the activation of the apoptosis and autophagy events. However, several deregulated processes or pathways were never connected to doxorubicin-induced skeletal muscle toxicities previously, such as activation of glycogen and lipid metabolism, or inhibition of enzyme involved in remodeling of the extracellular matrix. The implication of these processes in the muscle toxicity of anticancer drugs make sense as they have been shown to be important in muscle homeostasis and plasticity. For instance, dysregulation of lipid homeostasis or glycogen synthesis (i.e. *gbe1* fold change = 2.27) were shown to cause or be part in muscle atrophy present in various pathologies [42] [43] [44]. Obviously, loss of extracellular matrix and cell adhesion

may also participate into the muscular atrophy as these components are essential for the structure and the function of the muscle [45]. Interestingly, these loss of interaction with the matrix or adjacent cell may contribute to the complex regulation of the Hippo pathway and YAP that we also observed as they are essential effectors of the mechanotransduction processes that are often initiated at the membrane [34].

In addition to these deregulated pathways, the activity of several transcription factors is seen modulated by doxorubicin in the muscle. For instance, a complex regulation of muscle developmental and differentiation processes seems to take place as MyoD1, which favors proliferation of myoblasts, is induced, while MyoG, which induced differentiation into myotube, is repressed [30, 31]. NFKB and FOXP3 are induced which could be expected as they are nuclear effector for the signaling pathway induced by cytokines that are often association with cancer-related muscular atrophy [4, 46]. More surprisingly, HIF1A appears to be both induced and repressed. Although we cannot exclude that it may represent an artefact of the bioinformatics analyses, it may also be explained by a complex regulation of the specificity of HIF1A to target selected genes.

Doxorubicin induces Trim63 (Murf1) expression via the p53 and TAp63.

The transcriptomic approach showed an enrichment of p53/TAp63 target genes and regulators in response to doxorubicin in the skeletal muscle. The induction of p53 was previously shown and is consistent with activation of apoptosis and autophagy [14]. Similarly, activation of p53 was shown muscle atrophy in other contexts, such as limb immobilization [24], ALS [25], muscle unloading [47], or spinal muscular myopathy [48]. However, abolishing p53 function by gene inactivation did not rescue muscle atrophy, suggesting that compensatory mechanisms may exist [49]. Hence, the activation of TAp63 by doxorubicin we described here, and that we precedingly showed in ALS [25], can represent a such compensatory mechanism by inducing pro-apoptotic genes in place of p53. In this context, it is interesting to note that pifithrin that has been shown to inhibit the activity of several p53 family members can reduce the muscle atrophy induced by anticancer drugs [50].

In addition, activation of p53 and TAp63 could also explain some of the deregulation of the muscle metabolisms. For instance, p63 has been shown to be involved in the lipid metabolism [51], for example by inducing *apod* [52], which is a gene that is induced by doxorubicin in our experiments.

In addition to this function of p53 and TAp63 in apoptosis, we also provide evidence that the expression of the pro-atrophic ubiquitin ligase Trim63 is controlled by the p53 and TAp63 in the skeletal muscle upon treatment with doxorubicin. Both transcription factors bind to the *trim63* promoter and are involved in *trim63* mRNA upregulation caused by doxorubicin in C2C12 cells. In addition, the deletion of TAP63 in mice impact on the expression of *trim63*, increasing it. This suggests that the compensatory mechanisms induced by the loss of TAp63 end up to favor *trim63* expression upon doxorubicin treatment. For instance, TAp73 could be also part of these compensatory mechanisms as we observed its upregulation in TAp63 KO mice (data not shown). Interestingly, p53 or TAp63 do not play a role in *atrogin-1* expression, neither in vitro or in vivo, demonstrating a selective role of these two transcription factors on the ubiquitin ligase that induces degradation of the muscle structural protein and not on the one targeting the muscle specific transcription factors [39].

This selective effect on trim63 and not atrogin-1 may also explain why we did not observe significant differences in muscle structure and mass between the WT and the TAp63 KO mice upon doxorubicin treatment. However, this can also be due to the intervention of p53 or even TAp73. This would be consistent with our results and the previous studies showing that p53, p63 and p73 proteins play a role in muscle cell proliferation and differentiation [18].

Role of YAP in muscle atrophy and expression of trim63

Doxorubicin causes a differential regulation of YAP co-regulated genes. For instance, *cyr61*, *puma* and *ankrd1* are being upregulated, while *ctgf*, *igfbp5* and *axl* are downregulated. This complex regulation can be partly explained by the fact that YAP interact with multiple transcription factors that control the expression of these different target genes. For instance, *puma* and *bax* are co-regulated by YAP and TAp73 [53]. Additional interaction between the p53 family and YAP have been shown [54]. For instance, activation of YAP lead to increase TAp63 activity with production of ROS [55]. This latter study might explain our results showing that the expression of TAp63 and its target genes *aen* is downregulated by YAP silencing upon doxorubicin treatment in myoblastic cells. Overall, additional studies will be necessary to understand the mechanism that regulates the association of YAP with its different partners and how it impacts the expression of selected genes.

This complex regulation might also explain why the role of YAP and the Hippo pathway is still controversial in skeletal muscle [56]. For instance, a dominant active form of YAP, that cannot be

phosphorylated at serine 127 to be sequestered into the cytoplasm, reduces the expression of MyoG during myogenesis. This is consistent with our finding of an increase in YAP nuclear localization upon doxorubicin treatment. More interestingly, transgenic mice with such dominant active mutant display muscle atrophy with elevated expression of trim63 [57], supporting our findings that upon doxorubicin YAP participates into the activation of trim63 transcription. However, overexpression of a normal YAP produces the inverse phenotype [58], even if its expression was induced upon denervation that causes muscle atrophy. Interestingly, it was also found in this study a decreased in phosphorylation at serine 112 of YAP during myoblast maturation. Hence, it appears that the function of YAP in muscle plasticity involved its tight regulations by phosphorylation events that will also mitigate its association with different transcription factors.

In conclusion, this study highlights the complexity of the signaling pathways involved in muscle atrophy caused by anticancer treatments and point the importance of the p53 family in this process, including via the regulation of the pro-atrophic gene trim63. Our results also provide evidence of possible interactions between the p53 family and the Hippo pathway via the YAP co-activator during muscle atrophy, shaping a complex network linking mechanotransduction events with metabolism and cell survival processes.

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References

[1] K. Mukund, S. Subramaniam, Skeletal muscle: A review of molecular structure and function, in health and disease, Wiley Interdiscip Rev Syst Biol Med, (2019) e1462.

[2] R.Y. Cao, J. Li, Q. Dai, Q. Li, J. Yang, Muscle Atrophy: Present and Future, Advances in experimental medicine and biology, 1088 (2018) 605-624.

[3] O. Rom, A.Z. Reznick, The role of E3 ubiquitin-ligases MuRF-1 and MAFbx in loss of skeletal muscle mass, Free Radic Biol Med, 98 (2016) 218-230.

[4] J.M. Argiles, S. Busquets, F.J. Lopez-Soriano, The pivotal role of cytokines in muscle wasting during cancer, Int J Biochem Cell Biol, 37 (2005) 1609-1619.

[5] J.M. Argiles, F.J. Lopez-Soriano, B. Stemmler, S. Busquets, Novel targeted therapies for cancer cachexia, Biochem J, 474 (2017) 2663-2678.

[6] J.M. Argiles, B. Stemmler, F.J. Lopez-Soriano, S. Busquets, Inter-tissue communication in cancer cachexia, Nat Rev Endocrinol, 15 (2018) 9-20.

[7] W.D. Dewys, C. Begg, P.T. Lavin, P.R. Band, J.M. Bennett, J.R. Bertino, M.H. Cohen, H.O. Douglass, Jr., P.F. Engstrom, E.Z. Ezdinli, J. Horton, G.J. Johnson, C.G. Moertel, M.M. Oken, C. Perlia, C. Rosenbaum, M.N. Silverstein, R.T. Skeel, R.W. Sponzo, D.C. Tormey, Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group, Am J Med, 69 (1980) 491-497.

[8] T. Le Bricon, S. Gugins, L. Cynober, V.E. Baracos, Negative impact of cancer chemotherapy on protein metabolism in healthy and tumor-bearing rats, Metabolism, 44 (1995) 1340-1348.

[9] V.E. Baracos, Management of muscle wasting in cancer-associated cachexia: understanding gained from experimental studies, Cancer, 92 (2001) 1669-1677.

[10] D.S. Hydock, C.Y. Lien, B.T. Jensen, C.M. Schneider, R. Hayward, Characterization of the effect of in vivo doxorubicin treatment on skeletal muscle function in the rat, Anticancer Res, 31 (2011) 2023-2028.

[11] L.A. Gilliam, J.S. Moylan, E.W. Patterson, J.D. Smith, A.S. Wilson, Z. Rabbani, M.B. Reid, Doxorubicin acts via mitochondrial ROS to stimulate catabolism in C2C12 myotubes, Am J Physiol Cell Physiol, 302 (2012) C195-202.

[12] V. Adams, N. Mangner, A. Gasch, C. Krohne, S. Gielen, S. Hirner, H.J. Thierse, C.C. Witt, A. Linke, G. Schuler, S. Labeit, Induction of MuRF1 is essential for TNF-alpha-induced loss of muscle function in mice, J Mol Biol, 384 (2008) 48-59.

[13] J. De Larichaudy, A. Zufferli, F. Serra, A.M. Isidori, F. Naro, K. Dessalle, M. Desgeorges, M. Piraud, D. Cheillan, H. Vidal, E. Lefai, G. Nemoz, TNF-alpha- and tumor-induced skeletal muscle atrophy involves sphingolipid metabolism, Skeletal muscle, 2 (2012) 2.

[14] M. Schwarzkopf, D. Coletti, D. Sassoon, G. Marazzi, Muscle cachexia is regulated by a p53-PW1/Peg3-dependent pathway, Genes Dev, 20 (2006) 3440-3452.

[15] A. Porrello, M.A. Cerone, S. Coen, A. Gurtner, G. Fontemaggi, L. Cimino, G. Piaggio, A. Sacchi, S. Soddu, p53 regulates myogenesis by triggering the differentiation activity of pRb, J Cell Biol, 151 (2000) 1295-1304.

[16] G. Fontemaggi, A. Gurtner, S. Strano, Y. Higashi, A. Sacchi, G. Piaggio, G. Blandino, The transcriptional repressor ZEB regulates p73 expression at the crossroad between proliferation and differentiation, Mol Cell Biol, 21 (2001) 8461-8470.

[17] M. Paris, M. Rouleau, M. Puceat, D. Aberdam, Regulation of skin aging and heart development by TAp63, Cell Death Differ, 19 (2012) 186-193.

[18] H. Cam, H. Griesmann, M. Beitzinger, L. Hofmann, R. Beinoraviciute-Kellner, M. Sauer, N. Huttinger-Kirchhof, C. Oswald, P. Friedl, S. Gattenlohner, C. Burek, A. Rosenwald, T. Stiewe, p53 family members in myogenic differentiation and rhabdomyosarcoma development, Cancer Cell, 10 (2006) 281-293.

[19] K. Bensaad, A. Tsuruta, M.A. Selak, M.N. Vidal, K. Nakano, R. Bartrons, E. Gottlieb, K.H. Vousden, TIGAR, a p53-inducible regulator of glycolysis and apoptosis, Cell, 126 (2006) 107-120.

[20] P.S. Pardo, A.M. Boriek, The physiological roles of Sirt1 in skeletal muscle, Aging (Albany NY), 3 (2011) 430-437.

[21] X. Su, Y.J. Gi, D. Chakravarti, I.L. Chan, A. Zhang, X. Xia, K.Y. Tsai, E.R. Flores, TAp63 is a master transcriptional regulator of lipid and glucose metabolism, Cell Metab, 16 (2012) 511-525.

[22] F. Murray-Zmijewski, D.P. Lane, J.C. Bourdon, p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress, Cell Death Differ, 13 (2006) 962-972.

[23] R.A. Romano, K. Smalley, C. Magraw, V.A. Serna, T. Kurita, S. Raghavan, S. Sinha, DeltaNp63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation, Development, 139 (2012) 772-782.

[24] D.K. Fox, S.M. Ebert, K.S. Bongers, M.C. Dyle, S.A. Bullard, J.M. Dierdorff, S.D. Kunkel, C.M. Adams, p53 and ATF4 mediate distinct and additive pathways to skeletal muscle atrophy during limb immobilization, American journal of physiology. Endocrinology and metabolism, 307 (2014) E245-261.

[25] Y. von Grabowiecki, P. Abreu, O. Blanchard, L. Palamiuc, S. Benosman, S. Meriaux, V. Devignot, I. Gross, G. Mellitzer, J.L. Gonzalez de Aguilar, C. Gaiddon, Transcriptional activator TAp63 is upregulated in muscular atrophy during ALS and induces the pro-atrophic ubiquitin ligase Trim63, eLife, 5 (2016).

[26] V. Vidimar, C. Licona, R. Ceron-Camacho, E. Guerin, P. Coliat, A. Venkatasamy, M. Ali, D. Guenot, R. Le Lagadec, A.C. Jung, J.N. Freund, M. Pfeffer, G. Mellitzer, G. Sava, C. Gaiddon, A redox ruthenium compound directly targets PHD2 and inhibits the HIF1 pathway to reduce tumor angiogenesis independently of p53, Cancer Lett, 440-441 (2019) 145-155.

[27] C. Gaiddon, Y. Larmet, E. Trinh, A.L. Boutillier, B. Sommer, J.P. Loeffler, Brain-derived neurotrophic factor exerts opposing effects on beta2- adrenergic receptor according to depolarization status of cerebellar neurons, J Neurochem, 73 (1999) 1467-1476.

[28] D. Emig, N. Salomonis, J. Baumbach, T. Lengauer, B.R. Conklin, M. Albrecht, AltAnalyze and DomainGraph: analyzing and visualizing exon expression data, Nucleic Acids Res, 38 (2010) W755-762.

[29] Y. von Grabowiecki, C. Licona, L. Palamiuc, P. Abreu, V. Vidimar, D. Coovar, G. Mellitzer, C. Gaiddon, Regulation of a Notch3-Hes1 pathway and protective effect by a tocopherol-omega alkanol chain derivative in muscle atrophy, J Pharmacol Exp Ther, (2014).

[30] B.D. Harfmann, E.A. Schroder, K.A. Esser, Circadian rhythms, the molecular clock, and skeletal muscle, J Biol Rhythms, 30 (2015) 84-94.

[31] Y.Y. Cho, K. Yao, A.M. Bode, H.R. Bergen, 3rd, B.J. Madden, S.M. Oh, S. Ermakova, B.S. Kang, H.S. Choi, J.H. Shim, Z. Dong, RSK2 mediates muscle cell differentiation through regulation of NFAT3, J Biol Chem, 282 (2007) 8380-8392.

[32] J. Xiao, Q. Shi, W. Li, X. Mu, J. Peng, M. Li, M. Chen, H. Huang, C. Wang, K. Gao, J. Fan, ARRDC1 and ARRDC3 act as tumor suppressors in renal cell carcinoma by facilitating YAP1 degradation, Am J Cancer Res, 8 (2018) 132-143.

[33] X. Shen, X. Sun, B. Sun, T. Li, G. Wu, Y. Li, L. Chen, Q. Liu, M. Cui, Z. Zhou, ARRDC3 suppresses colorectal cancer progression through destabilizing the oncoprotein YAP, FEBS Lett, 592 (2018) 599-609.

[34] Z. Meng, T. Moroishi, K.L. Guan, Mechanisms of Hippo pathway regulation, Genes Dev, 30 (2016) 1-17.

[35] Y. Aylon, M. Oren, Tumor Suppression by p53: Bring in the Hippo!, Cancer Cell, 32 (2017) 397-399.

[36] X. Su, M. Napoli, H.A. Abbas, A. Venkatanarayan, N.H. Bui, C. Coarfa, Y.J. Gi, F. Kittrell, P.H. Gunaratne, D. Medina, J.M. Rosen, F. Behbod, E.R. Flores, TAp63 suppresses mammary tumorigenesis through regulation of the Hippo pathway, Oncogene, (2016).

[37] C.A. Goodman, J.M. Dietz, B.L. Jacobs, R.M. McNally, J.S. You, T.A. Hornberger, Yes-Associated Protein is up-regulated by mechanical overload and is sufficient to induce skeletal muscle hypertrophy, FEBS Lett, 589 (2015) 1491-1497.

[38] K.I. Watt, C.A. Goodman, T.A. Hornberger, P. Gregorevic, The Hippo Signaling Pathway in the Regulation of Skeletal Muscle Mass and Function, Exerc Sport Sci Rev, 46 (2018) 92-96.

[39] A.N. Kavazis, A.J. Smuder, S.K. Powers, Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle, Journal of applied physiology, 117 (2014) 223-230.

[40] A.P. Yu, X.M. Pei, T.K. Sin, S.P. Yip, B.Y. Yung, L.W. Chan, C.S. Wong, P.M. Siu, Acylated and unacylated ghrelin inhibit doxorubicin-induced apoptosis in skeletal muscle, Acta physiologica, 211 (2014) 201-213.

[41] A.J. Smuder, A.N. Kavazis, K. Min, S.K. Powers, Exercise protects against doxorubicin-induced markers of autophagy signaling in skeletal muscle, J Appl Physiol, (2011).

[42] S. Dalal, Lipid metabolism in cancer cachexia, Ann Palliat Med, 8 (2019) 13-23.

[43] P. de Lange, A. Lombardi, E. Silvestri, F. Goglia, A. Lanni, M. Moreno, Peroxisome Proliferator-Activated Receptor Delta: A Conserved Director of Lipid Homeostasis through Regulation of the Oxidative Capacity of Muscle, PPAR Res, 2008 (2008) 172676.

[44] M.A. Tarnopolsky, Myopathies Related to Glycogen Metabolism Disorders, Neurotherapeutics, 15 (2018) 915-927.

[45] M.F. Goody, R.B. Sher, C.A. Henry, Hanging on for the ride: adhesion to the extracellular matrix mediates cellular responses in skeletal muscle morphogenesis and disease, Dev Biol, 401 (2015) 75-91.

[46] L. Lu, J. Barbi, F. Pan, The regulation of immune tolerance by FOXP3, Nature reviews. Immunology, 17 (2017) 703-717.

[47] P.M. Siu, S.E. Alway, Id2 and p53 participate in apoptosis during unloading-induced muscle atrophy, Am J Physiol Cell Physiol, 288 (2005) C1058-1073.

[48] M. Van Alstyne, C.M. Simon, S.P. Sardi, L.S. Shihabuddin, G.Z. Mentis, L. Pellizzoni, Dysregulation of Mdm2 and Mdm4 alternative splicing underlies motor neuron death in spinal muscular atrophy, Genes Dev, 32 (2018) 1045-1059.

[49] M.S. Tsai, Y.T. Chiu, S.H. Wang, H.M. Hsieh-Li, H. Li, Abolishing Trp53-dependent apoptosis does not benefit spinal muscular atrophy model mice, Eur J Hum Genet, 14 (2006) 372-375.

[50] P.G. Komarov, E.A. Komarova, R.V. Kondratov, K. Christov-Tselkov, J.S. Coon, M.V. Chernov, A.V. Gudkov, A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy, Science, 285 (1999) 1733-1737.

[51] E. Candi, A. Smirnov, E. Panatta, A.M. Lena, F. Novelli, M. Mancini, G. Viticchie, M.C. Piro, N. Di Daniele, M. Annicchiarico-Petruzzelli, G. Melino, Metabolic pathways regulated by p63, Biochem Biophys Res Commun, 482 (2017) 440-444.

[52] Y. Sasaki, H. Negishi, R. Koyama, N. Anbo, K. Ohori, M. Idogawa, H. Mita, M. Toyota, K. Imai, Y. Shinomura, T. Tokino, p53 family members regulate the expression of the apolipoprotein D gene, J Biol Chem, 284 (2009) 872-883.

[53] S. Strano, O. Monti, N. Pediconi, A. Baccarini, G. Fontemaggi, E. Lapi, F. Mantovani, A. Damalas, G. Citro, A. Sacchi, G. Del Sal, M. Levrero, G. Blandino, The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA Damage, Mol Cell, 18 (2005) 447-459.

[54] N. Furth, Y. Aylon, M. Oren, p53 shades of Hippo, Cell Death Differ, 25 (2018) 81-92.

[55] H. Huang, W. Zhang, Y. Pan, Y. Gao, L. Deng, F. Li, F. Li, X. Ma, S. Hou, J. Xu, P. Li, X. Li, G. Hu, C. Li, H. Chen, L. Zhang, H. Ji, YAP Suppresses Lung Squamous Cell Carcinoma Progression via Deregulation of the DNp63-GPX2 Axis and ROS Accumulation, Cancer Res, 77 (2017) 5769-5781.

[56] O. Gnimassou, M. Francaux, L. Deldicque, Hippo Pathway and Skeletal Muscle Mass Regulation in Mammals: A Controversial Relationship, Frontiers in physiology, 8 (2017) 190.

[57] R.N. Judson, S.R. Gray, C. Walker, A.M. Carroll, C. Itzstein, A. Lionikas, P.S. Zammit, C. De Bari, H. Wackerhage, Constitutive expression of Yes-associated protein (Yap) in adult skeletal muscle fibres induces muscle atrophy and myopathy, PLoS One, 8 (2013) e59622.

[58] K.I. Watt, B.J. Turner, A. Hagg, X. Zhang, J.R. Davey, H. Qian, C. Beyer, C.E. Winbanks, K.F. Harvey, P. Gregorevic, The Hippo pathway effector YAP is a critical regulator of skeletal muscle fibre size, Nature communications, 6 (2015) 6048.

Figure legends

Figure 1. *Gastrocnemius muscle develop a dynamic transcriptomic program in response to the cytotoxicity induced by doxorubicin*. **A.** Schematic representation of the protocol followed to analyze the transcriptome of gastrocnemius of mice treated with doxorubicin (18mg/Kg, single dose). **B.** Evolution of non-treated and doxorubicin treated mice weight (mg). * indicates p<0.05 (n=9). **C-F.** Pathways (**C. E.**) and transcription factors-associated genes (**D. F.**) found increased (**C. D**.) or decreased (**E. F.**) enriched upon doxorubicine treatment. Deregulated genes were identified by RNA sequencing. Graphs display -log 10 FDR and z score for indicated deregulated pathways with *adjp* < 0.05. Deregulated pathways were identified using STRING, Reactome, DAVID and Altanalysis software.

Figure 2. *Doxorubicin induces muscle atrophy markers in muscle tissue*. **A.** Doxorubicin induces expression of p53 family target genes (*gadd45*, *nqo1*, *aen*, *eda2r*, *ddit4* and *peg3*) in gastrocnemius muscles. Graph represent fold change with *adjp* value relative to the control obtained in the RNA sequencing experiment. * indicates p < 0.05 respectively (n=3). **B.** Doxorubicin induces significant loss of mice weight in a time-dependent manner. Mice were weighted before doxorubicin injection (Ct) or 1 day (D1), 3 days (D3) and 5 days (D5) after doxorubicin injection. **C. D. & F.** mRNA level of *trim63* (**C**) and *atrogin1* (**D**) and *tap63* in gastrocnemius muscle (**F**) 3 days (D3) after treatment with doxorubicin. Gastrocnemius muscle were removed, and mRNA were analyzed by qPCR. *tbp* was used as housekeeping gene. Graphs represent means with SD relative to the control. * indicates p < 0.05 respectively (n=3). **E.** Doxorubicin induces the expression of p53 protein time-dependently in gastrocnemius muscle. Skeletal muscle was removed before doxorubicin injection (Ct) or 1 day (D1), 3 days (D3) and 5 days (D5) after doxorubicin treatment and protein level was analyzed by western blot. Actin was used a house-keeping gene.

Figure 3. *TAp63 participates in doxorubicin-induced muscle atrophy*. **A.** Schematic representation of the genetic approach used to assess the expression of TAp63 in skeletal muscle. **B.** Muscle tissues of mice treated for 3 days with doxorubicin were labelled for Dapi (blue) and native fluorescence of the Tomato protein was observed (red). A nucleus with high expression of Tomato is indicated by a white arrow. **C.** Doxorubicin induces TAp63 promoter activation in skeletal muscle. Immunoreactive nuclei (white arrow in B) for Tomato (TAp63 activated promoter) were counted in section of gastrocnemius from non-treated (NT) mice or mice treated for 3 days (D3) with doxorubicin. Graphs represent means with SD relative to the control, and * indicates p< 0.05 (n=3). **D.** Doxorubicin induces the

upregulation of the pro-atrophic factor *trim63* mRNA in gastrocnemius muscle. Gastrocnemius muscle of wild-type of KO TAp63 mice were isolated before treatment (Ct) or after 3 days (D3) of doxorubicin, were extracted and RT-qPCR were performed on *trim63* mRNA (n=3). Graphs represent means with SD relative to the control and * indicates $p< 0.05$.

Figure 4. *Doxorubicin induces a correlated upregulation of TAp63 and Trim63 expression in C2C12 myoblastic cells.* **A.** Doxorubicin induces expression of p53 and p63 target genes (*nqo1*, *noxa*, *aen*, *bax* and *cebpd*) in myoblastic cell line C2C12. C2C12 cells were cultured in a non-treated (NT) or 1 day-treated (dox) media and mRNA were analyzed by qPCR. Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3). **B. D. & E.** Doxorubicin induces an important dose and time-dependent induction of skeletal muscle atrophy mediators. C2C12 cells were cultured in a non-treated media (ct) or doxorubicin-treated media at 0.4 or 0.6μM during 1 hour, 6 hours, 18 hours or 24 hours. mRNA levels were analyzed by qPCR for *tap63* (**B**) *trim63* (**C**) and *atrogin-1* (**E**). **C.** Doxorubicin induces protein level of p53, p63 and Trim63 in C2C12 cells. Proteins were extracted from C2C12 cells in control condition (ct) or after doxorubicin treatment with 0.4 or 0.6μM during 6 hours, 18 hours or 24 hours and p53, p63 and Trim63 protein expression were analyzed by Western blot. Actin was used a house-keeping gene.

Figure 5. *TAp63 induces trim63 expression in response to doxorubicin*. **A.** Overexpression of p53 family members potentializes doxorubicin-induced *trim63* expression. C2C12 mRNA were extracted after no transfection (ct) or p53 family members overexpression p53, TAp63 or ΔNp63 in non-treated (Ct) or treated for 1 day with doxorubicin (Dox). *trim63* mRNA level was analyzed by qPCR. Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3). *tbp* was used a house-keeping gene. **B-E.** Inhibition of p53 family members negatively induce *trim63* expression in response to doxorubicin. mRNA level of *tp53* (**B**), *tap63* (**C**) and *trim63* (**D & E**) were analyzed after extraction from C2C12 cells transfected by siRNA against p53 (sip53) (**B.&D.**) or TAp63 (siTAp63) (**C.&E.**) or not (ct) before (Ct) or after doxorubicin-treatment (Dox). Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3).

Figure 6. *p53 and p63 bind to the trim63 promoter* **A.** Schematic representation of the *trim63* promoter indicating the location of putative p53/p63 binding sites. **B.** *trim63* promoter regions (RE1/2 and RE4) obtained after chromatin immunoprecipitation (ChIP) using p53 or p63 antibodies

were amplified by qPCR. C2C12 cells cultured in normal (Ct) or doxorubicin-treated (Dox). Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3).

Figure 7. A. Expression fold change of *arrdc2*, *ankrd1*, *cyr61*, *igfbp5*, *axl*, *ccnd1*, *plau* and *ctgf* obtained by RNA sequencing in gastrocnemius muscle of mice treated with doxorubicin. Graphs represent means with *adjp* relative to the control. * indicates $p < 0.05$ respectively (n=3). **B.** Gastrocnemius muscle were removed in control mice (Ct) or after 3 days (D3) or 5 days (D5) of doxorubicin treatment in mice, and mRNA of YAP target genes (*cyr61* and *ctgf*) were analyzed by RT-qPCR. Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3). **C.** C2C12 cells were cultured in a non-treated (Ct) or doxorubicin-treated media (Dox) for 1 day and RNA were extracted for RT-qPCR analysis of *puma*, *cyr61*, *ctgf*, *igfbp5*, *yap* and *taz* expression. Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3). **D.** YAP and pYAP (TAP phosphorylated at serine 127) protein expression and localization was analyzed by immunocytofluorescence, stained with specific YAP and phopho-serine 127-YAP antibodies, and DAPI, in control (Ct) or after doxorubicin treatment (Dox) of C2C12 cells. **E.** Expression of *trim63*, *cyr61* and *ctgf* mRNA were analyzed in normal condition (ct) or YAP inhibition with verteporfin (Ver) in response to doxorubicin (Dox) or not (Ct). Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3). **F.** Expression of *yap*, cyr61, *trim63*, *tap63* and *aen* were analyzed after RNA extraction from C2C12 cells transfected for 48 hours by siRNA against YAP (siYAP) or not (ct), and treated with doxorubicin for 24 hours (Dox) or not (Ct). Graphs represent means with SD relative to the control. * indicates $p < 0.05$ respectively (n=3).

Figure 1

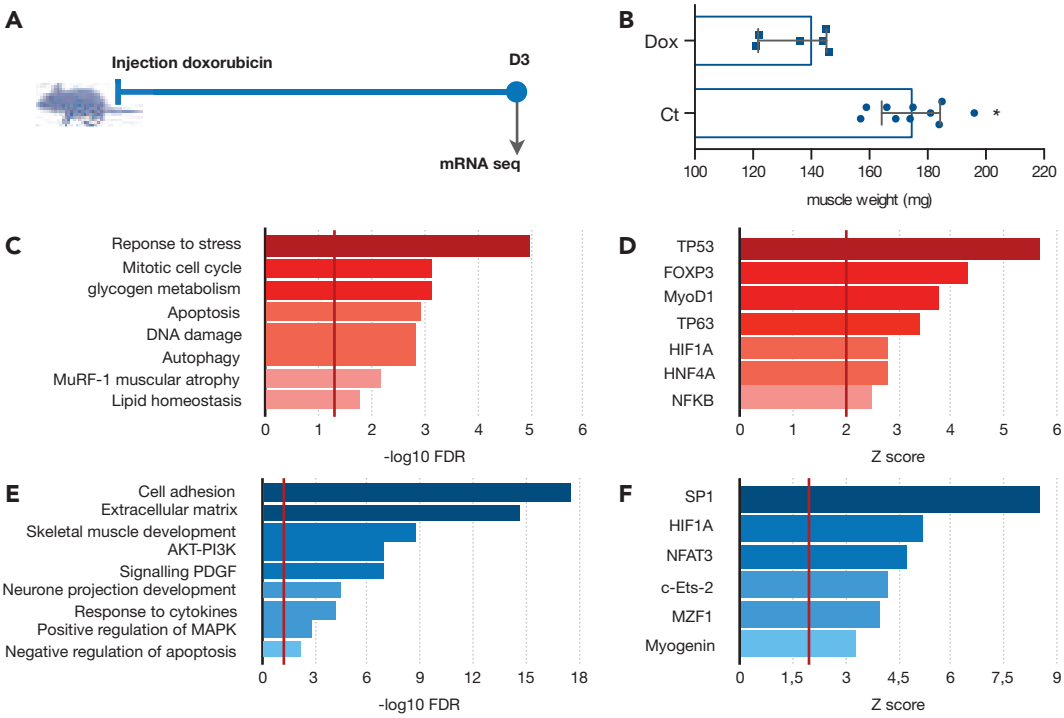
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Figure 2

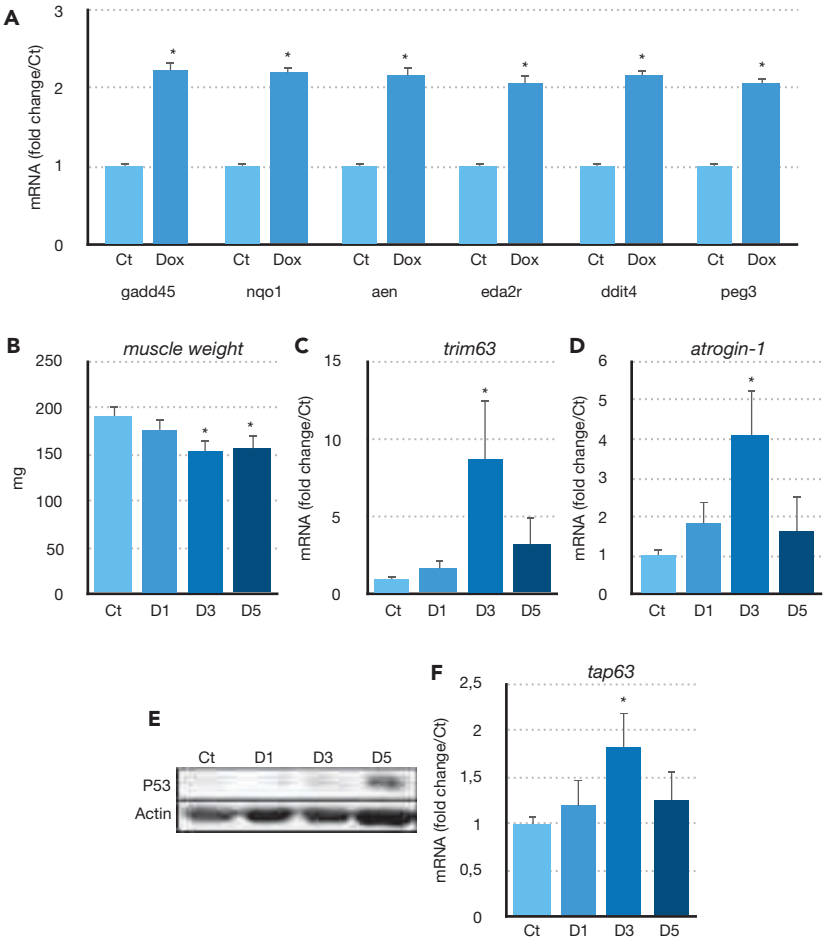
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Figure 3

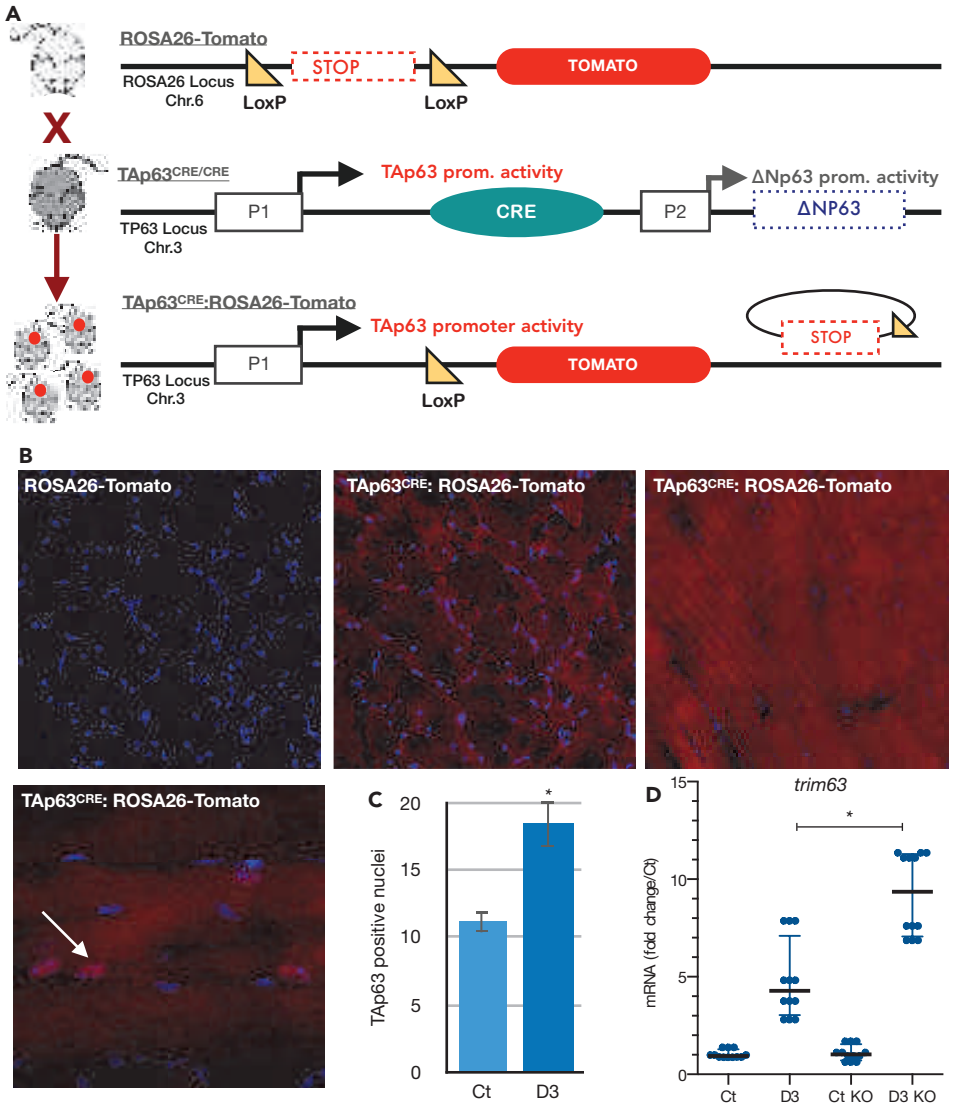
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Figure 4

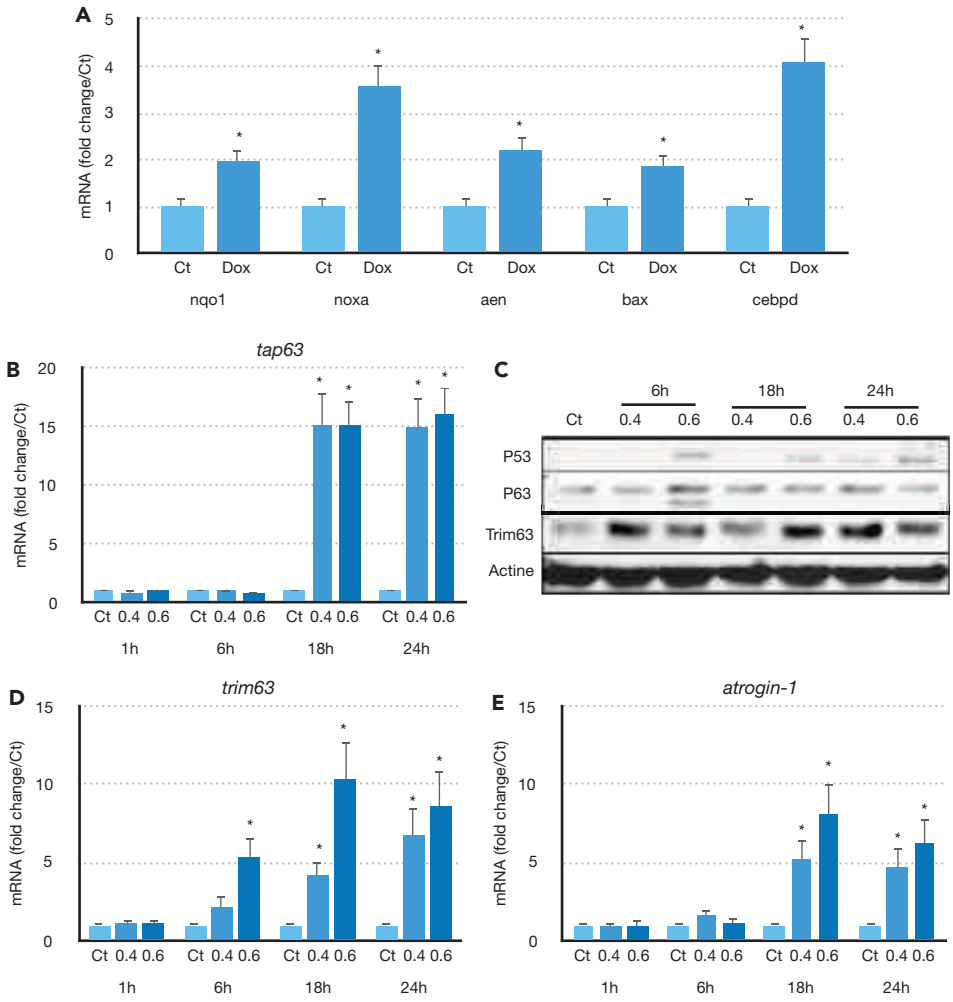
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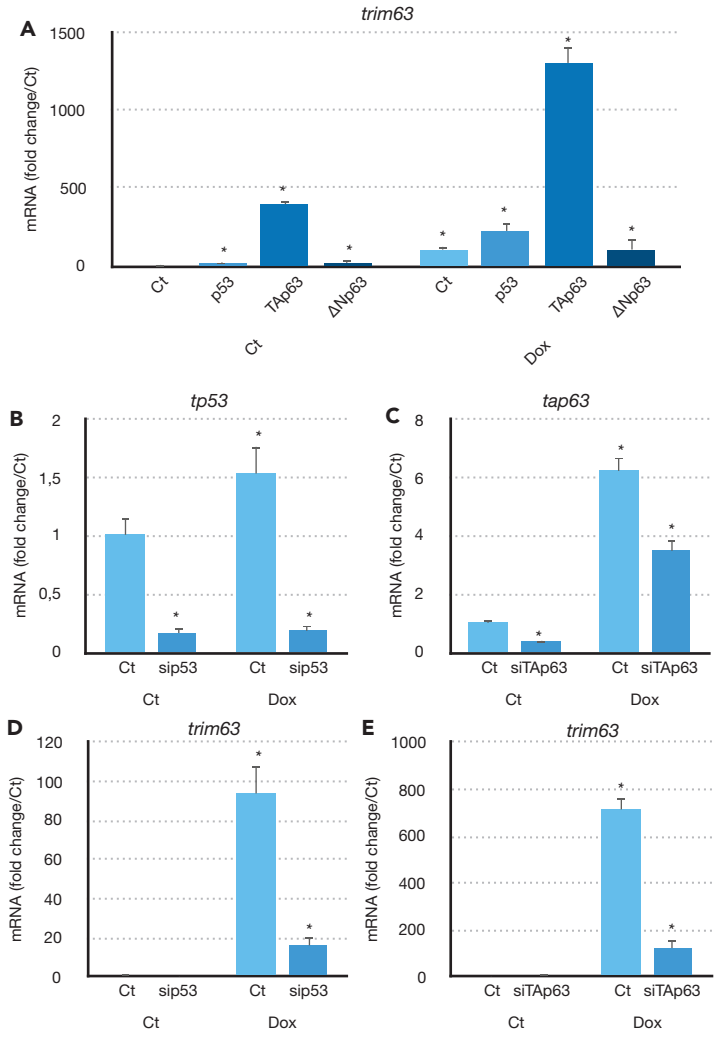
Figure 5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20
0
Ct
sip53
Ct
sip53
Ct
Dox
E
trim63
mRNA (fold change/Ct)
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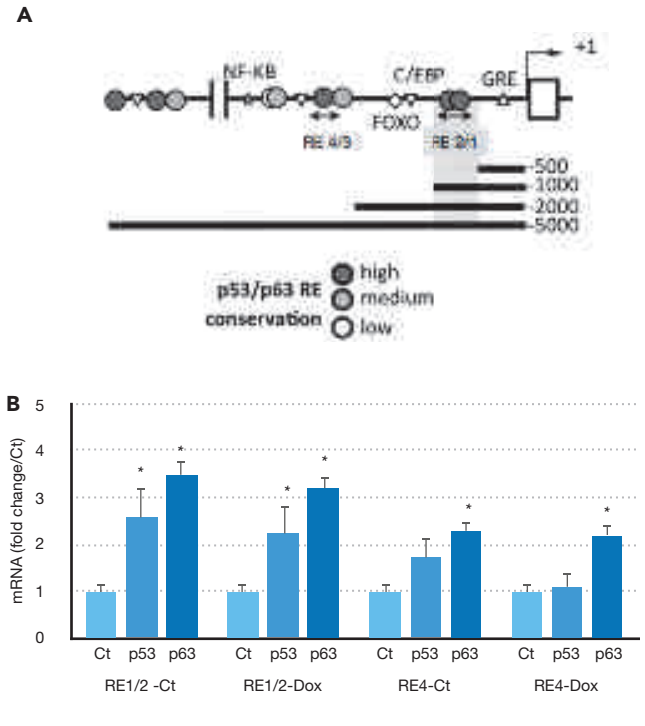
800
600
400
200
0
Ct
siTAp63
Ct
siTAp63
Ct
Dox

Figure 6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GRE
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FOXO
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high
medium
low
B
mRNA (fold change/Ct)
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RE1/2-Dox
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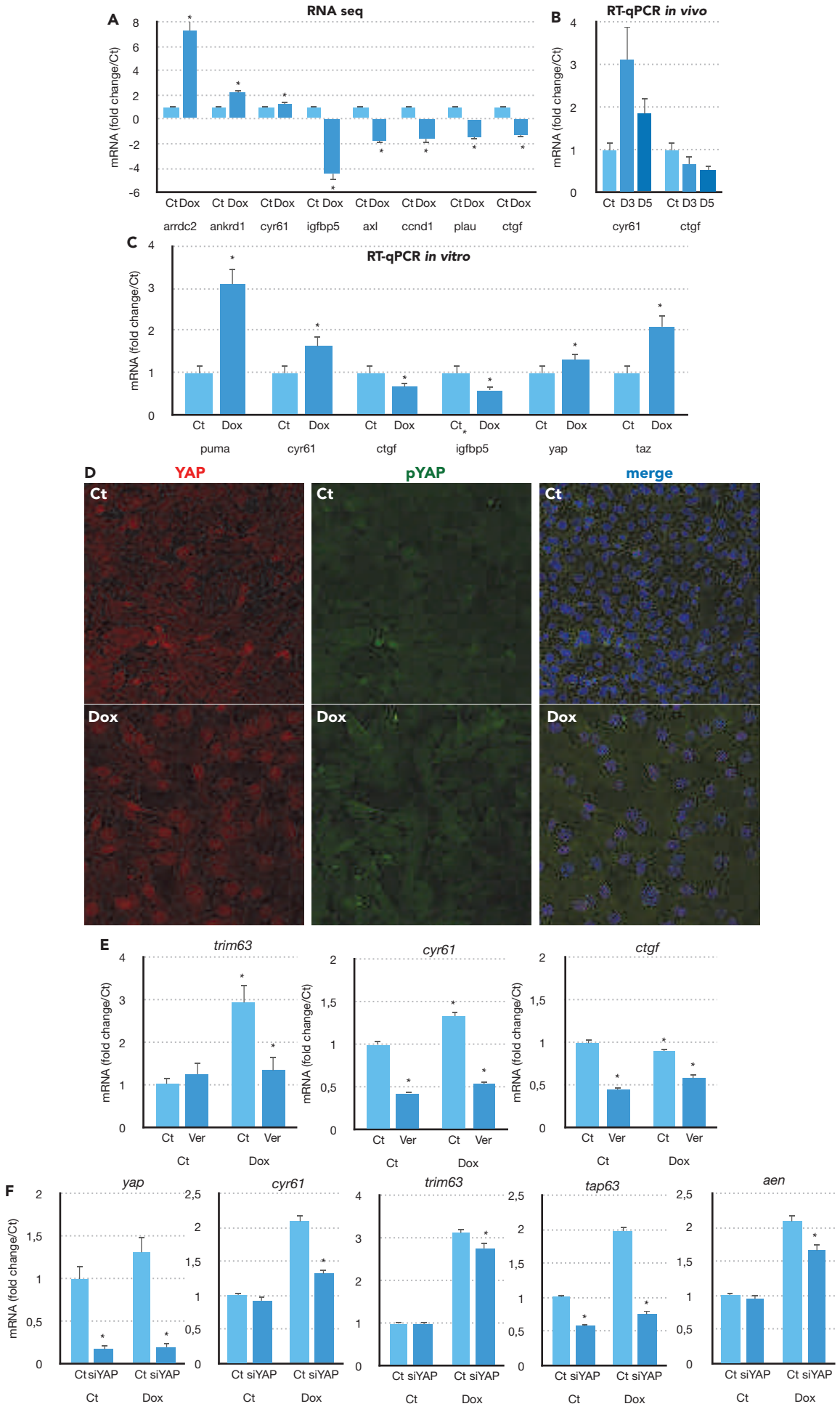
RE4-Dox

Figure 7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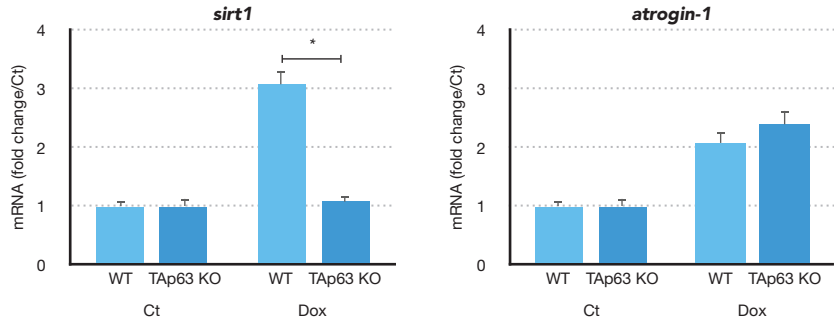
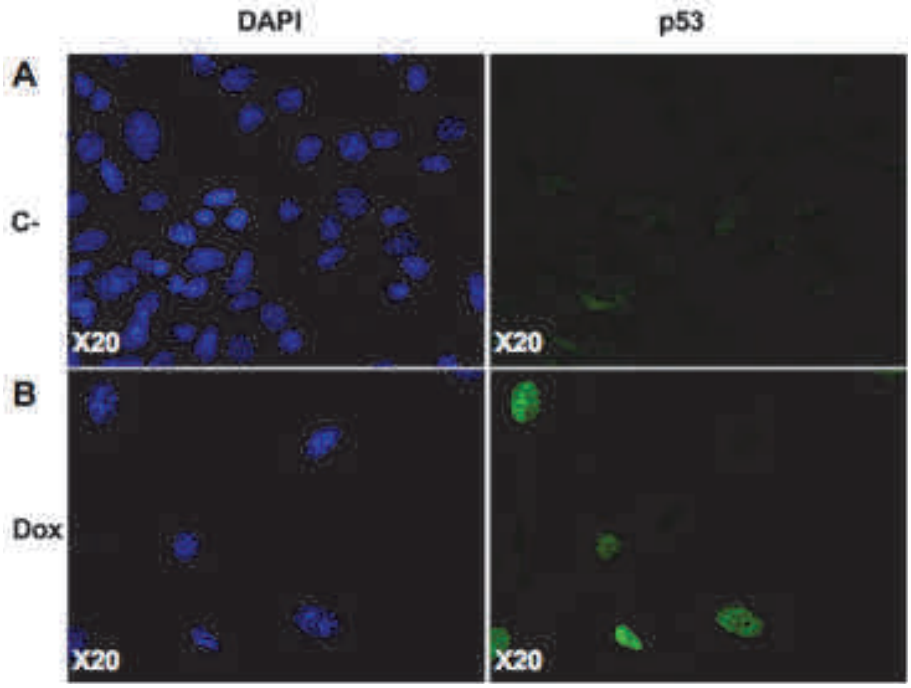


Supplementary Figure 1: Effect of doxorubicin on TAp63 KO mice. mRNA were prepared from WT and TAp63 KO mice non-treated or treated for 3 days with doxorubicin (Dox) at 18mg/Kg. RT-qPCR were then performed to measure *sirt1* and *atrogin-1* expression. Graph represent means and error bars (n=6). * indicates p<0.001 as measured by anova and tukey post test.



Supplementary Figure 2: Effect of doxorubicin on p53. C2C12 cells were non-treated (**A**) or treated (**B**) with doxorubicin (0.6 µM) for 24h and p53 was detected by immunohistochemistry. Cells were fixed and stained with antibody against p53 and DNA was stained with DAPI.

Suplementary Figure 3: Silencing of p53 and p63 on C2C12 cells. P53 and p63 protein expression was detected by Western blot after 48h of transfection with either siRNA control (C-) or siRNA (si) for p53 (**A**) or p63 (**B**).

CONCLUSION

During my thesis, I have investigated the involvement of the p53 family during muscle atrophy and gastrointestinal disorders mediated by chemotherapeutic treatments. Indeed, I highlighted new roles for the p53 family members in these organs in this context of anticancer treatment. Taken together, my results proved that the p53 family is directly responsible of Trim63 induction during cancer-associated cachexia after doxorubicin treatment. Moreover, Trim63 also appears as modulated by the Hippo pathway. It suggests that targeting the p53 family members and/or the Hippo pathway would represent therapeutic tools to counteract cancer-associated cachexia.

The role of the p53 family members was also demonstrated for the first time in the enteric nervous system. I showed that cisplatin administration activates the p53 family which in turn participate to the ENS neuronal loss responsible of gastrointestinal disorders. Once again, the possibility of targeting the p53 family expression to modulate the occurrence of cisplatin side effect may represent a therapeutic revolution.

DISCUSSION

Context: Cancers remain a major public health problem, particularly their associated treatment widely described, despite their efficacy, to induce important toxicities. More importantly, chemotherapies are known to provoke side effects that severely impact patient quality of life, notably platinum based compounds that generate gastrointestinal disorders. They appear through diarrhea, vomiting and constipation explained by alteration of the enteric nervous system (ENS), entity regulating all the gastrointestinal function [23]. Anthracylin compounds are also responsible of side effects, especially doxorubicin, which generates strong muscle atrophy [30]. Although these toxicities are highly studied, their underlying mechanisms remain poorly understood, highlighting the need to investigate them for therapeutic approaches development.

Involvement of the p53 family in anticancer treatment toxicities

Muscle atrophy: My thesis projects aimed to especially understand the molecular mechanisms related to gastrointestinal disorders and muscle atrophy. In this sense, we performed transcriptomic analysis on cisplatin-treated ENS and doxorubicin-treated skeletal muscle tissues, and interestingly highlighted a common misregulated pathway: the p53 family. Activation of the p53 family was observed in our skeletal muscle samples, pertinent with the literature where numerous studies reported the physiologic involvement of the p53 family members within the muscle homeostasis [256][304]. Indeed, we especially observed an upregulation of TAp73 and TAp63 consistent with their role in the regulation of the balance between anabolic/catabolic processes in skeletal muscle, already described [284].

The p53 family activity is also required during skeletal muscle pathology such as ALS, where the laboratory previously reported that both TAp63 and TAp73 are activated. In addition, we proved that during muscle atrophy, p53 and TAp63 specifically transactivate Trim63 promoter, a pro-atrophic factor belonging to the atrogene family [6]. This result is coherent with Yang and colleagues' study, which reported that p53 interacted with atrogenes in colon cancer associated cachexia [306].

However, it is important to notice that the link between the p53 family activation and the doxorubicin treatment was only demonstrated in breast cancer cells [308], suggesting that such molecular response to doxorubicin could happen in skeletal muscle.

Gastrointestinal disorders: In another part, my work highlights for the first time the involvement of the p53 family within the ENS. Indeed, the p53 family is essential for the central nervous system (CNS) development and homeostasis, however its function within the ENS is, nowadays, absolutely not known.

We know that the p53 family is required in response to cisplatin [4] and that cisplatin induces gastrointestinal problem by ENS alteration [1]. In this respect, I hypothesized that the p53 family activation could modulate cisplatin side effects. My work demonstrated that cisplatin induced the p53 family members expression. First, I focused on p53, unfortunately its expression profile expression was difficult to characterize because a basal protein level was detected in ENS sections but mRNA level of p53 did not change according the treatment. This is consistent with the literature that demonstrated that p53 is mainly post-translationally regulated [309]. In addition, p53 negatively regulates neuron proliferation and survival in neurons of the CNS, coherent with its increased expression after cisplatin, suggesting its involvement in ENS.

The p53 family member p73 is mostly described in the CNS to regulate maintenance and renewal of neuronal stem cells and differentiation. In this work I demonstrated that p73 is expressed in the ENS and that cisplatin treatment induces an elevated proportion of p73 positive cells, associated with an increase of TAp73 mRNA level. Interestingly, in a longer time course, p73 labeling is decreased while its mRNA level stays augmented. This suppose that the population of p73 positive cells is decreased but that they still strongly expressed p73.

The ENS is represented by ganglionic structures composed of neurons and glial cells which have a protective role towards neurons. Interestingly, I showed that p73 is expressed in the ENS, more precisely within enteric neurons nucleus. Its expression is increased after cisplatin treatment suggesting its potential role in enteric neuron homeostasis. It is consistent with its TA isoform, TAp73, whose role is to regulate the differentiation and function of post-mitotic neurons of the CNS as well as oligodendrocyte differentiation [118]. In our case, no expression of p73 was found outside enteric neurons suggesting that enteric glial cells are not expressing p73. However, it does not mean that enteric glial are not affected by cisplatin. Indeed, we showed that SOX10, an enteric glial stem cell marker, is expressed in response to cisplatin, suggesting that enteric progenitors might be activated by cisplatin administration. The hypothesis is either glial cells are impacted by cisplatin and their population needs to be regenerated, or that enteric neurons only are altered by cisplatin and the neuroprogenitor pool gives new glial cells to protect enteric neurons.

As p53 and p73 seem to have similar function between CNS and ENS, we investigated the expression of the last member: p63. Its mRNA level was increased by cisplatin in enteric plexus, however, no conclusive results about its protein expression was obtained due to ineffective antibodies. Literature also proposes numerous studies evaluating the mRNA level of the p53 family members and showing that their protein expressions are quite difficult to obtain. Nonetheless, we obtained some evidences of p63 involvement within the ENS, more particularly in enteric neurons, through our transgenic model: TAp63-Tomato mice. Indeed, we observed in

myenteric layers the native fluorescence of the Tomato protein; whose expression is controlled by the activation of TAp63 promoter; associated with specific differentiated neuronal markers (synaptobrevin and HuC/D).

However, this tool does not give any information about expression differences according treatment or control conditions. For example, Tomato expression within cisplatin-treated ENS only demonstrates the activity of TAp63 promoter, suggesting that it could have been physiologically activated before any cisplatin treatment, and that cells still display tomato expression over the time, even after cisplatin injection. This is pertinent with data in the literature describing p63 as a major regulator or CNS neurogenesis, more precisely in cell cycle and differentiation inhibition through TAp63 activation [121]. Our result may indicate that TAp63 would be involved in the neurogenesis of enteric neurons.

In addition, literature reports that p63 is essential to the development of the ectodermal tissue, malformed when p63 is deleted. Interestingly, the ENS derived from that tissue [132]. Based on this information, we used another transgenic mouse model, deleted for TAp63. We did not observe any malformation within the ENS structures in mice embryos when TAp63 is missing, however, it appears in adult mice that TAp63 deletion modulates the expression of TAp73 in response to cisplatin. It would suggest the importance of TAp63 within the ENS function, which could be compensated by other p53 family members, such TAp73.

Cell death modulation by the p53 family

We know that the p53 family is involved in numerous cellular process, notably in cell cycle arrest and apoptotic cell death by induction of specific target genes such *Noxa*, *Bax* or *Puma* in response to cytotoxic agents [114]. Platinum compounds, for example, generate DNA adducts whereas antibiotic compounds, such doxorubicin inhibits the topoisomerase I and leads to DNA damages.

ENS: Cisplatin has been described through its side effects on the gastrointestinal tract to provoke a reduction of the enteric neuron population associated with changes in the ganglionic size in the ENS [1]. However, the way this alteration happens was not investigated. Indeed, it was not studied if it results from proliferation defects or cell death. According literature informations, we hypothesized that the p53 family could mediate cell cycle arrest in response to cisplatin, however we demonstrated that proliferative markers such ki67, were observed in our ENS sections. Interestingly ki67 expression is localized in the vicinity of the enteric ganglia, that may suggest that enteric progenitors respond to cisplatin cytotoxic effect through an induction of their proliferation.

However, even if ki67 expression is increased in response to cisplatin we must be careful to assess that no obvious proliferation defects occur. In this sense, to evaluate platinum cytotoxicity, I focused my work on

the potential enteric apoptosis induced by cisplatin, and indeed, I demonstrated that cisplatin treatment induces apoptotic markers in the ENS such as nucleus condensation, cleaved caspase-3 expression and AIF relocalization. Furthermore, the expression of pro-apoptotic genes such as *Noxa*, *Bax* et *Puma* were significantly and transiently induced by cisplatin treatment. More interestingly, in KO-TAp63 mice, cisplatin-mediated induction of these target genes was maintained across the time, suggesting that TAp63 would act negatively on their expression and not participate into the apoptotic process. Surprisingly, that result is contradictive with the literature that demonstrated that TAp63, which is the pro-apoptotic isoform of p63, acts in favor to neuron apoptosis in the CNS [121].This could be explained by the fact that our KO-TAp63 mice do not express TAp63 since their embryonic development, allowing the possibility that compensatory mechanisms would have been established in response to TAp63 deletion, and by the way, hide the real involvement of TAp63 in our condition.

Moreover, we could not confirm the presence of DNA damages through γH2AX expression studies because of non-conclusive results. We cannot exclude that other type of cell death occur within ENS after cisplatin. Indeed, literature reports that nucleus condensation associated to AIF relocalization are characteristic of the parthanatos cell death also known as the PARP-dependent cell death [310]. PARP is an important actor in the DNA damage response. Studies demonstrated that PARP cleavage into PARP-1 protein, influences the occurrence of apoptosis, while simple PARP activation leads to necrosis through ATP depletion. It suggests that parthanatos or necrosis could also occur in ENS after cisplatin. This is pertinent with data reporting that the cisplatin dose influences the cell death response. Indeed, low concentration of cisplatin generates apoptotic response while a higher concentration induces necrosis [311]. This point needs to be clarified by investigating cleaved PARP (PARP-1), ATP content as well as necrotic marker such as RIP3 for example.

The p53 family is also known to participate in the senescent process [312], however, we did not get any positive results for senescence markers expression in ENS after cisplatin, as well in an early time point of treatment as a longer one. This is pertinent because cisplatin is not described to provoke senescence and because this is a cell-death mainly characteristic of aging cells even if it was recently reported to also be induced by stress conditions in young cells [313].

Cisplatin mode of action also generates reactive oxygen species (ROS) which will indirectly induce DNA damages [20]. ROS are known to provoke mitochondrial damages leading to the relocalization of some markers such AIF from the mitochondria to the nucleus. Interestingly, AIF is a mitochondrial protein involved in the respiratory chain which is translocated to the nucleus in order to induce DNA cleavage [314]. Interestingly, we demonstrated AIF relocalization in ENS after cisplatin treatment, more precisely within enteric neurons by its colocalization with specific neuronal markers, suggesting that mitochondria could be affected by cisplatin administration by ROS production. However, this point remains to be further investigated, for example by

immunochemistry, through the use of the dihydroethydine dye allowing the observation of ROS production. Moreover, ENS contains more than 15 neurons subtypes which let suppose that some specific subtypes would be more affected than others. Indeed, literature showed that enteric neuronal alteration is associated with an increase of nNOS and PGP9.5 markers which could give an idea of the neuronal class impacted [97][100].

Also, we know that patients display neuropathy and gastrointestinal disorders months and years after cisplatin treatment arrest. However, we found in our models a decrease of this cell death response at the longer time point (21days). We also know that cisplatin cytotoxicity depends on cumulative dose. Despite the fact that we used a single dose in order to generate an acute effect, similar to repetitive lower dose in clinic, it seems that differences remain. It needs to be clarified by investigating other time points during cisplatin treatment.

Skeletal muscle: As mentioned before, the p53 family is also described to be activated in response to doxorubicin in breast cancer cells [308]. Doxorubicin is an anticancer treatment that induces severe skeletal muscle atrophy in cancer patients by over-activating pro-atrophic factors such as Trim63 and Atrogin1 belonging to the atrogenes family. Trim63 and Atrogin 1 are regulated in this context by many signaling pathways, notably the IGF-PI3K-Akt-FoxO pathway [211] which was interestingly found upregulated in our transcriptomic analysis after doxorubicin treatment. More importantly, the p53 family is even more upregulated in the same condition, suggesting the link of the p53 family activation and doxorubicin in skeletal muscle. This is what we confirmed by using our TAp63-Tomato mouse model expressing the Tomato protein under TAp63 promoter activation. We showed that doxorubicin induced an increase of the Tomato-positive muscle cells confirming the importance of TAp63 within skeletal muscle after doxorubicin administration, through its promoter activation. According the literature, it suggests that doxorubicin would activate the p53 family. Furthermore, as mentioned before, we demonstrated the interaction of TAp63 onto Trim63 promoter suggesting that doxorubicin activates the p53 family which in turn can modulate Trim63 expression [6]. In this sense, we demonstrated that the increase of Trim63 expression was proportionally associated with the activation of TAp63 and TAp73, confirmed by TAp63 overexpression that stimulates even more Trim63. However, TAp63 silencing does not entirely abolish Trim63 supposing that another signaling pathway would be involved in this regulation. In this respect, we pointed out from the literature, one signaling pathway mainly involved in muscle homeostasis and induced in doxorubicin-associated muscle cells senescence: the hippo pathway [241].

Hippo signaling pathway takes part to atrophy modulation

The hippo family is composed of YAP and TAZ which assume mechanotransductive properties through the control of organ size and development [8][227]. The literature showed its involvement notably in skeletal muscle tissue where YAP negatively modulates the expression of Trim63 in order to promote muscle mass. In

addition, as described before, because of its nucleus accumulation, YAP was linked to doxorubicin-induced senescence in muscle cells [241].

We confirmed through study of YAP target genes: *Cyr61* and *Ctgf*, that the Hippo pathway is induced by doxorubicin because of an increase of their mRNA level. According these data, our hypothesis was based on the fact that YAP would also modulate Trim63 expression after doxorubicin treatment, which is already induced by TAp63. In particular, we though that YAP would negatively regulate Trim63 as described in the literature, in order to counteract its pro-atrophic function. Surprisingly, it appears that YAP inhibition does not have the positive effect on Trim63 that we expected. Indeed, we demonstrated by using Verteporfin (a chemical inhibitor of YAP) or RNA interference, that Trim63 expression is decreased too. However, it goes right with literature where the role of YAP is discussed. Indeed, one study is assuming that YAP inhibits Trim63 [7] while another one is demonstrating that YAP constitutive activation drives to Trim63 upregulation, consistent with our results [8].

Based on that, we could imagine that YAP could have the same influence on Trim63 as the p53 family. This could explain that the expression of the following genes: *Puma*, *Bax*, and *Noxa*, known to be target genes of p53 family members, were upregulated in response to doxorubicin. However, they are also described to be target genes of the hippo pathway which modulate the p53 family members activity. Indeed, YAP stimulates the expression of *Noxa* in order to induce apoptosis [315] and induces BAX expression after interacting with p73 [316]. This suppose either that these genes are expressed through the Hippo pathway or the p53 family only, or that both pathways interact to each other to modulate their expression. Indeed, YAP has been described to stabilize p73 by interfering with its specific negative regulator: Itch (Itchy, E3 ubiquitin protein ligase) [317].

It will be a necessary point to investigate to determine if YAP physically interacts with p73 or p63 to modulate their activity on Trim63 during doxorubicin treatment. For now, my results highlight the complex regulation of Trim63 expression in a doxorubicin-dependent context, by the p53 family and the Hippo pathway.

Enteric nervous system plasticity and inflammation

Our transcriptomic analysis interestingly revealed that inflamosome markers and neuronal late plasticity processes were induced by cisplatin treatment in ENS samples.

The plasticity of the ENS has been suggested by the ability of enteric glial cells to adapt their phenotype in response to reactive stimulus and their capacity to revert into glial cells progenitors when needed [73]. This fact could explain the presence of enteric glial cells progenitor marker: SOX10, associated with an increase of the proliferation process that we found, supported by an activation of neuronal and progenitor signaling pathways found in the RNA sequencing report.

About inflammation, this process has been widely studied and considered as one mediator of ENS-related pathologies. The inflammatory response is notably mediated by COX2, a key enzyme responsible of inflammatory molecules production such as prostaglandins, whose expression is found in activated macrophages [318]. However, its expression has never been described in the ENS, what we did. Indeed, we showed that cisplatin may cause ENS inflammation through the expression of COX2 observed in enteric ganglia after cisplatin treatment. This result has been reinforced by demonstrating under myenteric plexus layer, muscularis macrophages marker expression (IBA1, Ionized calcium binding adaptor molecule 1) known to regulate enteric neuron activity [81]. Furthermore, when a switch of polarization occurs in these macrophages, enteric neurons are drived to apoptosis, that generates inflammation and participate to gastrointestinal disorders [82].This interesting point needs to be further investigated by proving the role of these macrophages during cisplatin-related neuronal cell death and by determining the precise role of enteric glial cells during this process.

Taken together, all my results highlight potential therapeutic strategies to solve the persistent and severe problem of anticancer treatment side effects. Indeed, we could imagine that targeting the p53 family and blocking its activation in response to cisplatin, for example by using the pifithrin, would diminish or suppress enteric nervous system alteration. If cisplatin-induced inflammation is responsible of ENS decline through COX2 expression, it would be benefic to use anti-COX2 molecules (Celecoxib and Etoricoxib) to counteract this pro-inflammatory response. Furthermore, as anti-COX2 drugs already exist and are used in clinic, it would be very easy and interesting to investigate this potential strategy.
Also, neuroprogenitor pool stimulation by embryogenic factors could also be a potential application to optimize the recovery of the ENS and to replenish the population of functional and healthy neurons.

Therapeutic solutions for cancer-associated cachexia could also be imagined. For example, based on my results we could hope that by discovering de precise link between the p53 family and the Hippo pathway, it would be possible to target this interaction in order to block the over-activation of atrophic factors such as Trim63.

Additional investigations are needed to completely understand the precise molecular mechanisms during gastrointestinal and muscle disorders, however, my thesis work succeeded to highlight key actors of these toxicities.

REFERENCES

[1] G. Vera *et al.*, « Enteric neuropathy evoked by repeated cisplatin in the rat », *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.*, vol. 23, n° 4, p. 370-378, e162-163, avr. 2011.

[2] B. J. N. Sishi, B. Loos, J. van Rooyen, et A.-M. Engelbrecht, « Doxorubicin induces protein ubiquitination and inhibits proteasome activity during cardiotoxicity », *Toxicology*, vol. 309, p. 23-29, juill. 2013.

[3] M. Schwarzkopf, D. h Coletti, G. Marazzi, et D. S. Sassoon, « Chronic p 53 activity leads to skeletal muscle atrophy and muscle stem cell perturbation », 2008.

[4] S. Benosman *et al.*, « Multiple neurotoxic stresses converge on MDMX proteolysis to cause neuronal apoptosis », *Cell Death Differ.*, vol. 14, n° 12, p. 2047-2057, déc. 2007.

[5] S. Benosman *et al.*, « Complex regulation of p73 isoforms after alteration of amyloid precursor polypeptide (APP) function and DNA damage in neurons », *J. Biol. Chem.*, vol. 286, n° 50, p. 43013-43025, déc. 2011.

[6] Y. von Grabowiecki *et al.*, « Transcriptional activator TAp63 is upregulated in muscular atrophy during ALS and induces the pro-atrophic ubiquitin ligase Trim63 », *eLife*, vol. 5, févr. 2016.

[7] C. A. Goodman, T. A. Hornberger, et A. G. Robling, « Bone and skeletal muscle: Key players in mechanotransduction and potential overlapping mechanisms », *Bone*, vol. 80, p. 24-36, nov. 2015.

[8] R. N. Judson *et al.*, « Constitutive expression of Yes-associated protein (Yap) in adult skeletal muscle fibres induces muscle atrophy and myopathy », *PloS One*, vol. 8, n° 3, p. e59622, 2013.

[9] E. Lapi *et al.*, « PML, YAP, and p73 are components of a proapoptotic autoregulatory feedback loop », *Mol. Cell*, vol. 32, n° 6, p. 803-814, déc. 2008.

[10] S. Strano *et al.*, « Physical interaction with Yes-associated protein enhances p73 transcriptional activity », *J. Biol. Chem.*, vol. 276, n° 18, p. 15164-15173, mai 2001.

[11] D. Levy, Y. Adamovich, N. Reuven, et Y. Shaul, « The Yes-associated protein 1 stabilizes p73 by preventing Itch-mediated ubiquitination of p73 », *Cell Death Differ.*, vol. 14, n° 4, p. 743-751, avr. 2007.

[12] « Fondation contre le Cancer ». [En ligne]. Disponible sur: https://www.cancer.be/. [Consulté le: 17-août-2019].

[13] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, et A. Jemal, « Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries », *CA. Cancer J. Clin.*, vol. 68, n° 6, p. 394-424, nov. 2018.

[14] Y. T. Lee, Y. J. Tan, et C. E. Oon, « Molecular targeted therapy: Treating cancer with specificity », *Eur. J. Pharmacol.*, vol. 834, p. 188-196, sept. 2018.

[15] L. Galluzzi *et al.*, « Molecular mechanisms of cisplatin resistance », *Oncogene*, vol. 31, n° 15, p. 1869-1883, avr. 2012.

[16] P. Ramos et M. Bentires-Alj, « Mechanism-based cancer therapy: resistance to therapy, therapy for resistance », *Oncogene*, vol. 34, n° 28, p. 3617-3626, juill. 2015.

[17] F. Castinetti *et al.*, « Endocrine side-effects of new anticancer therapies: Overall monitoring and conclusions », *Ann. Endocrinol.*, vol. 79, n° 5, p. 591-595, oct. 2018.

[18] K. M. Tewey, T. C. Rowe, L. Yang, B. D. Halligan, et L. F. Liu, « Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II », *Science*, vol. 226, n° 4673, p. 466-468, oct. 1984.

[19] R. Moudgil et E. T. H. Yeh, « Mechanisms of Cardiotoxicity of Cancer Chemotherapeutic Agents: Cardiomyopathy and Beyond », *Can. J. Cardiol.*, vol. 32, n° 7, p. 863-870.e5, 2016.

[20] S. Dasari et P. B. Tchounwou, « Cisplatin in cancer therapy: molecular mechanisms of action », *Eur. J. Pharmacol.*, vol. 740, p. 364-378, oct. 2014.

[21] R. Zajączkowska, M. Kocot-Kępska, W. Leppert, A. Wrzosek, J. Mika, et J. Wordliczek, « Mechanisms of Chemotherapy-Induced Peripheral Neuropathy », *Int. J. Mol. Sci.*, vol. 20, n° 6, mars 2019.

[22] A. Chiorazzi, S. Semperboni, et P. Marmiroli, « Current View in Platinum Drug Mechanisms of Peripheral Neurotoxicity », *Toxics*, vol. 3, n° 3, p. 304-321, août 2015.

[23] R. Oun, Y. E. Moussa, et N. J. Wheate, « The side effects of platinum-based chemotherapy drugs: a review for chemists », *Dalton Trans. Camb. Engl. 2003*, vol. 47, n° 19, p. 6645-6653, mai 2018.

[24] I. Arany et R. L. Safirstein, « Cisplatin nephrotoxicity », *Semin. Nephrol.*, vol. 23, n° 5, p. 460-464, sept. 2003.

[25] S. Işeri, F. Ercan, N. Gedik, M. Yüksel, et I. Alican, « Simvastatin attenuates cisplatin-induced kidney and liver damage in rats », *Toxicology*, vol. 230, n° 2-3, p. 256-264, févr. 2007.

[26] M. Jiang et Z. Dong, « Regulation and pathological role of p53 in cisplatin nephrotoxicity », *J. Pharmacol. Exp. Ther.*, vol. 327, n° 2, p. 300-307, nov. 2008.

[27] A. Sharma, R. Houshyar, P. Bhosale, J.-I. Choi, R. Gulati, et C. Lall, « Chemotherapy induced liver abnormalities: an imaging perspective », *Clin. Mol. Hepatol.*, vol. 20, n° 3, p. 317-326, sept. 2014.

[28] M. Ha, L. Wei, X. Guan, L. Li, et C. Liu, « p53-dependent apoptosis contributes to di-(2-ethylhexyl) phthalate-induced hepatotoxicity », *Environ. Pollut. Barking Essex 1987*, vol. 208, n° Pt B, p. 416-425, janv. 2016.
[29] Y. Huo *et al.*, « Protective role of p53 in acetaminophen hepatotoxicity », *Free Radic. Biol. Med.*, vol. 106, p. 111-117, 2017.
[30] T. P. Braun *et al.*, « Muscle atrophy in response to cytotoxic chemotherapy is dependent on intact glucocorticoid signaling in skeletal muscle », *PloS One*, vol. 9, n° 9, p. e106489, 2014.
[31] H. Sakai *et al.*, « Effect of acute treadmill exercise on cisplatin-induced muscle atrophy in the mouse », *Pflugers Arch.*, vol. 469, n° 11, p. 1495-1505, 2017.
[32] H. Sakai *et al.*, « Dexamethasone exacerbates cisplatin-induced muscle atrophy », *Clin. Exp. Pharmacol. Physiol.*, vol. 46, n° 1, p. 19-28, janv. 2019.
[33] S. S. Ahmad, M. A. Reinius, H. M. Hatcher, et T. V. Ajithkumar, « Anticancer chemotherapy in teenagers and young adults: managing long term side effects », *BMJ*, vol. 354, p. i4567, sept. 2016.
[34] P. Frères *et al.*, « [Cancer therapy-induced cardiotoxicity] », *Rev. Med. Liege*, vol. 71, n° 9, p. 382-387, sept. 2016.
[35] S. E. Lipshultz *et al.*, « Female sex and higher drug dose as risk factors for late cardiotoxic effects of doxorubicin therapy for childhood cancer », *N. Engl. J. Med.*, vol. 332, n° 26, p. 1738-1743, 29 1995.
[36] L. C. M. Kremer, E. C. van Dalen, M. Offringa, et P. A. Voûte, « Frequency and risk factors of anthracycline-induced clinical heart failure in children: a systematic review », *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.*, vol. 13, n° 4, p. 503-512, avr. 2002.
[37] P. A. Henriksen, « Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention », *Heart Br. Card. Soc.*, vol. 104, n° 12, p. 971-977, 2018.
[38] K. Renu, A. V G, T. P. P B, et S. Arunachalam, « Molecular mechanism of doxorubicin-induced cardiomyopathy - An update », *Eur. J. Pharmacol.*, vol. 818, p. 241-253, janv. 2018.
[39] T. Chen, Z. Deng, R. Zhao, H. Shen, et W. Li, « SYKT Alleviates Doxorubicin-Induced Cardiotoxicity via Modulating ROS-Mediated p53 and MAPK Signal Pathways », *Evid.-Based Complement. Altern. Med. ECAM*, vol. 2018, p. 2581031, 2018.
[40] D.-X. Zhang *et al.*, « ERK1/2/p53 and NF-κB dependent-PUMA activation involves in doxorubicin-induced cardiomyocyte apoptosis », *Eur. Rev. Med. Pharmacol. Sci.*, vol. 20, n° 11, p. 2435-2442, 2016.
[41] J. Liu, W. Mao, B. Ding, et C. Liang, « ERKs/p53 signal transduction pathway is involved in doxorubicin-induced apoptosis in H9c2 cells and cardiomyocytes », *Am. J. Physiol. Heart Circ. Physiol.*, vol. 295, n° 5, p. H1956-1965, nov. 2008.
[42] H. Yamamoto, K. Ishihara, Y. Takeda, W. Koizumi, et T. Ichikawa, « Changes in the mucus barrier during cisplatin-induced intestinal mucositis in rats », *BioMed Res. Int.*, vol. 2013, p. 276186, 2013.
[43] H. R. Kourie et J. Klastersky, « Immune checkpoint inhibitors side effects and management », *Immunotherapy*, vol. 8, n° 7, p. 799-807, 2016.
[44] S. Sheth, D. Mukherjea, L. P. Rybak, et V. Ramkumar, « Mechanisms of Cisplatin-Induced Ototoxicity and Otoprotection », *Front. Cell. Neurosci.*, vol. 11, p. 338, 2017.
[45] S. Amptoulach et N. Tsavaris, « Neurotoxicity caused by the treatment with platinum analogues », *Chemother. Res. Pract.*, vol. 2011, p. 843019, 2011.
[46] G. Codacci-Pisanelli, L. Del Pup, M. Del Grande, et F. A. Peccatori, « Mechanisms of chemotherapy-induced ovarian damage in breast cancer patients », *Crit. Rev. Oncol. Hematol.*, vol. 113, p. 90-96, mai 2017.
[47] B. Lawrenz, N. Mahajan, et H. M. Fatemi, « The effects of cancer therapy on women's fertility: what do we know now? », *Future Oncol. Lond. Engl.*, vol. 12, n° 14, p. 1721-1729, juill. 2016.
[48] A. J. Levine, R. Tomasini, F. D. McKeon, T. W. Mak, et G. Melino, « The p53 family: guardians of maternal reproduction », *Nat. Rev. Mol. Cell Biol.*, vol. 12, n° 4, p. 259-265, 2011.
[49] J. M. Hotaling, N. Laufer, et Z. Rosenwaks, « Introduction: Cancer biomarkers and fertility », *Fertil. Steril.*, vol. 109, n° 1, p. 4-5, 2018.
[50] K. M. Bellizzi *et al.*, « Positive and negative psychosocial impact of being diagnosed with cancer as an adolescent or young adult », *Cancer*, vol. 118, n° 20, p. 5155-5162, oct. 2012.
[51] L. K. Zeltzer *et al.*, « Psychological status in childhood cancer survivors: a report from the Childhood Cancer Survivor Study », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 27, n° 14, p. 2396-2404, mai 2009.
[52] S. M. Sawyer *et al.*, « Fulfilling the Vision of Youth-Friendly Cancer Care: A Study Protocol », *J. Adolesc. Young Adult Oncol.*, vol. 5, n° 3, p. 267-277, 2016.
[53] O. Kanat, H. Ertas, et B. Caner, « Platinum-induced neurotoxicity: A review of possible mechanisms », *World J. Clin. Oncol.*, vol. 8, n° 4, p. 329-335, août 2017.
[54] M. A. Maj, J. Ma, K. N. Krukowski, A. Kavelaars, et C. J. Heijnen, « Inhibition of Mitochondrial p53 Accumulation by PFT-μ Prevents Cisplatin-Induced Peripheral Neuropathy », *Front. Mol. Neurosci.*, vol. 10, p. 108, 2017.

[55] K. Krukowski, C. H. Nijboer, X. Huo, A. Kavelaars, et C. J. Heijnen, « Prevention of chemotherapy-induced peripheral neuropathy by the small-molecule inhibitor pifithrin-μ », *Pain*, vol. 156, n° 11, p. 2184-2192, nov. 2015.
[56] H. Nakamae *et al.*, « Notable effects of angiotensin II receptor blocker, valsartan, on acute cardiotoxic changes after standard chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone », *Cancer*, vol. 104, n° 11, p. 2492-2498, déc. 2005.
[57] J. Donnez *et al.*, « Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births », *Ann. Med.*, vol. 43, n° 6, p. 437-450, 2011.
[58] T. Koll *et al.*, « Supportive Care in Older Adults with Cancer: Across the Continuum », *Curr. Oncol. Rep.*, vol. 18, n° 8, p. 51, 2016.
[59] S. G. Mohile *et al.*, « Geriatric Assessment-Guided Care Processes for Older Adults: A Delphi Consensus of Geriatric Oncology Experts », *J. Natl. Compr. Cancer Netw. JNCCN*, vol. 13, n° 9, p. 1120-1130, sept. 2015.
[60] W. E. Haley, « Family caregivers of elderly patients with cancer: understanding and minimizing the burden of care », *J. Support. Oncol.*, vol. 1, n° 4 Suppl 2, p. 25-29, déc. 2003.
[61] S. Grunberg *et al.*, « Single-dose fosaprepitant for the prevention of chemotherapy-induced nausea and vomiting associated with cisplatin therapy: randomized, double-blind study protocol--EASE », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 29, n° 11, p. 1495-1501, avr. 2011.
[62] D. G. Warr *et al.*, « Efficacy and tolerability of aprepitant for the prevention of chemotherapy-induced nausea and vomiting in patients with breast cancer after moderately emetogenic chemotherapy », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 23, n° 12, p. 2822-2830, avr. 2005.
[63] M.-C. Chen, W.-L. Hsu, P.-A. Hwang, Y.-L. Chen, et T.-C. Chou, « Combined administration of fucoidan ameliorates tumor and chemotherapy-induced skeletal muscle atrophy in bladder cancer-bearing mice », *Oncotarget*, vol. 7, n° 32, p. 51608-51618, 09 2016.
[64] M. Bobiński *et al.*, « The Effect of Fucoidan, a Potential New, Natural, Anti-Neoplastic Agent on Uterine Sarcomas and Carcinosarcoma Cell Lines: ENITEC Collaborative Study », *Arch. Immunol. Ther. Exp. (Warsz.)*, vol. 67, n° 2, p. 125-131, avr. 2019.
[65] J. B. Furness, « The enteric nervous system and neurogastroenterology », *Nat. Rev. Gastroenterol. Hepatol.*, vol. 9, n° 5, p. 286-294, mars 2012.
[66] G. Burzynski, I. T. Shepherd, et H. Enomoto, « Genetic model system studies of the development of the enteric nervous system, gut motility and Hirschsprung's disease », *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.*, vol. 21, n° 2, p. 113-127, févr. 2009.
[67] J. I. Lake et R. O. Heuckeroth, « Enteric nervous system development: migration, differentiation, and disease », *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 305, n° 1, p. G1-24, juill. 2013.
[68] U. Rauch *et al.*, « Expression of intermediate filament proteins and neuronal markers in the human fetal gut », *J. Histochem. Cytochem. Off. J. Histochem. Soc.*, vol. 54, n° 1, p. 39-46, janv. 2006.
[69] M. M. Hao et H. M. Young, « Development of enteric neuron diversity », *J. Cell. Mol. Med.*, vol. 13, n° 7, p. 1193-1210, juill. 2009.
[70] M. Colucci *et al.*, « Intestinal dysmotility and enteric neurochemical changes in a Parkinson's disease rat model », *Auton. Neurosci. Basic Clin.*, vol. 169, n° 2, p. 77-86, août 2012.
[71] C. Olsson et S. Holmgren, « The control of gut motility », *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, vol. 128, n° 3, p. 481-503, mars 2001.
[72] C. Giaroni, F. De Ponti, M. Cosentino, S. Lecchini, et G. Frigo, « Plasticity in the enteric nervous system », *Gastroenterology*, vol. 117, n° 6, p. 1438-1458, déc. 1999.
[73] S. Valès, M. Touvron, et L. Van Landeghem, « Enteric glia: Diversity or plasticity? », *Brain Res.*, vol. 1693, n° Pt B, p. 140-145, 15 2018.
[74] M. J. Saffrey, « Cellular changes in the enteric nervous system during ageing », *Dev. Biol.*, vol. 382, n° 1, p. 344-355, oct. 2013.
[75] A. E. Lomax, E. Fernández, et K. A. Sharkey, « Plasticity of the enteric nervous system during intestinal inflammation », *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.*, vol. 17, n° 1, p. 4-15, févr. 2005.
[76] J. Belkind-Gerson *et al.*, « Colitis induces enteric neurogenesis through a 5-HT4-dependent mechanism », *Inflamm. Bowel Dis.*, vol. 21, n° 4, p. 870-878, avr. 2015.
[77] S. W. Moore, « Down syndrome and the enteric nervous system », *Pediatr. Surg. Int.*, vol. 24, n° 8, p. 873-883, août 2008.
[78] T. A. Heanue et V. Pachnis, « Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies », *Nat. Rev. Neurosci.*, vol. 8, n° 6, p. 466-479, juin 2007.
[79] M. Anitha *et al.*, « Glial-derived neurotrophic factor modulates enteric neuronal survival and proliferation through neuropeptide Y », *Gastroenterology*, vol. 131, n° 4, p. 1164-1178, oct. 2006.

[80] B. Chandrasekharan, B. G. Nezami, et S. Srinivasan, « Emerging neuropeptide targets in inflammation: NPY and VIP », *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 304, n° 11, p. G949-957, juin 2013.
[81] K. G. Margolis et M. D. Gershon, « Enteric Neuronal Regulation of Intestinal Inflammation », *Trends Neurosci.*, vol. 39, n° 9, p. 614-624, 2016.
[82] L. Becker, L. Nguyen, J. Gill, S. Kulkarni, P. J. Pasricha, et A. Habtezion, « Age-dependent shift in macrophage polarisation causes inflammation-mediated degeneration of enteric nervous system », *Gut*, vol. 67, n° 5, p. 827-836, 2018.
[83] A. K. Chow et B. D. Gulbransen, « Potential roles of enteric glia in bridging neuroimmune communication in the gut », *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 312, n° 2, p. G145-G152, févr. 2017.
[84] P. Derkinderen, T. Rouaud, T. Lebouvier, S. Bruley des Varannes, M. Neunlist, et R. De Giorgio, « Parkinson disease: the enteric nervous system spills its guts », *Neurology*, vol. 77, n° 19, p. 1761-1767, nov. 2011.
[85] Á. Sánchez-Ferro *et al.*, « In vivo gastric detection of α-synuclein inclusions in Parkinson's disease », *Mov. Disord. Off. J. Mov. Disord. Soc.*, vol. 30, n° 4, p. 517-524, avr. 2015.
[86] A. Mulak et B. Bonaz, « Brain-gut-microbiota axis in Parkinson's disease », *World J. Gastroenterol.*, vol. 21, n° 37, p. 10609-10620, oct. 2015.
[87] T. Clairembault, L. Leclair-Visonneau, M. Neunlist, et P. Derkinderen, « Enteric glial cells: new players in Parkinson's disease? », *Mov. Disord. Off. J. Mov. Disord. Soc.*, vol. 30, n° 4, p. 494-498, avr. 2015.
[88] D. Devos *et al.*, « Colonic inflammation in Parkinson's disease », *Neurobiol. Dis.*, vol. 50, p. 42-48, févr. 2013.
[89] S. Semar *et al.*, « Changes of the enteric nervous system in amyloid-β protein precursor transgenic mice correlate with disease progression », *J. Alzheimers Dis. JAD*, vol. 36, n° 1, p. 7-20, 2013.
[90] J. Chen, E. Pitmon, et K. Wang, « Microbiome, inflammation and colorectal cancer », *Semin. Immunol.*, vol. 32, p. 43-53, 2017.
[91] C.-M. Zhao *et al.*, « Denervation suppresses gastric tumorigenesis », *Sci. Transl. Med.*, vol. 6, n° 250, p. 250ra115, août 2014.
[92] D. D. Von Hoff *et al.*, « Toxic effects of cis-dichlorodiammineplatinum(II) in man », *Cancer Treat. Rep.*, vol. 63, n° 9-10, p. 1527-1531, oct. 1979.
[93] R. J. Gibson et D. M. K. Keefe, « Cancer chemotherapy-induced diarrhoea and constipation: mechanisms of damage and prevention strategies », *Support. Care Cancer Off. J. Multinatl. Assoc. Support. Care Cancer*, vol. 14, n° 9, p. 890-900, sept. 2006.
[94] C. S. Lee, E. J. Ryan, et G. A. Doherty, « Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: the role of inflammation », *World J. Gastroenterol.*, vol. 20, n° 14, p. 3751-3761, avr. 2014.
[95] V. A. Carozzi, A. Canta, et A. Chiorazzi, « Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? », *Neurosci. Lett.*, vol. 596, p. 90-107, juin 2015.
[96] V. Stojanovska, S. Sakkal, et K. Nurgali, « Platinum-based chemotherapy: gastrointestinal immunomodulation and enteric nervous system toxicity », *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 308, n° 4, p. G223-232, févr. 2015.
[97] L. Wafai, M. Taher, V. Jovanovska, J. C. Bornstein, C. R. Dass, et K. Nurgali, « Effects of oxaliplatin on mouse myenteric neurons and colonic motility », *Front. Neurosci.*, vol. 7, p. 30, 2013.
[98] S. E. Carbone, V. Jovanovska, S. J. H. Brookes, et K. Nurgali, « Electrophysiological and morphological changes in colonic myenteric neurons from chemotherapy-treated patients: a pilot study », *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.*, vol. 28, n° 7, p. 975-984, 2016.
[99] A. M. Robinson, V. Stojanovska, A. A. Rahman, R. M. McQuade, P. V. Senior, et K. Nurgali, « Effects of Oxaliplatin Treatment on the Enteric Glial Cells and Neurons in the Mouse Ileum », *J. Histochem. Cytochem. Off. J. Histochem. Soc.*, vol. 64, n° 9, p. 530-545, 2016.
[100] R. M. McQuade, V. Stojanovska, E. Donald, R. Abalo, J. C. Bornstein, et K. Nurgali, « Gastrointestinal dysfunction and enteric neurotoxicity following treatment with anticancer chemotherapeutic agent 5-fluorouracil », *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.*, vol. 28, n° 12, p. 1861-1875, déc. 2016.
[101] D. Thorpe, A. Stringer, et R. Butler, « Chemotherapy-induced mucositis: the role of mucin secretion and regulation, and the enteric nervous system », *Neurotoxicology*, vol. 38, p. 101-105, sept. 2013.
[102] Y. Saegusa *et al.*, « Changes in the mucus barrier of the rat during 5-fluorouracil-induced gastrointestinal mucositis », *Scand. J. Gastroenterol.*, vol. 43, n° 1, p. 59-65, janv. 2008.
[103] A. M. Stringer *et al.*, « Irinotecan-induced mucositis is associated with changes in intestinal mucins », *Cancer Chemother. Pharmacol.*, vol. 64, n° 1, p. 123-132, juin 2009.
[104] C. I. Hagl, S. Heumüller-Klug, E. Wink, L. Wessel, et K.-H. Schäfer, « The human gastrointestinal tract, a potential autologous neural stem cell source », *PloS One*, vol. 8, n° 9, p. e72948, 2013.
[105] R. M. McQuade, V. Stojanovska, J. C. Bornstein, et K. Nurgali, « PARP inhibition in platinum-based chemotherapy: Chemopotentiation and neuroprotection », *Pharmacol. Res.*, vol. 137, p. 104-113, 2018.

[106] J. Escalante, R. M. McQuade, V. Stojanovska, et K. Nurgali, « Impact of chemotherapy on gastrointestinal functions and the enteric nervous system », *Maturitas*, vol. 105, p. 23-29, nov. 2017.
[107] Y.-X. Zhang, W.-Y. Pan, et J. Chen, « p53 and its isoforms in DNA double-stranded break repair », *J. Zhejiang Univ. Sci. B*, vol. 20, n° 6, p. 457-466, juin 2019.
[108] J.-C. Bourdon *et al.*, « p53 isoforms can regulate p53 transcriptional activity », *Genes Dev.*, vol. 19, n° 18, p. 2122-2137, sept. 2005.
[109] F. Murray-Zmijewski, D. P. Lane, et J.-C. Bourdon, « p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress », *Cell Death Differ.*, vol. 13, n° 6, p. 962-972, juin 2006.
[110] S. M. Joruiz et J.-C. Bourdon, « p53 Isoforms: Key Regulators of the Cell Fate Decision », *Cold Spring Harb. Perspect. Med.*, vol. 6, n° 8, 01 2016.
[111] Y.-X. Zhang, W.-Y. Pan, et J. Chen, « p53 and its isoforms in DNA double-stranded break repair », *J. Zhejiang Univ. Sci. B*, vol. 20, n° 6, p. 457-466, juin 2019.
[112] C. Lanni, M. Racchi, M. Memo, S. Govoni, et D. Uberti, « p53 at the crossroads between cancer and neurodegeneration », *Free Radic. Biol. Med.*, vol. 52, n° 9, p. 1727-1733, mai 2012.
[113] J. Pflaum, S. Schlosser, et M. Müller, « p53 Family and Cellular Stress Responses in Cancer », *Front. Oncol.*, vol. 4, p. 285, 2014.
[114] J.-C. Bourdon, « p53 Family isoforms », *Curr. Pharm. Biotechnol.*, vol. 8, n° 6, p. 332-336, déc. 2007.
[115] R.-A. Romano *et al.*, « ΔNp63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation », *Dev. Camb. Engl.*, vol. 139, n° 4, p. 772-782, févr. 2012.
[116] M. T. Wilhelm *et al.*, « Isoform-specific p73 knockout mice reveal a novel role for delta Np73 in the DNA damage response pathway », *Genes Dev.*, vol. 24, n° 6, p. 549-560, mars 2010.
[117] R. Killick *et al.*, « p73: a multifunctional protein in neurobiology », *Mol. Neurobiol.*, vol. 43, n° 2, p. 139-146, avr. 2011.
[118] M. V. Niklison-Chirou, R. Killick, R. A. Knight, P. Nicotera, G. Melino, et M. Agostini, « How Does p73 Cause Neuronal Defects? », *Mol. Neurobiol.*, vol. 53, n° 7, p. 4509-4520, 2016.
[119] L. Holembowski *et al.*, « While p73 is essential, p63 is completely dispensable for the development of the central nervous system », *Cell Cycle Georget. Tex*, vol. 10, n° 4, p. 680-689, févr. 2011.
[120] A. Yang *et al.*, « p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development », *Nature*, vol. 398, n° 6729, p. 714-718, avr. 1999.
[121] G. I. Cancino *et al.*, « p63 Regulates adult neural precursor and newly born neuron survival to control hippocampal-dependent Behavior », *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 33, n° 31, p. 12569-12585, juill. 2013.
[122] M. P. Fatt, G. I. Cancino, F. D. Miller, et D. R. Kaplan, « p63 and p73 coordinate p53 function to determine the balance between survival, cell death, and senescence in adult neural precursor cells », *Cell Death Differ.*, vol. 21, n° 10, p. 1546-1559, oct. 2014.
[123] C. D. Pozniak, F. Barnabé-Heider, V. V. Rymar, A. F. Lee, A. F. Sadikot, et F. D. Miller, « p73 is required for survival and maintenance of CNS neurons », *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 22, n° 22, p. 9800-9809, nov. 2002.
[124] V. De Laurenzi *et al.*, « Two new p73 splice variants, gamma and delta, with different transcriptional activity », *J. Exp. Med.*, vol. 188, n° 9, p. 1763-1768, nov. 1998.
[125] K. Miyazaki *et al.*, « A novel HECT-type E3 ubiquitin ligase, NEDL2, stabilizes p73 and enhances its transcriptional activity », *Biochem. Biophys. Res. Commun.*, vol. 308, n° 1, p. 106-113, août 2003.
[126] F. Talos *et al.*, « p73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis », *Cell Death Differ.*, vol. 17, n° 12, p. 1816-1829, déc. 2010.
[127] M. Fujitani *et al.*, « TAp73 acts via the bHLH Hey2 to promote long-term maintenance of neural precursors », *Curr. Biol. CB*, vol. 20, n° 22, p. 2058-2065, nov. 2010.
[128] M. K. Wetzel *et al.*, « p73 regulates neurodegeneration and phospho-tau accumulation during aging and Alzheimer's disease », *Neuron*, vol. 59, n° 5, p. 708-721, sept. 2008.
[129] M. P. Mattson et U. Ashery, « No more brain tangles with DeltaNp73 », *Trends Biochem. Sci.*, vol. 34, n° 1, p. 6-8, janv. 2009.
[130] S. M. de la Monte, Y. K. Sohn, et J. R. Wands, « Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease », *J. Neurol. Sci.*, vol. 152, n° 1, p. 73-83, nov. 1997.
[131] C. Turnquist *et al.*, « p53 isoforms regulate astrocyte-mediated neuroprotection and neurodegeneration », *Cell Death Differ.*, vol. 23, n° 9, p. 1515-1528, 01 2016.
[132] W. B. Jacobs *et al.*, « p63 is an essential proapoptotic protein during neural development », *Neuron*, vol. 48, n° 5, p. 743-756, déc. 2005.
[133] W. L. Stoppel, D. L. Kaplan, et L. D. Black, « Electrical and mechanical stimulation of cardiac cells and tissue constructs », *Adv. Drug Deliv. Rev.*, vol. 96, p. 135-155, janv. 2016.

[134] S. Schiaffino et C. Reggiani, « Fiber types in mammalian skeletal muscles », *Physiol. Rev.*, vol. 91, n° 4, p. 1447-1531, oct. 2011.
[135] J. A. Hill, E. N. Olson, K. K. Griendling, R. N. Kitsis, J. T. Stull, et H. L. Sweeney, « Muscle : fundamental biology and mechanisms of disease », 2012.
[136] C. F. Bentzinger, Y. X. Wang, et M. A. Rudnicki, « Building muscle: molecular regulation of myogenesis », *Cold Spring Harb. Perspect. Biol.*, vol. 4, n° 2, févr. 2012.
[137] S. Tajbakhsh, « Skeletal muscle stem cells in developmental versus regenerative myogenesis », *J. Intern. Med.*, vol. 266, n° 4, p. 372-389, oct. 2009.
[138] M. Karalaki, S. Fili, A. Philippou, et M. Koutsilieris, « Muscle regeneration: cellular and molecular events », *Vivo Athens Greece*, vol. 23, n° 5, p. 779-796, oct. 2009.
[139] S. B. P. Chargé et M. A. Rudnicki, « Cellular and molecular regulation of muscle regeneration », *Physiol. Rev.*, vol. 84, n° 1, p. 209-238, janv. 2004.
[140] T. Fernandes, Ú. P. R. Soci, S. F. S. Melo, C. R. Alves, et E. M. Oliveira, « Signaling Pathways that Mediate Skeletal Muscle Hypertrophy: Effects of Exercise Training », *Skelet. Muscle - Myogenesis Clin. Relat.*, août 2012.
[141] I. H. Rosenberg, « Sarcopenia: origins and clinical relevance », *J. Nutr.*, vol. 127, n° 5 Suppl, p. 990S-991S, 1997.
[142] M. Iannuzzi-Sucich, K. M. Prestwood, et A. M. Kenny, « Prevalence of sarcopenia and predictors of skeletal muscle mass in healthy, older men and women », *J. Gerontol. A. Biol. Sci. Med. Sci.*, vol. 57, n° 12, p. M772-777, déc. 2002.
[143] I. M. Conboy et T. A. Rando, « Aging, stem cells and tissue regeneration: lessons from muscle », *Cell Cycle Georget. Tex*, vol. 4, n° 3, p. 407-410, mars 2005.
[144] E. Edström, M. Altun, M. Hägglund, et B. Ulfhake, « Atrogin-1/MAFbx and MuRF1 are downregulated in aging-related loss of skeletal muscle », *J. Gerontol. A. Biol. Sci. Med. Sci.*, vol. 61, n° 7, p. 663-674, juill. 2006.
[145] S. Clavel, A.-S. Coldefy, E. Kurkdjian, J. Salles, I. Margaritis, et B. Derijard, « Atrophy-related ubiquitin ligases, atrogin-1 and MuRF1 are up-regulated in aged rat Tibialis Anterior muscle », *Mech. Ageing Dev.*, vol. 127, n° 10, p. 794-801, oct. 2006.
[146] M. de Carvalho et M. Swash, « The onset of amyotrophic lateral sclerosis », *J. Neurol. Neurosurg. Psychiatry*, vol. 77, n° 3, p. 388-389, mars 2006.
[147] B. Léger *et al.*, « Human skeletal muscle atrophy in amyotrophic lateral sclerosis reveals a reduction in Akt and an increase in atrogin-1 », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 20, n° 3, p. 583-585, mars 2006.
[148] L. P. Rowland et N. A. Shneider, « Amyotrophic lateral sclerosis », *N. Engl. J. Med.*, vol. 344, n° 22, p. 1688-1700, mai 2001.
[149] P. Pasinelli *et al.*, « Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria », *Neuron*, vol. 43, n° 1, p. 19-30, juill. 2004.
[150] C. Vande Velde, T. M. Miller, N. R. Cashman, et D. W. Cleveland, « Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, n° 10, p. 4022-4027, mars 2008.
[151] M. I. Ivanova *et al.*, « Aggregation-triggering segments of SOD1 fibril formation support a common pathway for familial and sporadic ALS », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, n° 1, p. 197-201, janv. 2014.
[152] S. Grinspoon, K. Mulligan, et Department of Health and Human Services Working Group on the Prevention and Treatment of Wasting and Weight Loss, « Weight loss and wasting in patients infected with human immunodeficiency virus », *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, vol. 36, n° Suppl 2, p. S69-78, avr. 2003.
[153] J. M. Argilés, S. Busquets, et F. J. López-Soriano, « The pivotal role of cytokines in muscle wasting during cancer », *Int. J. Biochem. Cell Biol.*, vol. 37, n° 8, p. 1609-1619, août 2005.
[154] N. Cano, « [Malnutrition and chronic renal failure] », *Ann. Med. Interne (Paris)*, vol. 151, n° 7, p. 563-574, nov. 2000.
[155] M. B. Reid, « COPD as a muscle disease », *Am. J. Respir. Crit. Care Med.*, vol. 164, n° 7, p. 1101-1102, oct. 2001.
[156] R. Roubenoff *et al.*, « Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation », *J. Clin. Invest.*, vol. 93, n° 6, p. 2379-2386, juin 1994.
[157] S. D. Anker *et al.*, « Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study », *Lancet Lond. Engl.*, vol. 361, n° 9363, p. 1077-1083, mars 2003.
[158] J. M. Argilés, B. Alvarez, et F. J. López-Soriano, « The metabolic basis of cancer cachexia », *Med. Res. Rev.*, vol. 17, n° 5, p. 477-498, sept. 1997.
[159] J. M. Argilés, B. Stemmler, F. J. López-Soriano, et S. Busquets, « Inter-tissue communication in cancer cachexia », *Nat. Rev. Endocrinol.*, vol. 15, n° 1, p. 9-20, 2018.
[160] V. E. Baracos, L. Martin, M. Korc, D. C. Guttridge, et K. C. H. Fearon, « Cancer-associated cachexia », *Nat. Rev. Dis. Primer*, vol. 4, p. 17105, 18 2018.

[161] L. A. Sabourin, A. Girgis-Gabardo, P. Seale, A. Asakura, et M. A. Rudnicki, « Reduced differentiation potential of primary MyoD-/- myogenic cells derived from adult skeletal muscle », *J. Cell Biol.*, vol. 144, n° 4, p. 631-643, févr. 1999.
[162] J. M. Argilés, F. J. López-Soriano, M. Toledo, A. Betancourt, R. Serpe, et S. Busquets, « The cachexia score (CASCO): a new tool for staging cachectic cancer patients », *J. Cachexia Sarcopenia Muscle*, vol. 2, n° 2, p. 87-93, juin 2011.
[163] B. H. L. Tan et K. C. H. Fearon, « Cachexia: prevalence and impact in medicine », *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 11, n° 4, p. 400-407, juill. 2008.
[164] A. Lucia et M. Ramírez, « Muscling In on Cancer », *N. Engl. J. Med.*, vol. 375, n° 9, p. 892-894, sept. 2016.
[165] J. Bachmann *et al.*, « Pancreatic cancer related cachexia: influence on metabolism and correlation to weight loss and pulmonary function », *BMC Cancer*, vol. 9, p. 255, juill. 2009.
[166] N. Johns, N. A. Stephens, et K. C. H. Fearon, « Muscle wasting in cancer », *Int. J. Biochem. Cell Biol.*, vol. 45, n° 10, p. 2215-2229, oct. 2013.
[167] B. H. L. Tan *et al.*, « P-selectin genotype is associated with the development of cancer cachexia », *EMBO Mol. Med.*, vol. 4, n° 6, p. 462-471, juin 2012.
[168] K. Fearon, J. Arends, et V. Baracos, « Understanding the mechanisms and treatment options in cancer cachexia », *Nat. Rev. Clin. Oncol.*, vol. 10, n° 2, p. 90-99, févr. 2013.
[169] N. J. Espat, L. L. Moldawer, et E. M. Copeland, « Cytokine-mediated alterations in host metabolism prevent nutritional repletion in cachectic cancer patients », *J. Surg. Oncol.*, vol. 58, n° 2, p. 77-82, févr. 1995.
[170] D. a. C. Deans, S. J. Wigmore, H. Gilmour, S. Paterson-Brown, J. A. Ross, et K. C. H. Fearon, « Elevated tumour interleukin-1beta is associated with systemic inflammation: A marker of reduced survival in gastro-oesophageal cancer », *Br. J. Cancer*, vol. 95, n° 11, p. 1568-1575, déc. 2006.
[171] S. Perboni et A. Inui, « Anorexia in cancer: role of feeding-regulatory peptides », *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 361, n° 1471, p. 1281-1289, juill. 2006.
[172] E. Maury et S. M. Brichard, « Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome », *Mol. Cell. Endocrinol.*, vol. 314, n° 1, p. 1-16, janv. 2010.
[173] O. J. McAnena et J. M. Daly, « Impact of antitumor therapy on nutrition », *Surg. Clin. North Am.*, vol. 66, n° 6, p. 1213-1228, déc. 1986.
[174] T. Le Bricon, S. Gugins, L. Cynober, et V. E. Baracos, « Negative impact of cancer chemotherapy on protein metabolism in healthy and tumor-bearing rats », *Metabolism.*, vol. 44, n° 10, p. 1340-1348, oct. 1995.
[175] V. E. Baracos, « Management of muscle wasting in cancer-associated cachexia: understanding gained from experimental studies », *Cancer*, vol. 92, n° 6 Suppl, p. 1669-1677, sept. 2001.
[176] E. R. Camp *et al.*, « Inducible nuclear factor-kappaB activation contributes to chemotherapy resistance in gastric cancer », *J. Am. Coll. Surg.*, vol. 199, n° 2, p. 249-258, août 2004.
[177] J. S. Damrauer, M. E. Stadler, S. Acharyya, A. S. Baldwin, M. E. Couch, et D. C. Guttridge, « Chemotherapy-induced muscle wasting: association with NF-κB and cancer cachexia », *Eur. J. Transl. Myol.*, vol. 28, n° 2, p. 7590, avr. 2018.
[178] G. Takemura et H. Fujiwara, « Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management », *Prog. Cardiovasc. Dis.*, vol. 49, n° 5, p. 330-352, avr. 2007.
[179] J. H. Doroshow, G. Y. Locker, I. Ifrim, et C. E. Myers, « Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine », *J. Clin. Invest.*, vol. 68, n° 4, p. 1053-1064, oct. 1981.
[180] S. Zhang *et al.*, « Identification of the molecular basis of doxorubicin-induced cardiotoxicity », *Nat. Med.*, vol. 18, n° 11, p. 1639-1642, nov. 2012.
[181] A. R. Dresdale *et al.*, « Prospective randomized study of the role of N-acetyl cysteine in reversing doxorubicin-induced cardiomyopathy », *Am. J. Clin. Oncol.*, vol. 5, n° 6, p. 657-663, déc. 1982.
[182] R. Supriya *et al.*, « Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle », *Front. Physiol.*, vol. 7, p. 323, 2016.
[183] H. E. Mohamed, M. E. Asker, S. I. Ali, et T. M. A. el-Fattah, « Protection against doxorubicin cardiomyopathy in rats: role of phosphodiesterase inhibitors type 4 », *J. Pharm. Pharmacol.*, vol. 56, n° 6, p. 757-768, juin 2004.
[184] P. G. Arthur, M. D. Grounds, et T. Shavlakadze, « Oxidative stress as a therapeutic target during muscle wasting: considering the complex interactions », *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 11, n° 4, p. 408-416, juill. 2008.
[185] L. K. McLoon, G. Bauer, et J. Wirtschafter, « Quantification of muscle loss in the doxorubicin-treated orbicularis oculi of the monkey. Effect of local injection of doxorubicin into the eyelid », *Invest. Ophthalmol. Vis. Sci.*, vol. 32, n° 5, p. 1667-1673, avr. 1991.
[186] L. K. McLoon, J. D. Wirtschafter, et J. D. Cameron, « Muscle loss from doxorubicin injections into the eyelids of a patient with blepharospasm », *Am. J. Ophthalmol.*, vol. 116, n° 5, p. 646-648, nov. 1993.
[187] E. Cullu, I. Ozkan, N. Culhaci, B. Alparslan, E. Dikicioğlu, et S. O. Savk, « [Doxorubicin-induced chemomyectomy effects in rat skeletal muscle] », *Acta Orthop. Traumatol. Turc.*, vol. 37, n° 4, p. 323-329, 2003.

[188] S. H. Lecker *et al.*, « Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 18, n° 1, p. 39-51, janv. 2004.
[189] M. Sandri, « Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome », *Int. J. Biochem. Cell Biol.*, vol. 45, n° 10, p. 2121-2129, oct. 2013.
[190] K. L. Rock *et al.*, « Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules », *Cell*, vol. 78, n° 5, p. 761-771, sept. 1994.
[191] P. R. Cao, H. J. Kim, et S. H. Lecker, « Ubiquitin-protein ligases in muscle wasting », *Int. J. Biochem. Cell Biol.*, vol. 37, n° 10, p. 2088-2097, oct. 2005.
[192] R. J. Deshaies, « SCF and Cullin/Ring H2-based ubiquitin ligases », *Annu. Rev. Cell Dev. Biol.*, vol. 15, p. 435-467, 1999.
[193] J. A. Spencer, S. Eliazer, R. L. Ilaria, J. A. Richardson, et E. N. Olson, « Regulation of microtubule dynamics and myogenic differentiation by MURF, a striated muscle RING-finger protein », *J. Cell Biol.*, vol. 150, n° 4, p. 771-784, août 2000.
[194] T. Centner *et al.*, « Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain », *J. Mol. Biol.*, vol. 306, n° 4, p. 717-726, mars 2001.
[195] A. S. McElhinny, K. Kakinuma, H. Sorimachi, S. Labeit, et C. C. Gregorio, « Muscle-specific RING finger-1 interacts with titin to regulate sarcomeric M-line and thick filament structure and may have nuclear functions via its interaction with glucocorticoid modulatory element binding protein-1 », *J. Cell Biol.*, vol. 157, n° 1, p. 125-136, avr. 2002.
[196] S. Perera, B. Mankoo, et M. Gautel, « Developmental regulation of MURF E3 ubiquitin ligases in skeletal muscle », *J. Muscle Res. Cell Motil.*, vol. 33, n° 2, p. 107-122, juin 2012.
[197] T. Cardozo et M. Pagano, « The SCF ubiquitin ligase: insights into a molecular machine », *Nat. Rev. Mol. Cell Biol.*, vol. 5, n° 9, p. 739-751, sept. 2004.
[198] S. C. Bodine *et al.*, « Identification of ubiquitin ligases required for skeletal muscle atrophy », *Science*, vol. 294, n° 5547, p. 1704-1708, nov. 2001.
[199] L. M. Baehr, J. D. Furlow, et S. C. Bodine, « Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids », *J. Physiol.*, vol. 589, n° Pt 19, p. 4759-4776, oct. 2011.
[200] S. H. Lecker, V. Solomon, S. R. Price, Y. T. Kwon, W. E. Mitch, et A. L. Goldberg, « Ubiquitin conjugation by the N-end rule pathway and mRNAs for its components increase in muscles of diabetic rats », *J. Clin. Invest.*, vol. 104, n° 10, p. 1411-1420, nov. 1999.
[201] M. D. Gomes, S. H. Lecker, R. T. Jagoe, A. Navon, et A. L. Goldberg, « Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 98, n° 25, p. 14440-14445, déc. 2001.
[202] R. T. Jagoe, S. H. Lecker, M. Gomes, et A. L. Goldberg, « Patterns of gene expression in atrophying skeletal muscles: response to food deprivation », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 16, n° 13, p. 1697-1712, nov. 2002.
[203] S. W. Jones, R. J. Hill, P. A. Krasney, B. O'Conner, N. Peirce, et P. L. Greenhaff, « Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 18, n° 9, p. 1025-1027, juin 2004.
[204] V. Kedar, H. McDonough, R. Arya, H.-H. Li, H. A. Rockman, et C. Patterson, « Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin I », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, n° 52, p. 18135-18140, déc. 2004.
[205] B. A. Clarke *et al.*, « The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle », *Cell Metab.*, vol. 6, n° 5, p. 376-385, nov. 2007.
[206] C. Polge *et al.*, « Muscle actin is polyubiquitinylated in vitro and in vivo and targeted for breakdown by the E3 ligase MuRF1 », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 25, n° 11, p. 3790-3802, nov. 2011.
[207] L. A. Tintignac, J. Lagirand, S. Batonnet, V. Sirri, M. P. Leibovitch, et S. A. Leibovitch, « Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase », *J. Biol. Chem.*, vol. 280, n° 4, p. 2847-2856, janv. 2005.
[208] E. M. Füchtbauer et H. Westphal, « MyoD and myogenin are coexpressed in regenerating skeletal muscle of the mouse », *Dev. Dyn. Off. Publ. Am. Assoc. Anat.*, vol. 193, n° 1, p. 34-39, janv. 1992.
[209] A. N. Kavazis, A. J. Smuder, et S. K. Powers, « Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle », *J. Appl. Physiol. Bethesda Md 1985*, vol. 117, n° 3, p. 223-230, août 2014.
[210] S. Schiaffino, K. A. Dyar, S. Ciciliot, B. Blaauw, et M. Sandri, « Mechanisms regulating skeletal muscle growth and atrophy », *FEBS J.*, vol. 280, n° 17, p. 4294-4314, sept. 2013.
[211] W. H. Biggs, W. K. Cavenee, et K. C. Arden, « Identification and characterization of members of the FKHR (FOX O) subclass of winged-helix transcription factors in the mouse », *Mamm. Genome Off. J. Int. Mamm. Genome Soc.*, vol. 12, n° 6, p. 416-425, juin 2001.

[212] M. Sandri *et al.*, « Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy », *Cell*, vol. 117, n° 3, p. 399-412, avr. 2004.
[213] D. S. Waddell *et al.*, « The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene », *Am. J. Physiol. Endocrinol. Metab.*, vol. 295, n° 4, p. E785-797, oct. 2008.
[214] Y. Kamei *et al.*, « Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control », *J. Biol. Chem.*, vol. 279, n° 39, p. 41114-41123, sept. 2004.
[215] A. M. J. Sanchez *et al.*, « AMPK promotes skeletal muscle autophagy through activation of forkhead FoxO3a and interaction with Ulk1 », *J. Cell. Biochem.*, vol. 113, n° 2, p. 695-710, févr. 2012.
[216] D. R. Calnan et A. Brunet, « The FoxO code », *Oncogene*, vol. 27, n° 16, p. 2276-2288, avr. 2008.
[217] H. Wang, N. Kubica, L. W. Ellisen, L. S. Jefferson, et S. R. Kimball, « Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1 », *J. Biol. Chem.*, vol. 281, n° 51, p. 39128-39134, déc. 2006.
[218] T. N. Stitt *et al.*, « The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors », *Mol. Cell*, vol. 14, n° 3, p. 395-403, mai 2004.
[219] S. Aittomäki et M. Pesu, « Therapeutic targeting of the Jak/STAT pathway », *Basic Clin. Pharmacol. Toxicol.*, vol. 114, n° 1, p. 18-23, janv. 2014.
[220] K. A. S. Silva *et al.*, « Inhibition of Stat3 activation suppresses caspase-3 and the ubiquitin-proteasome system, leading to preservation of muscle mass in cancer cachexia », *J. Biol. Chem.*, vol. 290, n° 17, p. 11177-11187, avr. 2015.
[221] B. Beutler, J. Mahoney, N. Le Trang, P. Pekala, et A. Cerami, « Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells », *J. Exp. Med.*, vol. 161, n° 5, p. 984-995, mai 1985.
[222] Y. P. Li et M. B. Reid, « NF-kappaB mediates the protein loss induced by TNF-alpha in differentiated skeletal muscle myotubes », *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 279, n° 4, p. R1165-1170, oct. 2000.
[223] M. Bar-Shai, E. Carmeli, P. Ljubuncic, et A. Z. Reznick, « Exercise and immobilization in aging animals: the involvement of oxidative stress and NF-kappaB activation », *Free Radic. Biol. Med.*, vol. 44, n° 2, p. 202-214, janv. 2008.
[224] M. G. Rhoads, S. C. Kandarian, F. Pacelli, G. B. Doglietto, et M. Bossola, « Expression of NF-kappaB and IkappaB proteins in skeletal muscle of gastric cancer patients », *Eur. J. Cancer Oxf. Engl. 1990*, vol. 46, n° 1, p. 191-197, janv. 2010.
[225] R. B. Hunter et S. C. Kandarian, « Disruption of either the Nfkb1 or the Bcl3 gene inhibits skeletal muscle atrophy », *J. Clin. Invest.*, vol. 114, n° 10, p. 1504-1511, nov. 2004.
[226] D. Cai *et al.*, « IKKbeta/NF-kappaB activation causes severe muscle wasting in mice », *Cell*, vol. 119, n° 2, p. 285-298, oct. 2004.
[227] Y.-P. Li *et al.*, « TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 19, n° 3, p. 362-370, mars 2005.
[228] R. C. Langen, A. M. Schols, M. C. Kelders, E. F. Wouters, et Y. M. Janssen-Heininger, « Inflammatory cytokines inhibit myogenic differentiation through activation of nuclear factor-kappaB », *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.*, vol. 15, n° 7, p. 1169-1180, mai 2001.
[229] O. Schakman, H. Gilson, et J. P. Thissen, « Mechanisms of glucocorticoid-induced myopathy », *J. Endocrinol.*, vol. 197, n° 1, p. 1-10, avr. 2008.
[230] M. Fischer, P. Rikeit, P. Knaus, et C. Coirault, « YAP-Mediated Mechanotransduction in Skeletal Muscle », *Front. Physiol.*, vol. 7, p. 41, 2016.
[231] S. Dupont *et al.*, « Role of YAP/TAZ in mechanotransduction », *Nature*, vol. 474, n° 7350, p. 179-183, juin 2011.
[232] S. Wu, J. Huang, J. Dong, et D. Pan, « hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts », *Cell*, vol. 114, n° 4, p. 445-456, août 2003.
[233] S. Basu, N. F. Totty, M. S. Irwin, M. Sudol, et J. Downward, « Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis », *Mol. Cell*, vol. 11, n° 1, p. 11-23, janv. 2003.
[234] T. Moroishi, C. G. Hansen, et K.-L. Guan, « The emerging roles of YAP and TAZ in cancer », *Nat. Rev. Cancer*, vol. 15, n° 2, p. 73-79, févr. 2015.
[235] K. I. Watt *et al.*, « Yap is a novel regulator of C2C12 myogenesis », *Biochem. Biophys. Res. Commun.*, vol. 393, n° 4, p. 619-624, mars 2010.
[236] Y. Nagata, T. A. Partridge, R. Matsuda, et P. S. Zammit, « Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling », *J. Cell Biol.*, vol. 174, n° 2, p. 245-253, juill. 2006.

[237] P. Fernando, J. F. Kelly, K. Balazsi, R. S. Slack, et L. A. Megeney, « Caspase 3 activity is required for skeletal muscle differentiation », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 99, n° 17, p. 11025-11030, août 2002.
[238] C. Sun *et al.*, « Common and Distinctive Functions of the Hippo Effectors Taz and Yap in Skeletal Muscle Stem Cell Function », *Stem Cells Dayt. Ohio*, vol. 35, n° 8, p. 1958-1972, 2017.
[239] K. I. Watt *et al.*, « The Hippo pathway effector YAP is a critical regulator of skeletal muscle fibre size », *Nat. Commun.*, vol. 6, p. 6048, janv. 2015.
[240] G. Kelley, « Mechanical overload and skeletal muscle fiber hyperplasia: a meta-analysis », *J. Appl. Physiol. Bethesda Md 1985*, vol. 81, n° 4, p. 1584-1588, oct. 1996.
[241] K. Ma *et al.*, « Nuclear accumulation of Yes-Associated Protein (YAP) maintains the survival of doxorubicin-induced senescent cells by promoting survivin expression », *Cancer Lett.*, vol. 375, n° 1, p. 84-91, mai 2016.
[242] B. Wei, W. Dui, D. Liu, Y. Xing, Z. Yuan, et G. Ji, « MST1, a key player, in enhancing fast skeletal muscle atrophy », *BMC Biol.*, vol. 11, p. 12, févr. 2013.
[243] E. A. Isenring et L. Teleni, « Nutritional counseling and nutritional supplements: a cornerstone of multidisciplinary cancer care for cachectic patients », *Curr. Opin. Support. Palliat. Care*, vol. 7, n° 4, p. 390-395, déc. 2013.
[244] T. Hanada *et al.*, « Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells », *Biochem. Biophys. Res. Commun.*, vol. 301, n° 2, p. 275-279, févr. 2003.
[245] M. Granado, T. Priego, A. I. Martín, M. A. Villanúa, et A. López-Calderón, « Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats », *Am. J. Physiol. Endocrinol. Metab.*, vol. 288, n° 3, p. E486-492, mars 2005.
[246] M. J. Tisdale, « Cachexia in cancer patients », *Nat. Rev. Cancer*, vol. 2, n° 11, p. 862-871, nov. 2002.
[247] J. T. Dalton, R. P. Taylor, M. L. Mohler, et M. S. Steiner, « Selective androgen receptor modulators for the prevention and treatment of muscle wasting associated with cancer », *Curr. Opin. Support. Palliat. Care*, vol. 7, n° 4, p. 345-351, déc. 2013.
[248] K. A. S. Silva *et al.*, « Inhibition of Stat3 activation suppresses caspase-3 and the ubiquitin-proteasome system, leading to preservation of muscle mass in cancer cachexia », *J. Biol. Chem.*, vol. 290, n° 17, p. 11177-11187, avr. 2015.
[249] C. Vinel *et al.*, « The exerkine apelin reverses age-associated sarcopenia », *Nat. Med.*, vol. 24, n° 9, p. 1360-1371, 2018.
[250] Y. Miyamoto, D. L. Hanna, W. Zhang, H. Baba, et H.-J. Lenz, « Molecular Pathways: Cachexia Signaling-A Targeted Approach to Cancer Treatment », *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.*, vol. 22, n° 16, p. 3999-4004, août 2016.
[251] T. A. Nissinen *et al.*, « Systemic blockade of ACVR2B ligands prevents chemotherapy-induced muscle wasting by restoring muscle protein synthesis without affecting oxidative capacity or atrogenes », *Sci. Rep.*, vol. 6, p. 32695, sept. 2016.
[252] G. A. Fisher, « A phase III study of xilonix in refractory colorectal cancer patients with weight loss. », *J. Clin. Oncol.*, vol. 33, n° 3_suppl, p. 685-685, janv. 2015.
[253] A. Jatoi, « Fish oil, lean tissue, and cancer: is there a role for eicosapentaenoic acid in treating the cancer anorexia/weight loss syndrome? », *Crit. Rev. Oncol. Hematol.*, vol. 55, n° 1, p. 37-43, juill. 2005.
[254] A. Dewey, C. Baughan, T. Dean, B. Higgins, et I. Johnson, « Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils) for the treatment of cancer cachexia », *Cochrane Database Syst. Rev.*, n° 1, p. CD004597, janv. 2007.
[255] C. L. Loprinzi *et al.*, « Randomized comparison of megestrol acetate versus dexamethasone versus fluoxymesterone for the treatment of cancer anorexia/cachexia », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 17, n° 10, p. 3299-3306, oct. 1999.
[256] A. A. Mills, B. Zheng, X. J. Wang, H. Vogel, D. R. Roop, et A. Bradley, « p63 is a p53 homologue required for limb and epidermal morphogenesis », *Nature*, vol. 398, n° 6729, p. 708-713, avr. 1999.
[257] A. Yang *et al.*, « p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours », *Nature*, vol. 404, n° 6773, p. 99-103, mars 2000.
[258] A. Porrello *et al.*, « p53 regulates myogenesis by triggering the differentiation activity of pRb », *J. Cell Biol.*, vol. 151, n° 6, p. 1295-1304, déc. 2000.
[259] H. Cam *et al.*, « p53 family members in myogenic differentiation and rhabdomyosarcoma development », *Cancer Cell*, vol. 10, n° 4, p. 281-293, oct. 2006.
[260] U. M. Moll et N. Slade, « p63 and p73: roles in development and tumor formation », *Mol. Cancer Res. MCR*, vol. 2, n° 7, p. 371-386, juill. 2004.
[261] S. Cefalù *et al.*, « TAp63gamma is required for the late stages of myogenesis », *Cell Cycle*, vol. 14, n° 6, p. 894, mars 2015.

[262] N. Hüttinger-Kirchhof, H. Cam, H. Griesmann, L. Hofmann, M. Beitzinger, et T. Stiewe, « The p53 family inhibitor DeltaNp73 interferes with multiple developmental programs », *Cell Death Differ.*, vol. 13, nº 1, p. 174-177, janv. 2006.
[263] M. Rouleau *et al.*, « TAp63 is important for cardiac differentiation of embryonic stem cells and heart development », *Stem Cells Dayt. Ohio*, vol. 29, nº 11, p. 1672-1683, nov. 2011.
[264] G. Fontemaggi *et al.*, « The transcriptional repressor ZEB regulates p73 expression at the crossroad between proliferation and differentiation », *Mol. Cell. Biol.*, vol. 21, nº 24, p. 8461-8470, déc. 2001.
[265] M. Paris, M. Rouleau, M. Pucéat, et D. Aberdam, « Regulation of skin aging and heart development by TAp63 », *Cell Death Differ.*, vol. 19, nº 2, p. 186-193, févr. 2012.
[266] S. Gonfloni, V. Caputo, et V. Iannizzotto, « P63 in health and cancer », *Int. J. Dev. Biol.*, vol. 59, nº 1-3, p. 87-93, 2015.
[267] P. S. Zammit, T. A. Partridge, et Z. Yablonka-Reuveni, « The skeletal muscle satellite cell: the stem cell that came in from the cold », *J. Histochem. Cytochem. Off. J. Histochem. Soc.*, vol. 54, nº 11, p. 1177-1191, nov. 2006.
[268] Y. Tamir et E. Bengal, « p53 protein is activated during muscle differentiation and participates with MyoD in the transcription of muscle creatine kinase gene », *Oncogene*, vol. 17, nº 3, p. 347-356, juill. 1998.
[269] C.-Y. Li, J. Zhu, et J. Y. J. Wang, « Ectopic expression of p73alpha, but not p73beta, suppresses myogenic differentiation », *J. Biol. Chem.*, vol. 280, nº 3, p. 2159-2164, janv. 2005.
[270] M. Schwarzkopf, D. Coletti, D. Sassoon, et G. Marazzi, « Muscle cachexia is regulated by a p53-PW1/Peg3-dependent pathway », *Genes Dev.*, vol. 20, nº 24, p. 3440-3452, déc. 2006.
[271] L. A. Donehower *et al.*, « Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours », *Nature*, vol. 356, nº 6366, p. 215-221, mars 1992.
[272] J. D. White, C. Rachel, R. Vermeulen, M. Davies, et M. D. Grounds, « The role of p53 in vivo during skeletal muscle post-natal development and regeneration: studies in p53 knockout mice », *Int. J. Dev. Biol.*, vol. 46, nº 4, p. 577-582, 2002.
[273] M. H. Yun, P. B. Gates, et J. P. Brockes, « Regulation of p53 is critical for vertebrate limb regeneration », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, nº 43, p. 17392-17397, oct. 2013.
[274] B. Stocks, J. R. Dent, S. Joanisse, C. E. McCurdy, et A. Philp, « Skeletal Muscle Fibre-Specific Knockout of p53 Does Not Reduce Mitochondrial Content or Enzyme Activity », *Front. Physiol.*, vol. 8, p. 941, 2017.
[275] L. D. Mayo et D. B. Donner, « The PTEN, Mdm2, p53 tumor suppressor-oncoprotein network », *Trends Biochem. Sci.*, vol. 27, nº 9, p. 462-467, sept. 2002.
[276] P.-Y. Zeng et S. L. Berger, « LKB1 is recruited to the p21/WAF1 promoter by p53 to mediate transcriptional activation », *Cancer Res.*, vol. 66, nº 22, p. 10701-10708, nov. 2006.
[277] K. Imamura, T. Ogura, A. Kishimoto, M. Kaminishi, et H. Esumi, « Cell cycle regulation via p53 phosphorylation by a 5'-AMP activated protein kinase activator, 5-aminoimidazole- 4-carboxamide-1-beta-D-ribofuranoside, in a human hepatocellular carcinoma cell line », *Biochem. Biophys. Res. Commun.*, vol. 287, nº 2, p. 562-567, sept. 2001.
[278] R. G. Jones *et al.*, « AMP-activated protein kinase induces a p53-dependent metabolic checkpoint », *Mol. Cell*, vol. 18, nº 3, p. 283-293, avr. 2005.
[279] K. Bensaad *et al.*, « TIGAR, a p53-inducible regulator of glycolysis and apoptosis », *Cell*, vol. 126, nº 1, p. 107-120, juill. 2006.
[280] A. L. Okorokov et J. Milner, « An ATP/ADP-dependent molecular switch regulates the stability of p53-DNA complexes », *Mol. Cell. Biol.*, vol. 19, nº 11, p. 7501-7510, nov. 1999.
[281] F. Schwartzenberg-Bar-Yoseph, M. Armoni, et E. Karnieli, « The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression », *Cancer Res.*, vol. 64, nº 7, p. 2627-2633, avr. 2004.
[282] S. Matoba *et al.*, « p53 regulates mitochondrial respiration », *Science*, vol. 312, nº 5780, p. 1650-1653, juin 2006.
[283] T. Zhang et W. L. Kraus, « SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions », *Biochim. Biophys. Acta*, vol. 1804, nº 8, p. 1666-1675, août 2010.
[284] X. Su *et al.*, « TAp63 is a master transcriptional regulator of lipid and glucose metabolism », *Cell Metab.*, vol. 16, nº 4, p. 511-525, oct. 2012.
[285] P. S. Pardo et A. M. Boriek, « The physiological roles of Sirt1 in skeletal muscle », *Aging*, vol. 3, nº 4, p. 430-437, avr. 2011.
[286] D. K. Fox *et al.*, « p53 and ATF4 mediate distinct and additive pathways to skeletal muscle atrophy during limb immobilization », *Am. J. Physiol. Endocrinol. Metab.*, vol. 307, nº 3, p. E245-261, août 2014.
[287] S. E. Alway, H. Degens, G. Krishnamurthy, et A. Chaudhrai, « Denervation stimulates apoptosis but not Id2 expression in hindlimb muscles of aged rats », *J. Gerontol. A. Biol. Sci. Med. Sci.*, vol. 58, nº 8, p. 687-697, août 2003.

[288] R. Persinger, Y. Janssen-Heininger, S. S. Wing, D. E. Matthews, M. M. LeWinter, et M. J. Toth, « Effect of heart failure on the regulation of skeletal muscle protein synthesis, breakdown, and apoptosis », *Am. J. Physiol. Endocrinol. Metab.*, vol. 284, nº 5, p. E1001-1008, mai 2003.
[289] L. Dalla Libera *et al.*, « Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure », *Am. J. Physiol. Cell Physiol.*, vol. 286, nº 1, p. C138-144, janv. 2004.
[290] E. E. Dupont-Versteegden, « Apoptosis in muscle atrophy: relevance to sarcopenia », *Exp. Gerontol.*, vol. 40, nº 6, p. 473-481, juin 2005.
[291] A. McArdle, A. Maglara, P. Appleton, A. J. Watson, I. Grierson, et M. J. Jackson, « Apoptosis in multinucleated skeletal muscle myotubes », *Lab. Investig. J. Tech. Methods Pathol.*, vol. 79, nº 9, p. 1069-1076, sept. 1999.
[292] C. Leeuwenburgh, C. M. Gurley, B. A. Strotman, et E. E. Dupont-Versteegden, « Age-related differences in apoptosis with disuse atrophy in soleus muscle », *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 288, nº 5, p. R1288-1296, mai 2005.
[293] M. Hirose, M. Kaneki, H. Sugita, S. Yasuhara, et J. A. Martyn, « Immobilization depresses insulin signaling in skeletal muscle », *Am. J. Physiol. Endocrinol. Metab.*, vol. 279, nº 6, p. E1235-1241, déc. 2000.
[294] J. Du et W. E. Mitch, « Identification of pathways controlling muscle protein metabolism in uremia and other catabolic conditions », *Curr. Opin. Nephrol. Hypertens.*, vol. 14, nº 4, p. 378-382, juill. 2005.
[295] P. O. Hasselgren et J. E. Fischer, « Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation », *Ann. Surg.*, vol. 233, nº 1, p. 9-17, janv. 2001.
[296] D. S. Tews, W. Behrhof, et S. Schindler, « Expression patterns of initiator and effector caspases in denervated human skeletal muscle », *Muscle Nerve*, vol. 31, nº 2, p. 175-181, févr. 2005.
[297] S. Zarei *et al.*, « A comprehensive review of amyotrophic lateral sclerosis », *Surg. Neurol. Int.*, vol. 6, p. 171, 2015.
[298] L. A. A. Gilliam, J. S. Moylan, L. A. Callahan, M. P. Sumandea, et M. B. Reid, « Doxorubicin causes diaphragm weakness in murine models of cancer chemotherapy », *Muscle Nerve*, vol. 43, nº 1, p. 94-102, janv. 2011.
[299] D. S. Hydock, C.-Y. Lien, B. T. Jensen, C. M. Schneider, et R. Hayward, « Characterization of the effect of in vivo doxorubicin treatment on skeletal muscle function in the rat », *Anticancer Res.*, vol. 31, nº 6, p. 2023-2028, juin 2011.
[300] T. Morimoto *et al.*, « Myocardial regulation of p300 and p53 by doxorubicin involves ubiquitin pathways », *Circ. J. Off. J. Jpn. Circ. Soc.*, vol. 72, nº 9, p. 1506-1511, sept. 2008.
[301] L. A. A. Gilliam *et al.*, « Doxorubicin acts via mitochondrial ROS to stimulate catabolism in C2C12 myotubes », *Am. J. Physiol. Cell Physiol.*, vol. 302, nº 1, p. C195-202, janv. 2012.
[302] L. A. A. Gilliam *et al.*, « Doxorubicin acts through tumor necrosis factor receptor subtype 1 to cause dysfunction of murine skeletal muscle », *J. Appl. Physiol. Bethesda Md 1985*, vol. 107, nº 6, p. 1935-1942, déc. 2009.
[303] V. Tang, A. Dhirapong, A. P. Yabes, et R. H. Weiss, « TNF-alpha-mediated apoptosis in vascular smooth muscle cells requires p73 », *Am. J. Physiol. Cell Physiol.*, vol. 289, nº 1, p. C199-206, juill. 2005.
[304] D. C. Guttridge, M. W. Mayo, L. V. Madrid, C. Y. Wang, et A. S. Baldwin, « NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia », *Science*, vol. 289, nº 5488, p. 2363-2366, sept. 2000.
[305] D. Coletti, V. Moresi, S. Adamo, M. Molinaro, et D. Sassoon, « Tumor necrosis factor-alpha gene transfer induces cachexia and inhibits muscle regeneration », *Genes. N. Y. N 2000*, vol. 43, nº 3, p. 120-128, nov. 2005.
[306] Z. J. P. Yang *et al.*, « p53 suppresses muscle differentiation at the myogenin step in response to genotoxic stress », *Cell Death Differ.*, vol. 22, nº 4, p. 560-573, avr. 2015.
[307] E. Zacksenhaus, Z. Jiang, D. Chung, J. D. Marth, R. A. Phillips, et B. L. Gallie, « pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis », *Genes Dev.*, vol. 10, nº 23, p. 3051-3064, déc. 1996.
[308] T. Ebata *et al.*, « Substrate Stiffness Influences Doxorubicin-Induced p53 Activation via ROCK2 Expression », *BioMed Res. Int.*, vol. 2017, p. 5158961, 2017.
[309] C. Dai et W. Gu, « p53 post-translational modification: deregulated in tumorigenesis », *Trends Mol. Med.*, vol. 16, nº 11, p. 528-536, nov. 2010.
[310] A. N. Weaver et E. S. Yang, « Beyond DNA Repair: Additional Functions of PARP-1 in Cancer », *Front. Oncol.*, vol. 3, p. 290, nov. 2013.
[311] V. M. Gonzalez, M. A. Fuertes, C. Alonso, et J. M. Perez, « Is cisplatin-induced cell death always produced by apoptosis? », *Mol. Pharmacol.*, vol. 59, nº 4, p. 657-663, avr. 2001.
[312] A. Rufini, P. Tucci, I. Celardo, et G. Melino, « Senescence and aging: the critical roles of p53 », *Oncogene*, vol. 32, nº 43, p. 5129-5143, oct. 2013.
[313] J. P. de Magalhães et J. F. Passos, « Stress, cell senescence and organismal ageing », *Mech. Ageing Dev.*, vol. 170, p. 2-9, 2018.
[314] S. Elmore, « Apoptosis: A Review of Programmed Cell Death », *Toxicol. Pathol.*, vol. 35, nº 4, p. 495-516, 2007.

[315] K. Valis *et al.*, « Hippo/Mst1 stimulates transcription of the proapoptotic mediator NOXA in a FoxO1-dependent manner », *Cancer Res.*, vol. 71, n° 3, p. 946-954, févr. 2011.
[316] H. Zhang, S. Wu, et D. Xing, « YAP accelerates Aβ(25-35)-induced apoptosis through upregulation of Bax expression by interaction with p73 », *Apoptosis Int. J. Program. Cell Death*, vol. 16, n° 8, p. 808-821, août 2011.
[317] M. Rossi *et al.*, « The ubiquitin-protein ligase Itch regulates p73 stability », *EMBO J.*, vol. 24, n° 4, p. 836-848, févr. 2005.
[318] M. Giroux et A. Descoteaux, « Cyclooxygenase-2 Expression in Macrophages: Modulation by Protein Kinase C-α », *J. Immunol.*, vol. 165, n° 7, p. 3985-3991, oct. 2000.

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Role of the p53 family in chemotherapy related side effects on the enteric nervous system and skeletal muscle homeostasis

Résumé

Une des grandes complications des traitements anticancéreux s'explique par les nombreux effets secondaires liés aux composés de chimiothérapie classique dont les sels de platine et les anthracyclines. Ils causent respectivement une altération du système nerveux entérique (SNE) et une sévère atrophie musculaire pouvant être létales ; malheureusement ; leurs origines sont encore mal connues et peu de traitements sont efficaces ou développés pour les limiter.

L'objectif de ce travail a été d'identifier les mécanismes moléculaires responsables de la perte neuronale du SNE et de la dégradation abusive des protéines du muscle pour empêcher ces altérations et développer de nouvelles approches thérapeutiques. Ces travaux ont prouvé pour la première fois l'existence de la famille p53 dans le SNE via son implication dans la mort cellulaire liée au cisplatine, ainsi que son rôle majeur dans l'activation du processus catabolique dans le muscle lié à la doxorubicine.

Dans l'ensemble, ces travaux montrent l'importance de la famille p53 dans les toxicités des chimiothérapies et font émerger de potentiels outils thérapeutiques basés sur le ciblage de la famille p53.

Mots clés : Chimiothérapies, toxicité, famille p53, système nerveux entérique, muscle

Summary

One of the major complications of cancer treatments is the important side effects associated with conventional chemotherapy compounds including platinum salts and anthracyclines. They cause respectively an alteration of the enteric nervous system (ENS) and severe muscular atrophy which can be lethal; unfortunately, their origins are still poorly understood, and few treatments are effective or developed to limit them.

The objective of this work was to identify the molecular mechanisms responsible for the neuronal loss of the ENS and the excessive degradation of muscle proteins to prevent these alterations and develop new therapeutic approaches. This work proved for the first time the existence of the p53 family in the ENS through its involvement in cisplatin-related cell death, as well as its major role in activating the doxorubicin-related catabolic process in muscle.

Overall, these results show the importance of the p53 family in the toxicities of chemotherapy and point out the emergence of potential therapeutic tools based on the targeting of the p53 family.

Keywords: Chemotherapy, toxicity, p53 family, enteric nervous system, muscle