UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1943

Micro-environmental control of tumor differentiation and invasiveness

CAROLINE GÉLABERT

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2023

ISSN 1651-6206
ISBN 978-91-513-1800-4
URN urn:nbn:se:uu:diva-500362

Dissertation presented at Uppsala University to be publicly examined in Room A1:111a, BMC, Husargatan 3, Uppsala, Tuesday, 13 June 2023 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Senior Lecturer/Associate Professor Alexander Pietras (Division of Translational Cancer Research, Lund University).

Abstract

Gélabert, C. 2023. Micro-environmental control of tumor differentiation and invasiveness. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1943. 64 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1800-4.

The transforming growth factor β (TGFβ) family participates in embryonic development and adult tissue homeostasis. In early stages of tumorigenesis, TGFβ promotes cell cycle arrest and apoptosis; however, in advanced malignancies, TGFβ promotes tumor cell migration and metastasis via the induction of epithelial-tomesenchymal transition (EMT). A new aspect of the regulation of TGFβ signalling is the participation of non-coding RNAs, molecules that are not translated into proteins but are nevertheless important regulators of gene expression. The expression of the long non-coding RNA *LINC00707* was identified being down-regulated by TGFβ by engaging the transcription factor KLF6. *LINC00707* resides in the cytoplasm where it interacts with and sequesters the Smad proteins, which mediate TGFβ signals. Thus, when TGFβ signaling downregulates *LINC00707*, the Smad complex is free to enter the nucleus and regulate its target genes implicated in the EMT process. It is also important to consider the biology of cells in their microenvironment. The growth of solid tumors leads to regional deprivation of nutrients within a tumor. Glutamine deprivation in mesenchymal and epithelial hepatocellular carcinoma cell lines showed a large change in gene expression related to TGFβ signaling in cells adapted to glutamine deprivation, suggesting a dependence of TGFβ signaling on glutamine metabolism. In mesenchymal cells, we observed a mesenchymal-to-epithelial transition associated with reduced metabolic activity and a reduced generation of reactive oxygen species. Mitochondrial reactive oxygen species are known for their capacity to regulate various signalling pathways associated with diverse cellular responses. This work also identified the polarity protein Par3 as a negative regulator of mitochondrial activity in glioblastoma cells. Moreover, Par3 leads to suppressed invasiveness and sustained clonogenicity of glioblastoma cells.

In summary, this work describes novel regulatory mechanisms that affect different aspects of cancer biology in both epithelial (carcinoma) and nonepithelial (glial) tumor cells. A central component that unifies these new mechanisms of cancer cell regulation is the TGFβ signaling pathway. In addition to its novel findings, this work opens several questions whose investigation can provide deeper mechanistic understanding of the action of the key RNAs or proteins analysed in this thesis.

Keywords: Cancer, cell signaling, TGFβ, non-coding RNAs, metabolism

Caroline Gélabert, Department of Medical Biochemistry and Microbiology, Box 582, Uppsala University, SE-75123 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-1800-4
URN urn:nbn:se:uu:diva-500362 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-500362)

List of Papers

This thesis is based on the following papers, which are referred in the text by their Roman numerals.

I. **Gélabert, C.**, Papoutsoglou, P., Ahlström, E., Ameur, A., Heldin, C.-H., Caja, L., and Moustakas, A. (2023) The long non-coding RNA LINC00707 interacts with Smad proteins to regulate TGFβ signaling and cancer cell invasion. *Cell Communication and Signaling, re-submitted after revision.*

II. **Gélabert, C.**, Campisano S., Golán I.C., Beyene N.T., Heldin, C.-H., Chisari A., Sancho P., Moustakas, A. and Caja, L. (2023). Glutamine deprivation alters TGF-β signaling in hepatocellular carcinoma. *Cell Death and Disease, Manuscript*.

III. Dadras, M.S., Caja, L., Mezheyeuski, A., Liu, S., **Gélabert, C.**, Gomez-Puerto, M.C., Gallini, R., Rubin, C.-J., ten Dijke, P., Heldin, C.-H. and Moustakas, A. (2021) The polarity protein Par3 coordinates positively self-renewal and negatively invasiveness in glioblastoma. *Cell Death and Disease, 12: 932*.

The following paper is not included in the thesis:

Chisari A, Golán I, Campisano S, **Gélabert C**, Moustakas A, Sancho P, Caja L. (2021) Glucose and Amino Acid Metabolic Dependencies Linked to Stemness and Metastasis in Different Aggressive Cancer Types. *Frontiers in Pharmacology, 12: 723798, Review.*

Contents

Abbreviations

AP-1: activated protein-1
aPKC: atypical protein kinase C
ARTS: apoptosis-related protein in the TGFβ-signaling
ASK: apoptosis signal-regulating kinase 1
ASO: antisense nucleotides
ATF: activating transcription factor
ADP: adenosine diphosphate
AMP: adenosine monophosphate
ATP: adenosine triphosphate
Bcl: B-cell lymphoma 2
BDNF: brain-derived neurotrophic factor
BIM: *Bcl*-2-like *protein* 11
Bmf: Bcl2 modifying factor
Bmi-1: B lymphoma Mo-MLV insertion region 1 homolog
BMP: bone morphogenic protein
CAF: cancer-associated fibroblast
CASC: cancer susceptibility candidate
CCP: cytoplasmic carboxypeptidase
CKI: cyclin-dependent kinase inhibitor
CSAP: cilia and spindle-associated protein
CSC: cancer stem cell
CTHRC1: collagen triple helix repeat containing 1
DAP: death-associated protein
Daxx: Death domain-associated *protein* 6
DLG: disc large
DNA: deoxyribonucleic acid
E-Cadherin: epithelial cadherin
ECM: extracellular matrix
EGF: epidermal growth factor
EGFR: epidermal growth factor receptor
EMA: European medicine agency
EMT: epithelial-to-mesenchymal transition
EPR: epithelial cell program regulator
ERK: extracellular signal-regulated kinase
FAD: flavin adenine dinucleotide
FDA: food and drug administration
FKBP: FK506-binding protein
FOXP: forkhead box protein
GAG: glycosaminoglycan
GBM: glioblastoma

GCN: general control nonderepressible

GDF: growth differentiation factor

GLUT: glucose transporter

GPx: glutathione peroxidase

GSC: glioma stem cell

GSK: glycogen synthase kinase

GLS: glutaminase

GTP: guanosine triphosphate

HCC: hepatocellular carcinoma

HIFα: *Hypoxia-inducible factor 1-alpha*

IFN-γ: interferon-gamma

IL: interleukin

JAK: Janus kinase

JmjC: Jumonji-C

JNK: c-Jun N-terminal kinase

KLF6: Krüppel like factor 6

LAP: latency-associated peptide

LFAR1: liver fibrosis-associated lncRNA1

Lgl: lethal giant larvae

LKB: liver kinase B

LLC: large latent complex

lncRNA: long non-coding RNA

lncRNA-ATB: lncRNA activated by TGFβ

lncRNA CTBP1-AS2: lncRNA C-terminal binding protein1- antisense RNA 2

lncRNA MACC-AS1: lncRNA metastasis associated in colon cancer-antisense 1

lncRNA-TUG: lncRNA taurine up-regulated

LTBP: latent TGFβ-binding protein

MAPK: mitogen-activated protein kinase

MAP: microtubule-associated protein

MEK: MAPK/extracellular signal-regulated kinase

MET: mesenchymal-to-epithelial transition

MGMT: O^6-methylguanine DNA methyltransferase

MH: Mad Homology

MMP: metalloprotease

mRNA: messenger RNA

miRNA: micro RNA

MIS: Müllerian inhibiting substance

mTOR: mammalian target of rapamycin

NADH: nicotinamide adenine dinucleotide, hydrogenated/reduced

N-Cadherin: neural cadherin

NORAD: non-coding RNA activated by DNA damage

NRE: nuclear retention element

NSC: neural stem cell

NXF: nuclear RNA export factor

P21: protein of 21 kDa

P38: protein of 38 kDa

P4HA3: prolyl 4-hydroxylase alpha3

p53: protein of 53 kDa

PALS1: protein associated with Lin-Seven 1

Par: partition-defective
PATJ: Pals1-associated tight junction
PDGF: platelet-derived growth factor
PD-L1: programmed death-ligand 1
PHD2 : prolyl hydroxylase domain protein 2
PI3K: phosphoinositide 3′-kinase
piRNA: piwi interacting RNA
PTEN: phosphatase and tensin homolog
PTP1B: protein-tyrosine phosphatase 1B
PVT: plasmacytoma variant translocation
Raf: rapidly accelerated fibrosarcoma
Ras: rat sarcoma virus oncogene
REDD: regulated in development and DNA damage response
R-Smad: receptor-activated Smad
RNA: ribonucleic acid
ROS: reactive oxygen species
SCN1A: sodium voltage-gated channel alpha subunit 1
SDC4: syndecan-4
siRNA: small interfering RNA
SLC1A5: solute carrier family 1 member 5
SMASR: Smad3-associated lncRNA
SNHG6: small nucleolar RNA host gene
snRNP: small nuclear ribonucleoprotein
SOD: superoxide dismutase
STAT: signal transducer and activator of transcription
TAK1: TGFβ-activated kinase-1
TCA: tricarboxylic acid
TF: transcription factor
TGFβ: transforming growth factor beta
TGFβR: transforming growth factor beta receptor
T-bet: T-box expressed in T cells
Th: T helper
TIAM: T-lymphoma and metastasis 1
TLR: Toll-like receptor
TRAF: TNF receptor associated factor
TRX: thioredoxin
TTL: tubulin tyrosine ligase
VEGF: *vascular endothelial growth factor*
ZEB: zinc finger E-box-binding homeobox
ZO: zonula occludens

Petit résumé en francais
Short summary in French

Le corps humain est composé d´environ 50,000 milliards de cellules. Chacune de ces cellules est organisée comme un petit organisme composé de différents éléments aux fonctions bien précises : certains ont pour rôle de produire l´énergie nécessaire à la cellule pour survivre et proliférer si nécessaire, d´autres digèrent et dégradent les déchets produits par la cellule, ou encore d´autres détectent lorsque la cellule est en mauvaise santé et induit sa mort afin d´éviter les dommages au niveau de l´organisme. Le noyau de la cellule peut être vu comme son cerveau: il contient l´information génétique (sous forme de code A,T,C,G) qui permet à la cellule de produire les éléments nécessaires (appelés protéines) à son bon fonctionnement et de s´adapter à son environnement. D´une manière simplifiée, le code génétique est lu par la cellule, ce qui produit un molécule intermédiaire appelée ARN messager, elle-même lue et traduite en protéine. Le génome humain code pour environ 20,000 protéines et leur expression est finement régulée selon les besoins de la cellule.

Plus récemment, les avancées informatiques ont montré qu´une grande partie de l´ADN code pour un certain type d´ARN qui n´est jamais traduit en protéine : ces molécules sont appelées des ARN non codants. A quelques exceptions près, il était admis depuis longtemps que seuls les protéines, codées par les ARN messagers, avaient vraiment un rôle dans la cellule. Alors pourquoi les cellules dépenseraient-elles de l´énergie à produire des ARN non codants? De récentes études ont montré que ces ARN non codants sont en fait très importants pour la régulation de l´expression de l´ADN (pour que le bon gène soit exprimé au bon moment et au bon endroit) ou de la fonction de certaines protéines au sein de la cellule. Comme l´a très justement dit mon cousin Paul « les protéines c´est les ouvriers, les ARN non codants c´est la police ». C´est sur ce type de molécule que se porte le projet principal de cette thèse.

Je m´intéresse en particulier aux ARN non codants régulés par TGFβ, qui est une protéine capable d´induire l´expression de certains gènes. Ces gènes régulés par TGFβ sont principalement liés à la migration cellulaire, mécanisme très important lors du développement embryonnaire (migration des cellules pour former tel organe à tel endroit) ou lors d´une cicatrisation (il faut que les cellules migrent dans la blessure pour pouvoir la refermer). Ce-

pendant, l´activation de la migration cellulaire au sein d´une tumeur est liée à la dissémination métastatique des cellules tumorales, c´est à dire l´invasion de cellules tumorales dans la circulation sanguine et la colonisation d´un deuxième organe. C´est cette généralisation du cancer qui est létale pour le patient. J´ai montré dans une **première publication** que l´ARN non codant *LINC00707* affecte la migration cellulaire en interagissant et en séquestrant un ensemble de protéines appelé Smad dans le cytoplasme de la cellule, et ainsi l'empêche d´accéder au noyau de la cellule où ils ont pour rôle d´activer les gènes responsables de la migration cellulaire. Lorsque la cellule entre en contact avec TGFβ, l´expression de *LINC00707* est réduite, le complexe Smad est donc libéré et peut accéder à ses gènes cibles dans le noyau. La migration cellulaire est ainsi activée. Ce mécanisme a été démontré dans des lignées cellulaires issues de cancer du cerveau, prostate et peau.

En plus d´une capacité de migration très accrue par rapport aux cellules non cancéreuses, les cellules cancéreuses sont caractérisées par une prolifération incontrôlée, une résistance aux signaux de morts qui les rendent immortelles ainsi qu´une résistance aux radio- et chimiothérapies. Une caractéristique à laquelle je m´intéresse particulièrement dans un deuxième projet est l´utilisation de l´énergie par les cellules cancéreuses. La prolifération et migration consomment beaucoup d´énergie, les cellules cancéreuses ont donc besoin d´un gros apport d´énergie. La source d´énergie des cellules sont principalement le glucose et la glutamine. Ces nutriments sont disponibles dans la circulation sanguine, il est donc facilement imaginable que les cellules situées au bord de la tumeur ont facilement accès à ces nutriments car elles sont directement en contact avec la circulation sanguine. Qu´en est-il des cellules situées au cœur de la tumeur, où l´accès à l´oxygène et nutriments est limité ? Dans une **deuxième publication**, j´ai étudié l´effet du manque de glutamine en comparant les caractéristiques de cellules de cancer du foie exposées à de la glutamine ou privées de glutamine. J´ai montré que le manque de glutamine ralentit la prolifération des cellules, leur capacité à se renouveler et à migrer, et donc une diminution de l´agressivité tumorale. Cela peut s´expliquer par une diminution de la production de « dérivés réactifs de l´oxygène » qui peuvent être vus comme des toxines pour les cellules produites en parallèle de la prolifération cellulaire. Ces toxines sont associées à l´agressivité tumorale de par leur capacité à induire l´invasion tumorale, mécanisme démontré dans le cancer du cerveau dans une **troisième publication**.

Plus d´informations en anglais !

1 Introduction

The first cells ever observed were a group of vegetal cells in 1665 by the biologist Robert Hooke from a thin layer of cork that he described as an irregular honey-comb. He was actually looking at empty dead cell walls of plant tissues. Bacteria were simultaneously discovered in water samples by the biologist Anton van Leeuwenhoek who described their motility, which is a characteristic of living organisms. It took approximately a hundred years to observe the first animal cell and its nucleus under a microscope due to the fragility of animal tissues and the absence of wall around animal cells that makes them more difficult to observe. At this time, the quality of the microscopes did not allow them to describe the internal structure but the botanist and physiologist Schwann and Schleiden hypothesised that cells were the fundamental unit of life in the middle of the 19th century. The development of electron microscopy in the middle of the 20th century revealed the cell structure in detail and the existence of organelles inside the cells which initiated the understanding of cell function in parallel with the development of biochemistry, genetics and molecular biology.

Cell biology is essential to understand life and diseases. The human body is made of 10,000 to 100,000 billion cells and every single cell can be seen as a self-sustainable entity, containing all the necessary components for its well-being within 10 to 100 μm. Cells must be able to produce energy, degrade their waste, develop senses to monitor the environment and even sense when it is time to proliferate or die. They also need to adapt and interact with their environment and neighbouring cells. To do so, the function of each component of each cell is tightly controlled. Any mistake in regulation can possibly lead to a dysfunction at the organism level and generate different kind of diseases, for instance cancer that is a typical cell disease. The present work focuses on different aspects of cancer cell biology, from cell signaling to cell metabolism and regulation of oxidative stress.

1.1 Cancer

Cancer is a leading cause of death worldwide. Every year, 14 million of new cancer cases are diagnosed and 8 million people die of cancer worldwide (Torre et al., 2016). Cancer potentially develops in all organs but men are

mostly affected by prostate, lung, and colorectal whereas women are more affected by breast, lung, thyroid and colorectal cancer (Kim et al., 2018). Cancer is characterized by dynamic changes in the genome, genome-widespread epigenetic alterations and chromosomal aberrations leading to the gain or loss of molecular function reflecting changes to physiological processes. Typically, tumor cells develop from normal cells that contain DNA mutations and acquire new properties such as uncontrolled cell division. As long as the abnormal cells are under control by the immune system and do not spread away from the tissue of origin, they are considered as benign tumors. Malignant cancer cells are characterized by their capabilities of tissue invasion and sustained angiogenesis, self-sufficiency in growth signals, insensitivity to anti-growth and apoptosis signals and limitless replicative potential (Hanahan and Weinberg, 2011).

1.2 Glioblastoma

Glioblastoma (GBM) is the most common and malignant type of glial tumors in the brain. Yet, it is a rare malignancy with a prevalence estimated at 1/100,000 and usually diagnosed in 45 to 70-year-old people. The prognosis of GBM patients is poor, with a survival of approximately 12 months after diagnosis. The first care treatment of GBM involves surgical resection followed by radiotherapy and chemotherapy with temozolomide, a DNA alkylating agent that extends survival by approximately 2.5 months. Temozolomide promotes the hypermethylation and inactivation of the O^6-methylguanine DNA methyltransferase (MGMT) responsible for the repair of DNA double-stranded breaks, which causes cell cycle arrest in the G2/M phase and eventually cell death (Nagel et al., 2017). The classification of GBM includes three subtypes: classical, mesenchymal and proneural. They are characterized by their transcriptomic profile and genetic mutations: the mesenchymal subtype typically contains p53 and phosphatase and tensin homolog (PTEN) mutations, the classical subtype usually contains PTEN mutations only and the proneural subtype is characterized by phosphoinositide 3′-kinase (PI3K) mutations. The mesenchymal subtype is the most aggressive due to its high angiogenic and invasive capacities (Saito et al., 2019). There are three potential cells of origin of GBM: neural stem cells (NSCs) with a high regenerative plasticity and developmental potential (Jacques et al., 2010), NSC-derived astrocytes that generate mature astrocytes through symmetric division in adults (Chow et al., 2011) and oligodendrocyte precursor cells that is the main dividing cell population in the adult brain (Rebetz et al., 2008). Cell of origin can sometimes be traced as tumor cells retain some gene expression profile of the cells of origin but the cell of origin often remains unknown. Common features of these three subtypes (classical, mesenchymal and proneural) are their high proliferation rate

and chemoresistance. They are able to form secondary tumors inside the brain and very rarely outside the brain (Anderson et al., 2020).

1.3 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common tumor worldwide and comprises 75%-85% of cases of liver cancer. HCC usually affects people after 40 years of age (Bray et al., 2018). HCC are believed to originate from genetically damaged hepatocytes, the main type of cell present in the liver, or from lesions in hepatic progenitor cells (Tummala et al., 2017). HCC arises from liver cirrhosis, which is caused by environmental sources such as alcohol, bad diet or viral hepatitis. If detected early, HCC is easily removable by surgery but when detected in later stage, chemotherapy is needed. The most common chemical agents in chemotherapy against HCC are sorafenib, regorafenib and lenvatinib, three receptor tyrosine kinase inhibitors that are often combined or sequentially administrated. Sorafenib and regorafenib are small multi-tyrosine kinase inhibitors that block the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptor tyrosine kinase activities (Raza and Sood, 2014). In addition, regorafenib induces apoptosis via the inhibition of the STAT3 signaling by inducing src homology 2 domain-containing phosphatase 1 (SHP1) (Tai et al., 2014). Lenvatinib is similar but more efficient than sorafenib in targeting angiogenesis in HCC (Yamamoto et al., 2014). The overall survival of late HCC patients is still low because of its high metastatic capacities to the lung, bones and lymph nodes (Natsuizaka et al., 2005).

1.4 Prostate cancer

Prostate cancer is the most common type of cancer in men worldwide. Prostate tissue is made of three types of cells: luminal, basal and neuroendorcrine. Luminal or basal phenotypes are observed in prostate cancer, therefore, it is believed that both luminal (Wang et al., 2009) or basal (Wang et al., 2013) cells can be the cell of origin of prostate cancer. Prostate cancer is characterized by recurrent genetic alterations in the androgen pathway, the PI3K/Akt pathway (Taylor et al., 2010), the TGFβ/SMAD4 pathway (Ding et al., 2011), the loss of PTEN/p53 pathway that allows MYC to drive proliferation, leading to an increased cell proliferation and metastasis (Nowak et al., 2015). The prognosis for prostate cancer patients is rather good when the tumor is removed in early stage, but metastatic prostate cancer remains incurable despite the different available treatments. Radiotherapy and surgical castration are the most common prostate cancer treatment combined with chemotherapy. Five drugs are currently approved by the food and drug ad-

ministration (FDA): docetaxel, cabazitaxel, abiraterone, enzalutamide and sipuleucel-T. One strategy is to block cell division by binding and stabilizing microtubules (docetaxel or cabazitaxel in docetaxel-resistant tumors). The androgen pathway being dysregulated in prostate cancer, it is often targeted either by preventing the testosterone synthesis or by blocking the androgen receptor (abiraterone and enzalutamide). Sipuleucel-T is an immune-based treatment agent (Komura et al., 2018).

2 TGFβ signaling and function

2.1 The TGFβ family and its receptors

Transforming growth factor beta (TGFβ) is a prototype of a large family of secreted proteins involved in various cellular processes in the adult organism and during embryonic development. The human TGFβ family has thirty-three genes that encode for secreted cytokines that include the activins, the bone morphogenic proteins (BMPs), the growth differentiation factors (GDFs), inhibins, nodal, the Müllerian inhibiting substance (MIS) and the three TGFβ isoforms (TGFβ1, β2 and β3), the latter being collectively referred as TGFβs (Tzavlaki and Moustakas, 2020).

TGFβs are synthetized as a latent protein made of two parts; the latency-associated peptide (LAP) and a C-terminal polypeptide known as mature TGFβ. These two domains together form a large latent complex (LLC) that does not have any biological function. The TGFβ activation process involves the secretion of the LLC covalently bound with latent TGFβ binding proteins (LTBPs) to the extracellular matrix (ECM), where the LLC and LTBPs interact with additional proteins of the ECM such as fibrillins and fibronectins. Mechanical forces generated by the binding of the LAP to integrins and the binding to the ECM proteins result in the proteolysis of the LLC-associated proteins and release active TGFβ dimer to its receptors and co-receptors (Annes et al., 2004; Miyazono and Heldin, 1989; Shi et al., 2011).

The TGFβs bind to a complex of transmembrane kinase receptors, the TGFβ type I (TGFβRI) and type II (TGFβRII) receptors. Binding of a TGFβ ligand induces assembly of two type I and two type II receptors. There are seven human type I receptors and five type II receptors, and individual members of the TGFβ family bind to specific combinations of type I and type II receptors. TGFβ1, β2 and β3 can bind to the single TGFβRII, however, they associate with two different types of TGFβRI (also called ALK-1 and ALK-5) (Heldin and Moustakas, 2016). The interaction between TGFβ and the TGFβ receptors involves the help of co-receptors including betaglycan, a membrane-anchored proteoglycan with glycosaminoglycan (GAG) chains that brings the TGFβ to the TGFβ receptor II (Lopez-Casillas et al., 1994). Once the ligand bound to the complex of type I/type II receptors with the contribution of co-receptors, conformation of the receptor is modified allosterically which leads to phosphorylation of TGFβRI on serine residues by the TGFβRII kinase, causing the dissociation of the negative regulator of

the type I receptor, FK506-binding protein (FKPB12), and the activation of the type I receptor kinase (Huse et al., 1999). In general, the activated TGFβ-receptors are able to phosphorylate serine and threonine amino acids on their substrate proteins and thus initiate the cascade of signal transduction responsible for biological response (Huang et al., 2011).

2.2 TGFβ signaling pathways

The major signaling effectors of TGFβ are the SMAD proteins after phosphorylation of their C-terminal serines by the activated TGFβRI. The phosphorylated SMADs known as the "receptor-activated SMADs" (R-SMADs) dissociate from the receptor, two R-SMADs form a trimeric complex with the "co-SMAD", SMAD4, in order to translocate into the nucleus (Macias-Silva et al., 1996). Once in the nucleus, the SMAD complex, in collaboration with other transcription factors, is able to repress or activate target gene expression. The SMAD family comprises five R-SMADs (SMAD1, 2, 3, 5 and 8), one co-SMAD (SMAD4) and two inhibitory SMADs (SMAD6 and 7). SMAD6 and 7 are able to exert negative feedback by blocking R-SMAD phosphorylation or promoting receptor ubiquitylation, dephosphorylation and lysosomal degradation (Murayama et al., 2020). SMAD 2 and SMAD 3 act as R-SMADs for activin and TGFβ signaling, whereas SMAD1, SMAD5, and SMAD8 mediate responses to BMPs and GDFs (Miller et al., 2019). Structurally, the R-SMADs and co-SMAD can be divided in three main parts; an N-terminal Mad Homology 1 domain (MH1), a central linker and a C-terminal Mad homology 2 domain (MH2) (**Figure 1**). The MH1 domain contains a nuclear localization signal and binds to DNA (SMAD2, 3, 4). SMAD4 also contains its nuclear export signal in its MH1 domain. The MH2 domain, the most highly conserved domain, contains the epitopes for receptor interaction (SMAD2, 3) and SMAD oligomerization (SMAD2, 3, 4). SMAD2 and 3 also carry their nuclear export signal on their MH2 domain. The linker region contains phosphorylation sites and is important for the regulation of the stability, subcellular localization and activity of SMADs due to the presence of ubiquitin ligase-binding motifs on SMAD2 and 3 (Wu et al., 2001).

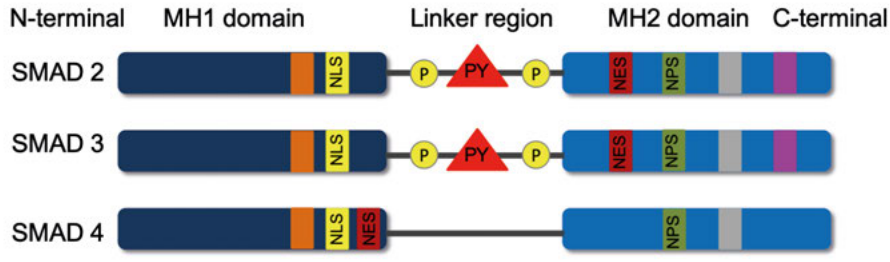

Figure 1 Schematic representation of the SMAD proteins

The orange box represents the DNA binding motif, the yellow box represents the nuclear localization signal (NLS), the red triangle represents the ubiquitin ligase motif (PY), the yellow circle represents the phosphorylation sites., the red box represents the nuclear export signal (NES), the green box represents the nucleopore signal (NPS), the grey box represents interaction sites between Smad2,3 and 4 and the pink box represents the interaction site with the TGFβ receptors.

The SMADs are not the only TGFβ-regulated signaling mediators. Other signaling proteins, known for their participation in other major pathways (e.g. receptor tyrosine kinases), are also often referred as "non-SMAD", and initiate parallel pathways that eventually cooperate with the SMADs (**Figure 2**) or crosstalk with additional signaling pathways such as Wnt or Notch. The mitogen-activated protein kinase (MAPK) family that include the extracellular-regulated kinases (ERK1 and 2), Jun N-terminal kinases (JNKs) and p38 MAPKs is an important family classified as non-SMAD pathway. ERK 1 and 2 are activated by the Ras - Raf - MEK1/2 cascade usually induced by mitogenic stimuli but also by TGFβ after phosphorylation of ShcA (Lee et al., 2007). The JNK and p38 MAPKs are activated by MAPK kinases (MAPK kinase 4-7 and MAPK kinase 3-6 respectively), activated themselves by the TGFβ-activated kinase-1 (TAK1) and the auto-ubiquitinated TNF Receptor Associated Factor 4 and 6 (TRAF4, TRAF6) upon TGFβ receptor oligomerization (Chen et al., 2015; Thakur et al., 2009; Yamashita et al., 2008). In addition to the JNK/ p38 pathways, the activation of TRAF6 results in recruitment and phosphorylation of AKT by PI3K (Hamidi et al., 2017). TGFβ, via PI3K, activates mTORC2, which in turn can phosphorylate and activate AKT promoting cell survival.

The different TGFβ-activated pathways can cooperate and regulate each other in order to fine-tune their activation or to interact with new partners. First, activated AKT prevents phosphorylation of SMAD3, thus attenuating SMAD3-dependent signaling. Another inhibitor of the SMAD pathway is TRAF4 that targets Smurf2 for polyubiquitylation and subsequent degradation of the TGFβ type I receptor and SMAD proteins, therefore acting as an inhibitor of TGFβ signaling (Zhang et al., 2013). The MAPK pathways can

be modulated by TGFβ and other stimuli (cytokines, cell-cell contacts etc) in a SMAD-independent manner, but reports also suggest SMAD-dependent mechanisms. One example of the crosstalk between the SMADs and MAPK pathways is the interaction between SMAD2/3 and c-Jun or Fos to activate the transcription factor Activated Protein-1 (AP-1) (Sundqvist et al., 2020; Zhang et al., 1998). Structurally, AP-1 is one of the transcription factors composed of heterodimers of Jun, Fos or the activating transcription factor (ATF) activated by the MAPK family that are involved in cell proliferation, death, migration, survival and eventually tumorigenesis when dysregulated. Finally, phosphorylated ERK1/2 are believed to boost the SMAD activity by extra phosphorylation of the SMAD in their linker region and therefore enhance downstream events (Hayashida et al., 2003).

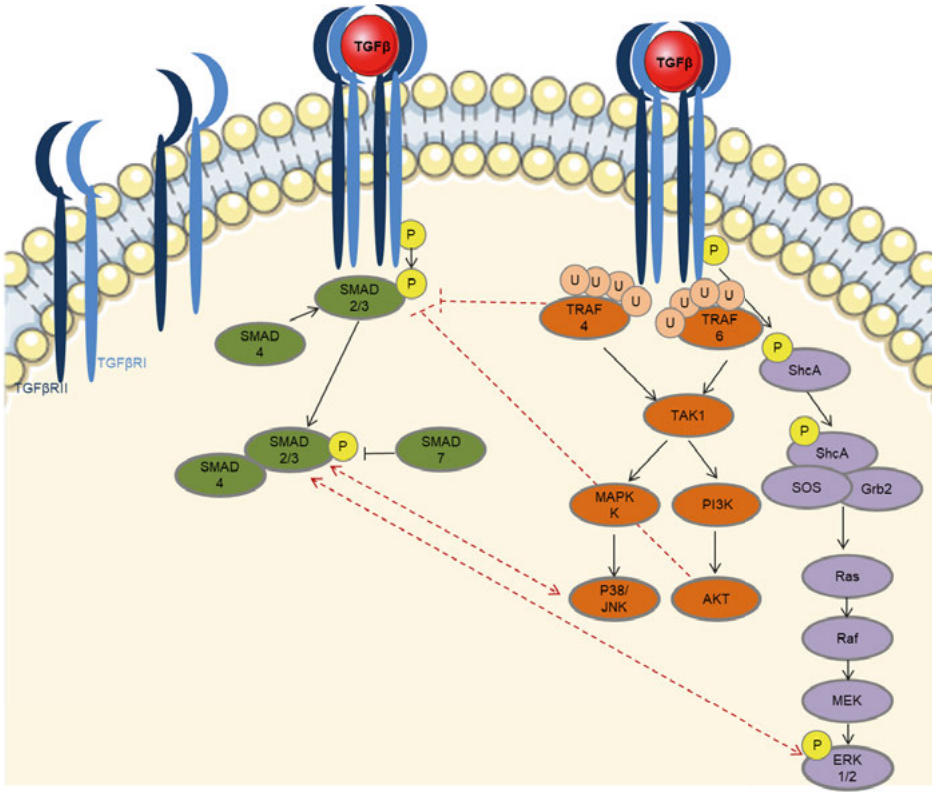

Figure 2 Schematic representation of the SMAD and non-SMAD TGFβ-activated pathways. The dotted red lines represent the possible cross-talk between the TGFβ-activated pathways.

Considering the wide range of TGFβ-regulated pathways associated with a wide spectrum of target genes, TGFβ plays a highly pleiotropic and complex role on cellular responses in a cell type-dependent manner.

2.3 TGFβ-mediated physiological responses

The TGFβ pathway regulates many cellular functions in physiological and pathological contexts. Under physiological conditions and during early stage of tumor development, TGFβ regulates cell cycle progression by inducing the cyclin-dependent kinase inhibitors (CKI) including p15, p16, p21 and p27, and causing growth arrest in the G1 phase of the cell cycle (Datto et al., 1995; Massague, 2008). The proteasomal pathway is also needed to degrade the CyclinD1 that promotes the G1-S transition (Zhang et al., 2002).

Apoptosis is an important process for embryo development, adult homeostasis and is very often dysregulated in cancer. TGFβ is a well-known inducer of apoptosis especially in hepatocytes and prostate epithelial cells. For instance, the SMAD complex is able to induce the pro-apoptotic members of the Bcl2 family BIM and Bmf (Ramjaun et al., 2007). BIM induction is regulated through SMAD3 that induces the MAPK phosphatase to attenuate ERK activation/phosphorylation and promote the accumulation of BIM (Ramesh et al., 2008). Another effector of TGFβ-dependent apoptosis induced by the Smad complex is the death-associated protein kinase (DAP-kinase) (Jang et al., 2002). In addition, downstream components of the apoptotic machinery such as Daxx (Perlman et al., 2001) or the apoptosis-related protein in the TGFβ-signaling (ARTS) (Larisch et al., 2000) positively modulate the TGFβ-induced apoptosis. The non-SMAD signaling cascade activated by TRAF6 also contributes to prostate cancer cell apoptosis (Hamidi et al., 2017).

TGFβ is a potent modulator of the differentiation of immune cells and a modulator of inflammatory responses. TGFβ inhibits the differentiation of effector T helper (T_h) cells also known as $CD4^+$ cells which are stimulated by antigens and release cytokines in order to recruit other immune cells. Mechanistically, TGFβ inhibits the expression of T-bet, the main regulator of T_h1 differentiation and interferon-γ (IFN-γ) activation (Park et al., 2007) via GATA-3, which is the main regulator of the differentiation of T_h2 helper cells. TGFβ inhibits FOXP3 that controls the differentiation of T cells (Gorelik et al., 2002). Due to its regulation of the cell cycle, TGFβ induces cell cycle arrest of B cells (which synthetize antibodies) and T cells (which recognize antigenic epitopes) by up-regulation of cell cycle inhibitors (p15, p21, p27) and therefore affect the performance of the acquired immune response (Wolfraim et al., 2004). On the contrary, TGFβ promotes the differentiation of another type of T helper cells, the T_h17 cells which target bacterial and fungal pathogens (David and Massague, 2018). Overall, the inhibitory effect of TGFβ may promote tumorigenesis by evasion of the immune surveillance. On the other hand, the suppressive effect of TGFβ on the expression of inflammatory cytokines such as IFN-γ and interleukins 2 (IL-2) in a SMAD-dependant manner (McKarns et al., 2004), suggests a protective role against tumor-promoting chronic inflammation.

TGFβ is a major inducer of epithelial-to-mesenchymal transition (EMT) (Xu et al., 2009). EMT is a biological process that allows an epithelial cell to undergo morphological and transcriptional changes that enable it to become a mesenchymal cell with increased migratory capacity and invasiveness (Xu et al., 2009). This process is needed in the context of embryo development, when undifferentiated cells spread through the embryo to form a new differentiated tissue at a specific place. In order to detach from the surrounding cells and migrate, cells need to rearrange their actin cytoskeleton into stress fibers, to break their cell-cell junctions and to reorganize the ECM. TGFβ represses the expression of epithelial genes such as E-cadherin and ZO-1 involved in cell-cell junctions, and simultaneously induces the expression of mesenchymal genes such as N-cadherin, fibronectin or vimentin involved in the cell-cell junctions, reorganization of ECM and reorganization of the cytoskeleton respectively. TGFβ signaling is directly responsible for the transcriptional induction of a panel of transcription factors (TFs) that provide the necessary stimuli to induce EMT (EMT-TFs), such as SNAI1, SNAI2, ZEB1, ZEB2, TWIST1 and TWIST2, many of which (SNAI1, ZEB1, ZEB2) associate with TGFβ signal transducers of the SMAD family and control their activity (Choi et al., 2007; Vervoort et al., 2013). Interestingly and in line with the importance of TGFβ during embryogenesis, the mesenchymal transition is also accompanied by a modification of the stemness characteristics of the cells. Cells undergoing EMT reset their epigenetic landscape to modify the expression of their differentiation genes regulated by specific EMT-TFs, especially TWIST1 or SNAI1, via the activation of a large number of genes leading to a complete reprogramming of the cells, including a gain of stem cell-like properties (Batlle et al., 2013; Schmidt et al., 2015; Tsirigoti et al., 2022). Although certain characteristics of the cells are permanently altered by the process of EMT (Schmidt et al., 2015; Tsubakihara et al., 2022), which can partially be explained by a stable epigenetic footprint (Bedi et al., 2014), the reverse process is called mesenchymal-to-epithelial transition (MET) and is associated with the reactivation of epithelial markers (Chao et al., 2010), a return in the cell cycle (Tsai et al., 2012) and the partial reactivation of differentiation genes (Li et al., 2017).

TGFβ can also remodel cell metabolism. The adaptation of metabolic substrates and enzymes is required for the cells to adjust their energy and metabolite production (Liu and Chen, 2022). Glucose metabolism is essential for cell survival. It has been shown that TGFβ increases the mRNA level of glucose transporter GLUT1 in mouse embryonic fibroblasts and mammary cancer cells (Kitagawa et al., 1991; Nilchian et al., 2020). Intracellular glucose is largely used to produce energy (ATP), mostly by oxidative phosphorylation. The intermediate product between glucose and the first metabolite able to enter the TCA cycle, the acetyl-coA, is pyruvate. The conversion of pyruvate to acetyl-coA is regulated by the enzyme pyruvate dehydrogenase, whose activity has been shown to be decreased by TGFβ in fibrotic hu-

man kidneys leading to a switch from oxidative phosphorylation to aerobic glycolysis that supports proliferation and protein synthesis (Smith and Hewitson, 2020). The process of EMT is accompanied with the reprogramming of lipid metabolism in order to increase fatty acid oxidation needed to sustain the increased mitochondrial oxidation of mesenchymal cells in hepatocellular carcinoma (Soukupova et al., 2021). In non-small cell lung cancers, the induction of EMT is correlated with the overexpression of the enzyme prolyl 4-hydroxylase α3 (P4HA3) by TGFβ, an enzyme that regulates the use of intracellular amino acids. P4HA3 converts proline to hydroxyproline, which contributes to the collagen accumulation needed for mesenchymal cells to invade (Nakasuka et al., 2021).

2.4 Aberrant TGFβ signaling in cancer

TGFβ plays a dual role in cancer development, from tumor suppressor during the initial stages of tumorigenesis to tumor promoter at later stages. The current model is that TGFβ loses its tumor suppressive role and gain tumor promoting abilities during tumorigenesis.

In the context of cancer, the process of EMT allows cancer cells to detach from the primary tumor and invade the surrounding tissues, leading to the colonisation of distant organs (Moustakas and Heldin, 2007). Once tumorigenesis has progressed, cancer cells also tend to acquire increasing resistance to the growth inhibitory response of TGFβ and start secreting abnormal levels of TGFβ, leading to tumor progression (Massague, 2008). Finally, although the pro-apoptotic effects of TGFβ are well-known, TGFβ is also described as anti-apoptotic in certain types of cancer, for instance in mouse breast cancer where TGFβ induces the anti-apoptotic long non-coding RNA lncRNA-SMAD7 (Arase et al., 2014).

In certain types of cancer, such as colorectal cancer or pancreatic cancer, TGFβ signaling is inactivated due to mutations on TGFβ receptors I and II (Bharathy et al., 2008) and SMADs (mostly SMAD4), events associated with an increased aggressiveness (Lin et al., 2019). On the contrary, TGFβ signaling is highly activated and promotes invasion and metastasis of many types of cancer, including prostate cancer (Vo et al., 2013).

In addition to the diverse mutations and inactivation of the TGFβ-signaling components, TGFβ cooperates with oncogenic pathways and facilitates the development of aggressive, less differentiated and invasive tumors. The combined action of epidermal growth factor (EGF) and TGFβ signaling is a classic example of oncogenic cooperation and context-dependence. Both EGF and TGFβ activate common signaling pathways (i.e RAS-MAPK or PI3K-AKT pathways), which in turn activate transcription factors such as Jun or Fos able to interact with the SMADs and trigger activation of a

TGFβ-induced SMAD-dependent breast cancer invasion program (Sundqvist et al., 2020).

3 Long non-coding RNAs

3.1 3.1 Non-coding RNAs: definition

RNAs are crucial molecules in cells. The central dogma of molecular biology is that RNAs are mediators of genetic information from DNA to protein. This is correct but does not fully describe the complexity of genetic systems. The protein-coding mRNAs represent only 2% of the total genome and encode for approximately 20,000 different proteins in human cells. The genes for non-coding RNAs, which represent the large majority of the total genome and excluding rRNA and tRNA genes, have been considered as junk DNA for many years. More recently, the development of high-throughput transcriptome analysis has revealed the importance and complexity of these molecules. The classification of the non-coding RNAs is arbitrarily based on their size: the small non-coding RNAs that include the microRNAs (about 20 nucleotides), the piRNAs (about 30 nucleotides), the circular RNAs (100 to 10,000 nucleotides) and the long non-coding RNAs that exceed 200 nucleotides (Hombach and Kretz, 2016).

3.2 3.2 Long non-coding RNAs: classification and fate

Long non-coding RNAs (lncRNAs) are expressed in bacteria, plant and animals and represent 16,000 to over 100,000 expressed genes in human cells (Harrow *et al.*, 2012; Iyer *et al.*, 2015). Similar to all mRNAs transcribed by RNA polymerase II, lncRNAs are capped by 7-methyl guanosine (m^7G) at their 5′ ends, spliced (often less efficiently than mRNAs), polyadenylated at their 3′ ends and sometimes carry small open reading frames but usually show limited protein coding potential (Chillon and Marcia, 2020). They carry their own promoter or share it with an adjacent protein-coding gene potential (Chillon and Marcia, 2020). In addition to their size which exceeds 200 nucleotides, lncRNAs are defined by their genomic location with respect to their neighbouring protein-coding genes. The antisense lnRNAs are transcribed from the opposite strand to that of the sense protein-coding gene and further subdivided according to their relative location to the protein coding gene: the 5’ end of the antisense lncRNAs can be close to the 5’ end of the sense mRNA, close to the 3’ tail of the mRNA or the 5’ or 3’ ends of the two RNAs can be partially or fully complementary. The intronic lncRNAs

are contained in the intronic sequences of protein-coding genes and do not overlap with exons. Divergent lncRNAs share a common promoter with a protein-coding gene but are transcribed in opposite direction. In the contrary, intergenic RNAs are located in between protein-coding genes and carry their own promoter. LncRNAs can function in *cis*, influencing the expression and/or chromatin state of nearby genes or in *trans*, regulating the expression of genes far away from where the lncRNA is transcribed (Vance and Ponting, 2014).

Once transcribed, the sequence of lncRNAs transcripts defines their localization in the cell. The nuclear localization of lncRNAs is usually determined either by a weak splicing leading to their temporal accumulation in the nucleus (Mele et al., 2017) or by the presence of nuclear retention element (NRE) which contains an U1 snRNA-binding site and C-rich motifs that recruits U1 snRNP, a small nuclear ribonucleoprotein able to associate with Polymerase II and resulting in the accumulation of lncRNAs on chromatin (Azam et al., 2019).

The ones that are not retained in the nucleus are spliced and exported to the cytoplasm according to the presence of the nuclear RNA export factor (NXF1) (Zuckerman et al., 2020). More rarely, nuclear-encoded lncRNAs can be translocated to the mitochondria via an unknown mechanism (Rackham et al., 2011) or loaded into extracellular vesicles by protein binding (Statello et al., 2018).

3.3 Long non-coding RNAs: molecular functions

Depending on their localization and their specific interactions with DNA, RNA and proteins, lncRNAs can modulate chromatin function, alter the stability and translation of cytoplasmic mRNAs and interfere with signaling pathways.

In the nucleus, *cis* or *trans* lncRNAs can regulate gene expression by direct interaction with the chromatin resulting in a conformation change of the chromatin. The RNA-DNA interaction is mediated by the formation of a DNA-RNA triplex and can induce both gene silencing and activation. The negative charge of RNA can neutralize the positively charged histone tails, leading to chromatin de-compaction and therefore gene expression (Dueva et al., 2019). RNA-mediated opening of chromatin therefore functions as a regulator of rapid switch of gene expression (Dueva et al., 2019). LncRNAs can also interact with enhancer RNAs and help for the recruitment of transcription factors that activate gene transcription (Postepska-Igielska et al., 2015). In addition to the triple-helix formation, lncRNAs can form R-loop structures with the chromatin, and the R-loop structure itself is recognized by specific transcription factors resulting in the activation or repression of the target gene (Boque-Sastre et al., 2015). Their protein-binding potential

allow them to interact with various transcription factors and act as a molecular scaffold (Holdt et al., 2013; Yap et al., 2010), molecular guide or decoy by sequestering chromatin modifiers from promoters of target genes (Jain et al., 2016). At the post-transcriptional level, lncRNAs can affect pre-mRNA splicing by interacting with splicing factors (Yin et al., 2012) or with the pre-mRNA itself (Yap et al., 2018).

In the cytoplasm, lncRNAs can modulate mRNA stability by pairing to complementary mRNA and subsequently recruiting proteins responsible for their degradation (Kretz et al., 2013). Another way for lncRNAs to affect cell function is to pair with and sponge miRNAs in order to reduce their availability to target mRNAs. Of note, the stoichiometry between the lncRNA and miRNA is critical in the lncRNA-mediated sponging mechanism, the effect on the target mRNA being noticeable only when the relative concentration of the miRNA is greatly inferior compared to the abundance of the competitive lncRNA (Salmena et al., 2011). Due to their ability to bind to protein, lncRNAs can positively or negatively affect protein translocation to the nucleus (Lee et al., 2016) or bind to proteins involved in the regulation of key signaling pathways (Xu et al., 2021) and therefore affect gene expression and biological responses.

3.4 Long non-coding RNAs in cancer

3.4.a Long non-coding RNAs: a potential therapeutic target

The abundance and diversity of non-coding RNAs makes them attractive therapeutic targets for cancer treatment and other diseases. Over the past decade, various RNA-based therapies have been developed and showed promising results in pre-clinical and clinical trials (Winkle et al., 2021). So far, most of the RNA-based therapies that are in development aim to mimic or inhibit miRNAs. Long non-coding RNAs are also being developed and tested, but the broader modes of action of long non-coding RNAs (transcriptional and post-translational regulation, protein interaction) compared with miRNAs require a deeper understanding of their biological functions. Current preclinical studies consist of the targeting of natural antisense transcripts that act as gene expression inhibitors in *cis*, two of them showing promising results; one in the modulation of the brain-derived neurotrophic factor (BDNF) (Modarresi et al., 2012; Padmakumar et al., 2021) involved in memory formation and the other one in the upregulation of the gene *SCN1A* which causes the brain disorder Dravet syndrome, when down-regulated (Hsiao et al., 2016).

Currently, 12 RNA-based therapeutics gained the FDA and/or the European Medicine Agency (EMA) approval. These 12 approved RNA-based therapies target the liver, muscle or nervous system via intravitreal or subcu-

taneous injection and rely on the use of siRNAs that cause gene down-regulation or antisense nucleotides (ASOs) that cause mRNA degradation, modify the pre-mRNA splicing or block protein translation (Winkle et al., 2021).

The advantage of using non-coding RNAs instead of chemical compounds is that RNAs are naturally occurring molecules in human cells and therefore cells have all the machineries needed for their processing. Another advantage is that non-coding miRNAs usually directly or indirectly target one pathway at multiple levels, thus giving a broader, yet specific, response. However, for now, most of the RNA-based clinical trials using siRNAs or ASOs are unsuccessful because of the lack of control of the drug delivery. RNA structures are very unstable, negatively charged and hydrophilic, making their diffusion through the lipid membrane very difficult (Akinc et al., 2008). The problem of the specificity of delivery to a certain cell type or organ as well as the lack of control in terms of quantity uptaken per cell, possibly causing off-target effects or overdosing, remain to be improved (Jin et al., 2015). Another challenge for RNA-based therapies is the immune response triggered by the detection of double- or single-stranded RNAs as a viral defence mechanism via the Toll-like receptor (TLRs) and the activation of various downstream immune responses (Alexopoulou et al., 2001). One way to limit the immunogenicity of naked RNAs is to neutralize their charge and therefore their interaction with proteins, including TLRs (Sledz et al., 2003). Other technologies are being developed and tested such as miRNA-mimics and anti-miRNAs but no lncRNA-based therapeutics have been approved for clinical trials yet.

3.4.b Long non-coding RNAs regulated by TGFβ

More and more long non-coding RNAs are described as effectors of TGFβ signaling in cancer. The vast majority of lncRNAs controlled by TGFβ regulate the epithelial-to-mesenchymal transition. The first lncRNA demonstrated to be positively regulated by TGFβ was the *lncRNA-ATB*. *LncRNA-ATB* overexpressed in lung cancer and associated with poor prognosis (Wei et al., 2018), upregulates the expression of miR-200c/ TWIST1 pathway by sponging *miR-200c*, a negative regulator of ZEB1/2, leading to an increased EMT in breast cancer (Li et al., 2018) and hepatocellular carcinoma (Yuan et al., 2014) cells.

Cell proliferation is also affected by lncRNAs regulated by TGFβ in cancer. The lncRNA *TUG1* is induced by TGFβ and increases cell proliferation capacities via the EMT pathway in pancreatic cancer by upregulating the expression of matrix metalloproteases (MMP) MMP2 and MMP9, two metalloproteases that contribute to cancer cell proliferation (Qin and Zhao, 2017). Another interesting example is the long non-coding RNA *EPR* that interacts with chromatin and attenuates cell proliferation by positively regu-

lating the cell cycle inhibitor p21/Cdkn1a, both transcriptionally and post-transcriptionally. *EPR* is downregulated by TGFβ/SMAD signaling, promoting cell proliferation (Rossi et al., 2019). The lncRNA *CTBP1-AS2* is up-regulated in colorectal cancer and activates the TGFβ/SMAD2/3 pathway by inhibiting its competitive miRNA miR-95-5p, which results in an inhibition of cell proliferation and invasion (Li et al., 2021).

The activation of TGFβ signaling itself is regulated by TGFβ-dependant lncRNAs by promoting (*lnc-LFAR1*) or inhibiting (*lnc-TSI*) the interaction between the SMADs and the TGFβ receptors (Wang et al., 2018; Zhang et al., 2017). Another way by which lncRNAs regulate TGFβ signaling is to modulate the power of the SMAD pathway. The lncRNA *NORAD* facilitates the nuclear transport of the SMAD complex in the nucleus by interacting with importin β1 (Kawasaki et al., 2018), the lncRNA *ELIT1* interacts with SMAD3 and acts as a co-factor by recruiting SMAD3 to the promoters of its target genes including *SNAI1* (Sakai et al., 2019) and the SMAD3-associated lncRNA *SMASR*, on the contrary, prevents SMAD3 phosphorylation and therefore the activation of its target genes (Xu et al., 2021). *SNHG6* reduces the expression of SMAD7, the inhibitor of the SMAD pathway (Wang et al., 2019). The expression of TGFβ1 or TGFβ2 is also directly affected by the lncRNA *PVT1* that up-regulates the transcription of TGFβ1 (Li et al., 2022; Zhang et al., 2018) and *CASC9* that stabilizes the mRNAs that encode for TGFβ1 or TGFβ2 (Luo et al., 2019).

In gastric cancer, the lncRNA *MACC-AS1* is induced by TGFβ secreted by mesenchymal stem cells. *MACC-AS1* antagonizes *miR-145-5p* that promotes fatty acid oxidation, leading to an increased tumor self-renewal and chemoresistance (Zhao et al., 2018).

3.4.c The long non-coding RNA *LINC00707*

The long non-coding RNA *LINC00707* is located on chromosome 10, is made of 3,087 nucleotides, contains 5 exons and expressed as a unique splice-variant (NCBI reference sequence: NR-038291.1, **Figure 3**).

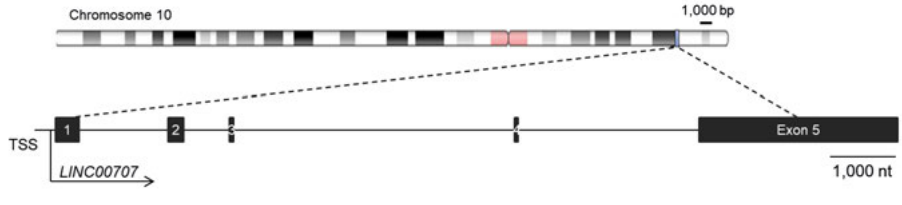

Figure 3. Genomic organization of the *LINC00707* gene on chromosome 10. Exons are shown as black boxes and introns as lines, the arrow indicates direction of transcription. TSS = transcription start site. Scale bar on the chromosome: 1,000 base pairs, scale bar on the *LINC00707* transcript: 1,000 nucleotides.

Various functions have been recently attributed to *LINC00707* in different normal and cancer cell models. *LINC00707* is upregulated and sponges miRNAs such as *miR-370-3p* in human bone marrow-derived mesenchymal stem cells to promote osteogenesis (Jia et al., 2019). In cancer, *LINC00707* sponges *miR- 485-5p*, an inhibitor of cell proliferation via its direct inhibition of O-GlcNAcylation which stabilizes the protein Bmi-1 to increase the proliferation of colorectal cancer cells (Wang et al., 2020), or *miR-382-5p* (Guo et al., 2021) and *miR-374c-5p* (Fang et al., 2022) to modulate the expression of their downstream target genes VEGFA and syndecan-4 (SDC4) respectively, leading to an increased proliferation and tumor growth of cervical cancer. *LINC00707* interacts with HuR, an RNA-binding proteins involved in the stabilization or degradation of mRNAs. The interaction between *LINC00707* and HuR reinforce the stabilization of the mRNAs VAV3 and F11R, encoding for proteins respectively implicated in cell adhesion and in the formation of tight junctions. The stabilization of these two mRNA promotes proliferation and metastasis in gastric cancer (Xie et al., 2019). In normal tissue, *LINC00707* is highly expressed in placenta and bladder but its functions in these organs remain unknown (Fagerberg et al., 2014).

4 Cell polarity and cancer aggressiveness

Cell polarity refers to the spatial organization of cells or of cell components either in shape or architecture. The apico-basal polarity of epithelial cells refers to the secretory, apical phase of the cells that face a lumen, and the basal side of the cell that interacts with the ECM, basement membrane. The apico-basal polarity is a result of cell-cell interaction and cell-ECM interactions and is tightly regulated in order to regulate their asymmetric division and directed cell migration (Rodriguez-Boulan and Macara, 2014). The development and maintenance of cell polarity is a result of multiple signals from polarity proteins, epithelial cadherin (E-cadherin) and focal adhesion contacts with the ECM. These signals organize the cytoskeleton and organelle localization and loss of these signals is associated with the transition from epithelial to mesenchymal phenotypes that develop front-to-back polarization (Aigner et al., 2007; Whiteman et al., 2008). Such invasive cells exhibit rather a front-rear polarity which directs their migration toward certain stimuli or chemoattractants.

Despite the different morphological organization, brain cells also need to be polarized in order to communicate via neurotransmitters, migrate or direct the neurite and dendrite projections (Sakakibara and Hatanaka, 2015). The polarization of the different cell types is controlled by different protein complexes, some of them being restricted to epithelial cells and others being involved in different cell types.

4.1 Par3/Par6/PKC complex

The apico-basal polarity of epithelial cells is defined by the asymmetric distribution of the evolutionary conserved complexes Crumbs/PALS1/PATJ (Crumbs complex) restricted to epithelial cells, the Scribble/lethal giant larvae (Lgl)/ Disc large (DLG) (Scribble complex) and the Par3/Par6/PKC complex (Par complex). The Crumbs and Par complexes are localized in the apical zone, near the tight junctions of the cells (Chen and Zhang, 2013; Tan et al., 2020) whereas the Scribble complex is localized on the basolateral side of the cells (Troyanovsky et al., 2021). Mechanistically, there is a feedback loop between the three polarity complexes in order to maintain the epithelial cell polarity: the protein Crumbs recruits PALS1 which recruits Par6

to mediate the phosphorylation of Par3 through atypical protein kinase C (aPKC) and the phosphorylation of LGL via the activation of small GTPases of the Rho family (Betschinger et al., 2003; Horikoshi et al., 2009).

The formation of the Par3/Par6 complex, the direct interaction between Par3 and aPKC, as well as the aPKC kinase activity are required for the apical domain formation. The Par complex participates in the formation of cell-cell tight junctions through the direct interaction of Par3 with the junctional adhesion molecules (JAMs) and small GTPases of the Rho family such as Cdc42 (Joberty et al., 2000); however, the formation of tight junctions does not entirely depend on the polarity complex. Loss of epidermal Par3 leads to disturbed skin barrier, altered expression and localization of tight junctions and increased thickness of the epidermis (Ali et al., 2016). In neurons, the aPKC of the Par complex is activated by the Wnt pathway which in turn inhibits GSK3β, MAPK2, and activate LKB1 and TIAM1 and leads to neuronal polarization (Hapak et al., 2018).

4.2 Cell polarity disruption in cancer

Tight junctions, asymmetric division and apico-basal polarity are very often altered in cancer. A genome-wide screen of polarity complex genes in various types of cancer has revealed that Par3 is commonly deleted in carcinomas and in glioblastomas, events associated with poor prognosis. The consequence of this deletion is an increased cancer cell aggressiveness due to cell polarity disruption, which is restored by Par3 rescue (Rothenberg et al., 2010). Loss of cell polarity proteins leads to the impairment of cell-cell junctions, which is one of the first steps of EMT prior to metastatic progress (Xue et al., 2013). Par3 silencing promotes tumorigenesis through induction of matrix metalloproteases, destruction of ECM, all mediated by an inappropriate aPKC-dependant JAK/STAT3 activation (McCaffrey et al., 2012). The Par3 complex also modulates EMT by regulating EMT markers. In normal conditions, one of the roles of aPKC is to degrade SNAI1, thus inhibiting EMT. In breast cancer, loss of apical–basal polarity prevents aPKC-mediated SNAI1 phosphorylation and stabilizes the SNAI1 protein to promote EMT and invasion (Jung et al., 2019).

5 Reactive oxygen species

5.1 Source and production

Cell metabolism involves mitochondrial activity to produce the energy needed for the cells to proliferate and survive. The mitochondrial respiratory chain consists of five multi-subunit protein complexes located in the mitochondrial intermembrane space (respiratory complexes I-V) and two factors (the cytochrome c and coenzyme Q10) (Rich and Marechal, 2010). The role of the mitochondrial respiratory chain is to produce adenosine triphosphate (ATP), a usable form of energy by the cells, by oxidation of glucose and other sugars. During this process, electrons are transferred from NADH, an intermediate of the TCA cycle, to oxygen by respiratory complexes in the inner mitochondrial membrane which create a transmembrane electrochemical gradient. First, the NADH produced by the TCA cycle is converted to NAD^+ by the complex I, this reaction releases two electrons. The complex II catalyses the conversion of succinate into fumarate followed by an oxidation of $FADH_2$ into FAD, which releases two more electrons. The four electrons produced by complex I and II are transferred to the coenzyme ubiquitone (Q) which is reduced to ubiquitol (QH_2), which in turn oxidizes the complex III by the transfer of two electrons. Complex III gives two electrons to cytochrome C which passes them to complex IV. Complex IV uses these electrons to catalyse the reduction of molecular oxygen (O_2) into water (H_2O). Each reaction (except the reaction of complex II) is coupled with proton translocation to the cytoplasm to maintain the charge difference necessary for the electron transportation. The electron transport chain and the movement of protons creates an electrochemical proton gradient known as mitochondrial membrane potential. Mitochondria dissipate the membrane potential by the re-entry of protons inside the mitochondria via complex V, which is coupled to the production of ATP by ADP.

During this process, it happens that NADH and/or oxygen are incompletely reduced and give rise to the superoxide radical $^{\bullet}O_2^-$ produced by the one-electron reduction of O_2 (**Figure 4**). Oxygen can also be reduced by two electrons (peroxide $^{\bullet}O_2^{-2}$), associate with hydrogen (hydroxyl radical $^{\bullet}OH$ and hydroxyl ion OH^-) or two hydroxyl ions can associate to form hydrogen peroxide H_2O_2. All these reactive molecules and free radicals derived from oxygen are called Reactive Oxygen Species (ROS) (Mazat et al., 2020). Mistakes in the mitochondrial chain resulting in ROS production are more likely

to happen in highly metabolically active cells, when mitochondria are overwhelmed by too high energetic demands. Endogenous ROS production involves not only the mitochondria, but also several enzymatic reactions including NADPH oxidases, xanthine oxidase, uncoupled endothelial nitric oxide synthase, arachidonic acid and its metabolic enzymes like lipoxygenase and cyclooxygenase (Gorrini et al., 2013).

ROS are unstable and easily transfer their extra electron, catalysing various reactions in cells (Zorov et al., 2014). To prevent the damage from ROS, cells possess several antioxidant enzymes such as superoxide dismutases (SOD) MnSOD and Cu/ZnSOD, which are located in the mitochondria and the cytosol, respectively, where they convert superoxide into hydrogen peroxide (Sheng et al., 2014). Hydrogen peroxide is then converted into water by the enzyme glutathione peroxidase (GPx) that is active both in the mitochondria and in the cytoplasm (**Figure 4**).

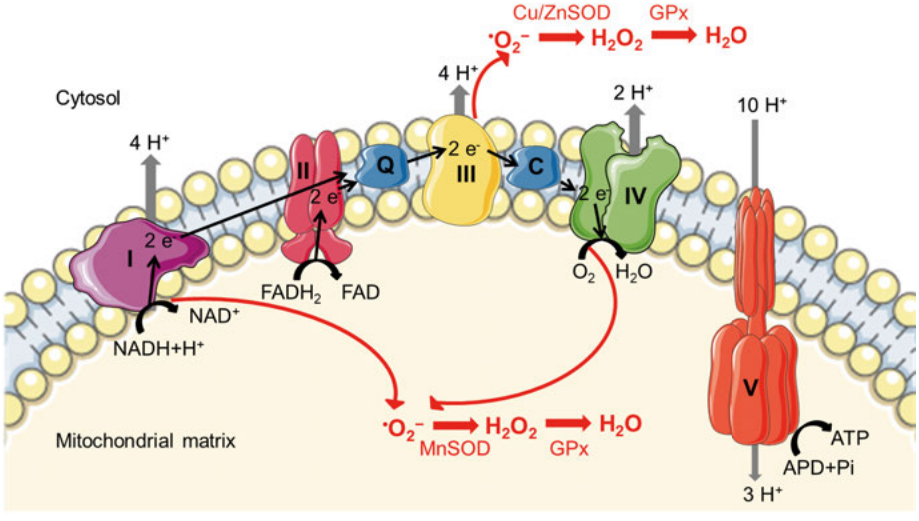

Figure 4 Schematic representation of mitochondrial ROS production by the electron transport chain and antioxidant enzymes.

The five complexes of the electron transport chain are indicated in roman numbers and the co-factors cytochrome C and coenzyme Q10 are indicated with the letters C and Q respectively. Grey arrows represent the transport of H^+ and straight black arrows represent the electron transport. Red arrows represent the generation of the superoxide radical $^{\bullet}O_2^-$ and its neutralization by the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx).

5.2 Reactive oxygen species in cancer

ROS have cellular signaling capabilities. Cancer cells contain elevated quantity of ROS due to their high metabolic and proliferation rate and ROS have been associated with various cell responses, both pro-tumorigenic and anti-tumorigenic.

On one hand, high level of ROS can cause oxidation of lipids and modify the permeability of cell membranes (Wong-Ekkabut et al., 2007), affect protein activity via the modification of cysteine residues within proteins (van der Reest et al., 2018), or DNA structure by inducing single- or double-stranded breakage and DNA damage response, base modifications, deoxyribose modification and DNA cross-linking (Fan et al., 2019). ROS are also able to directly modulate signaling pathways, for instance the hyperactivation of the mammalian target of rapamycin (mTOR) via the PI3K/AKT survival pathway by oxidizing and inactivating the phosphatases PTEN and PTP1B, negative regulators of PI3K/Akt signaling (Leslie et al., 2003). ROS also activate the MAPK pathways, inducing growth factor receptor activation and MAPK/ERK pro-proliferative signaling (Hashmi et al., 2018; Wang et al., 2011). Finally, ROS also promote tumor angiogenesis and metastasis by oxidation of prolyl hydroxylase domain protein 2 (PHD2) which leads to the oncogenic stabilization of HIF-1α protein during hypoxia (Jung et al., 2008; Park et al., 2010). In order to promote metastasis, and in addition to the previous examples (e.g., the MAPK and PI3K/AKT pathways), ROS regulate additional transcriptional activities (e.g., SNAI1) to enhance cancer cell migration and invasion (Alexandrova et al., 2006; Barnett et al., 2011; Basuroy et al., 2010).

On the other hand, ROS can promote anti-tumorigenic signaling. An excess of ROS in the cells promotes cell death through the intermediate of the apoptosis signal-regulating kinase 1 (ASK1) via activation of the ASK1/JNK and ASK1/p38 signaling pathways (Goldman et al., 2004). ROS- mediated oxidation of thioredoxin (TRX, a small redox protein) causes ASK1 activation (the TRX partner), thereby triggering the suppression of anti-apoptotic factors through the activation of the downstream MAPK pathway (Cheng et al., 2014; Madan et al., 2013). ROS-mediated activation of the JNK and p38 signaling pathways can also induce cell cycle arrest, preventing cancer cell growth and proliferation (Xie et al., 2016; Zhang et al., 2019).

6 Cancer metabolism

Cell metabolism is the conversion of available nutrients to usable energy for living cells. Metabolic reactions are categorized as catabolic, which is the break-down of compounds and usually associated with a release of energy, or anabolic, which is the build-up of compounds such as proteins, lipids or nucleic acids and usually consumes energy. One of the main nutrients that regulate cell metabolism is glutamine, an amino acid that initiates multiple cell processes.

6.1 Glutamine metabolism

Glutamine is an abundant amino acid available in the bloodstream or synthesized by the cells by catabolism of other amino acids. Glutamine is then catabolized via a process known as glutaminolysis after transport into the mitochondria and conversion into glutamate by glutaminases (GLS) (Yoo et al., 2020). Mitochondrial glutamate is then converted into α-ketoglutarate and free ammonia by glutamate dehydrogenase and feeds into the tricarboxylic acid (TCA) cycle in order to produce ATP (Yoo et al., 2020). Moreover, glutamine is used to synthesize other non-essential amino acids, glutathione or purine- and pyrimidine-based nucleotides (Yoo et al., 2020).

In addition to the use of glutamine to produce energy and new cell intermediates, glutamine can regulate post-translational modifications on tubulin, a major component of microtubules that controls structure and shape to all eukaryotic cells (Roll-Mecak, 2020). Microtubules are involved in the regulation of multiple processes such as proliferation, migration or intracellular cargo transport. In order to accommodate all these functions, microtubules associate with specific microtubule-associated proteins (MAPs) recruited by specific and dynamic post-translational modifications carried by tubulin proteins (Roll-Mecak, 2020). The reversible glutamylation on microtubules consists in the addition of a chain of glutamate on the C-terminal tails of tubulin and is controlled by evolutionarily conserved enzymes. The enzyme tubulin tyrosine ligase (TTL) together with its co-factor cilia and spindle-associated protein (CSAP) function as tubulin glutamate ligases, which add multiple glutamates to specific substrate proteins via a tyrosine (Bompard et al., 2018). Conversely, side chain glutamates are removed by tubulin deglu-

tamylases of the cytoplasmic carboxypeptidase (CCP) family (Rogowski et al., 2010).

When glutamate is not used by the cells to regulate post-translational modifications, α-ketaglutarate generated from glutaminolysis can be used as an energy donor via the TCA cycle, a precursor for amino acid biosynthesis or a regulator of epigenetic processes via protein modification and binding of other proteins. Indeed, α-ketaglutarate acts as a cofactor for the enzyme Jumonji-C that contains a histone demethylase domain (Tsukada et al., 2006). The activation of the Jumonji-C enzyme therefore triggers histone demethylation, and in parallel balances the reaction via the release of succinate and formaldehyde that antagonize the activity of the histone demethylase. Histone lysine demethylation can signal either gene expression activation or repression depending on the particular lysine residue that is methylated. Glutamine, a precursor of α-ketoglutarate, is therefore indirectly implicated in the regulation of epigenetic regulation of the chromatin (Pan et al., 2016).

6.2 Glutamine metabolism in cancer cells

6.2.a Metabolic adaptation

Tumor initiation and progression require the metabolic reprogramming of cancer cells in order to meet their increased bioenergetics, biosynthetic and redox demand. The metabolic reprogramming of cancer cells is today admitted as a hallmark of cancer (Hanahan and Weinberg, 2011). *In vivo*, important nutrients such as glucose and glutamine are available in the bloodstream and supplied by catabolism of carbohydrates and proteins respectively consumed via diet. In the context of solid tumors, accessibility of a given tumor cell to nutrients is modulated by its proximity to the vasculature: cells located in the periphery of the tumor and therefore adjacent to the vasculature have access to nutrients whereas cells in the core of the tumor have diminished access to nutrients and oxygen (Boroughs and DeBerardinis, 2015). In terms of metabolic adaptation, nutrient-deprived cells first decrease their demand for ATP in order to maintain an adequate ATP/ADP ratio (Gameiro and Struhl, 2018) or convert two molecules of ADP, the precursor of ATP, into one molecule each of AMP and ATP (Klepinin et al., 2020). In addition to a lower metabolic activity, nutrient-deprived cells can decrease their mTOR kinase activity in order to increase autophagy and catabolize existing proteins, thus providing an intracellular glutamine supply to sustain mitochondrial function (Duran et al., 2012). Nutrient deprivation-induced autophagy is triggered by a limited number of amino acids: glutamine, arginine, methionine and lysine. The lack of these amino acids is sensed by the cells and induces AKT activation by a GCN2/ATF4/REDD1 signaling pathway that activates mTORC2 (Jin et al., 2021). As a mechanism that sustains

cancer cell malignancy, TGFβ contributes to the induction of glutamine catabolism via up-regulation of the transporter solute carrier family 1 member 5 (SLC1A5), responsible for the transport of glutamine inside the cell and later inside mitochondria, as well as the up-regulation of the enzyme GLS1 that converts glutamine into glutamate to enhance α-ketoglutarate entry to the TCA cycle (Soukupova et al., 2017). Finally, certain Ras-transformed cancer cells counteract the lack of glutamine by the degradation of unsaturated fatty acids to support ATP production (Kamphorst et al., 2013).

6.2.b Glutamine deprivation on cancer aggressiveness

Regional glutamine deficiency in tumors has been studied by transient glutamine deprivation. It was reported that glutamine deprivation affects cancer-associated fibroblast (CAFs) motility, which are able to activate the signaling complex of AKT2/TRAF6/p62 that controls polarization, in order to direct CAF mobility toward glutamine (Mestre-Farrera et al., 2021). In cervical carcinoma HeLa and in breast cancer cells, extracellular matrix stiffness increases microtubule glutamylation (regulated by glutamine concentration) leading to increased cell invasion (Torrino et al., 2021). Glutamine metabolism also affects cell responses to TGFβ, the regulator of cell invasion via the process of EMT, as explained above. It was shown in pancreatic ductal cancer that glutamine depletion increases the level of SNAI2/Slug, the TGFβ-induced EMT-TF, which in turn induces the process of EMT via MEK/ERK signaling and ATF4 (Recouvreux et al., 2020). In ovarian cancer, however, glutamine depletion reduces the invasive capacity of the cells via the down-regulation of MMPs, mediated by the transcriptional regulator of MMP gene expression and key regulator of EMT in ovarian cancer, ETS1. The strategy to impair ETS1-induced gene expression varies in different cell lines, either via its down-regulation at the gene level or via its reduced translocation to the nucleus (Prasad and Roy, 2021) .

In addition to the modulation of cancer cell invasion via the regulation of polarity complexes, microtubule glutamylation and expression of epithelial markers, glutamine deprivation has been shown to induce epithelial differentiation of breast cancer stem cells into epithelial subtypes by a reduced nuclear localization of β-catenin. Interestingly, basal and luminal breast cancer cells respond differentially towards changes in extracellular and intracellular glutamine, basal cells with mesenchymal phenotype being more dependent on extracellular glutamine supply than luminal cells (Jariyal et al., 2021). Another surprising effect of glutamine deprivation is the regulation of immune escape by tumors. Renal carcinoma cells deprived in glutamine exhibit higher level of programmed death-ligand 1 (PD-L1) induced by the EGFR/ERK/c-Jun pathway which results in a decreased immune response associated with poor prognosis (Ma et al., 2020). Finally, and in agreement with the statement that glutamine controls the energy production of cancer

cells needed to sustain cell viability, the lack of glutamine induces apoptosis in glial and glioblastoma cell lines via the assembly of GLS1, the enzyme that converts glutamine to glutamate, into filaments. The filament organization of GLS1 is triggered by the lack of glutamate and leads to a decrease of asparagine synthesis that impairs the mitochondrial activity, causing ROS-induced apoptosis (Jiang et al., 2022).

7 Present investigation

The aim of this thesis was to identify new actors of TGFβ signaling, from non-coding RNAs to regulators of metabolism (papers I-III). These studies were conducted in a large panel of cell lines, tumoral or immortalized, listed in the following table. A particular emphasis was given to glioblastoma (paper III) that focuses on the effect of cell polarity complex disruption in invasion and stem-cell renewal.

Cell line	Organ	Cell type	Mutations	Gender	Reference
PC3U	Prostate	Cancer cell line - Epithelial	TP53, PTEN	Male	(Carroll et al., 1993; Fraser et al., 2012)
HaCaT	Skin	Immortalized cell line – Epithelial	TP53	Male	(Lehman et al., 1993)
U2987MG	Brain	Astrocytoma cell line	Unknown	Male	(Savary et al., 2013)
U3031MG	Brain	GBM cell line, Mesenchymal subtype	Loss of chromosome 10, PTEN	Female	(Dadras et al., 2021)
U3034MG	Brain	GBM cell line, Mesenchymal subtype	Loss of chromosome 10, PTEN	Male	(Dadras et al., 2021)
U3005MG	Brain	GBM cell line, Proneural subtype	Loss of chromosome 10, PTEN	Male	(Dadras et al., 2021)
HLF	Liver	Cancer cell line – Mesenchymal subtype	TERT, TP53	Male	(Hsu et al., 1993; Qiu et al., 2019)

SNU-499	Liver	Cancer cell line - HBV induced	ARID1A, AXIN1A, TP53	Male	(Kang et al., 1996; Qiu et al., 2019)
Hep3B	Liver	Cancer cell line – Epithelial – HBV induced	AXIN1, RB1	Male	(Yu et al., 2019)

7.1 PAPER I: TGFβ-induced down-regulation of *LINC00707* promotes signaling and migration

Background: TGFβ is a cytokine involved in various cellular processes in the adult organism and during embryonic development. In the context of cancer, TGFβ modulates and mediates the development of EMT, correlated with invasive growth and metastasis in different types of tumors. In addition, several non-coding RNAs have been reported to be regulated by TGFβ and thus contribute to EMT.

Summary: In this study, we describe the long intergenic non-coding RNA *00707* (*LINC00707*) as a TGFβ-responsive gene. By combining transcriptomic data from human keratinocytes and glioblastoma cells, we found that TGFβ signaling down-regulates the expression of *LINC00707*. These findings have been extended to additional cancer cell types (prostate, lung and breast). *LINC00707* is downregulated via the transcription factor Krüppel Like Factor 6 (KLF6) as a regulator of *LINC00707*. *In situ* hybridization, cell fragmentation and RNAscope experiments have shown that *LINC00707* resides less in the nucleus and more into the cytoplasm where it forms distinct and large clusters. In keratinocytes, RNA sequencing data and gene ontology analysis revealed that stimulation by TGFβ or silencing of *LINC00707* up-regulate the expression of genes involved in extracellular matrix organization and cell-substrate adhesion. In agreement with the gene ontology analysis, loss or gain-of-function experiments with *LINC00707* revealed enhanced or reduced invasiveness of cancer cells, respectively. Mechanistically, *LINC00707* interacts with and retains the SMAD complex in the cytoplasm. Upon TGFβ stimulation, *LINC00707* down-regulation releases the SMAD proteins and allows their accumulation in the cytoplasm and therefore the regulation of TGFβ target genes responsible for the modulation of the invasive capacities of cancer cells. These data provide a new component of TGFβ-mediated cancer cell invasion, implicating *LINC00707* as a negative regulator of this process.

7.2 PAPER II: Glutamine deprivation alters TGFβ signaling in hepatocellular carcinoma

Background: Tumor cells rely on glutamine to fulfill their metabolic demands and sustain their proliferation. The elevated consumption of glutamine and localization of the cells within the tumor can lead to intratumoral nutrient depletion, causing metabolic stress that potentially impacts tumor progression. Glutamine deprivation affects cancer cell aggressiveness via its effect on stemness, migration, on the regulation of the redox state or on cell proliferation. However, the adaptation to long term glutamine starvation and the differential response to glutamine starvation between mesenchymal or epithelial hepatocellular carcinoma cells, including the association with high or low TGFβ signaling remain unknown.

Summary: Our study shows that epithelial and mesenchymal hepatocellular carcinoma can adapt to long term starvation without undergoing senescence or apoptosis. However, glutamine metabolism is needed to maintain a certain proliferative rate, clonogenic and self-renewal capacities, the decrease of these features being associated with a decrease of ROS production. A large panel of genes is differentially expressed in glutamine-deprived cells, most of the up-regulated genes being related to cell differentiation and most of the down-regulated genes being related to extracellular matrix reorganization and cell adhesion. Consistently, the glutamine-deprived cells showed a weak response to TGFβ stimulation, associated with a decrease of cell adhesion and invasive capacities. More interestingly, glutamine metabolism seems to be needed to maintain a mesenchymal phenotype with invasive capacities. Indeed, the absence of glutamine in the HCC culture medium leads to a reinforcement of the epithelial phenotype or even a switch from the mesenchymal to the epithelial phenotype, associated with a dramatic decrease of mesenchymal gene expression and a loss of invasive capacity of the cells. We therefore conclude that glutamine deprivation pushes the cells to become more epithelial, this effect being logically more obvious in mesenchymal cells.

7.3 PAPER III: Par3 promotes glioblastoma stem cell self-renewal while inhibiting cell invasion

Background: This paper emphasizes the fact that brain malignancy is characterized by invasiveness to the surrounding tissue and by the presence of stem-like cells known as glioblastoma stem cells (GSCs). Whether mechanisms that generate and maintain the GSCs are also responsible for the invasiveness of GBM is unclear.

Summary: The protein Par3 is a cytoplasmic protein and member of the polarity complex Par3/Par6/aPKC. Furthermore, Par3 is commonly deleted in carcinomas and in glioblastomas, which is associated with poor prognosis. In this paper, we study the role of the Par3 protein (encoded by *PARD3*) in patient-derived GSCs. These cells were depleted from Par3 which resulted in a decreased neurosphere forming capacity and enhanced invasiveness. This phenotype was corroborated by genome-wide transcriptomic analysis, revealing gene profiles associated with low stemness and high invasiveness in GSCs with suppressed Par3 expression. The genome-wide transcriptomic analysis after Par3 silencing also revealed a low expression of mitochondrial enzymes that generate ATP. Accordingly, silencing Par3 reduced ATP production and concomitantly increased reactive oxygen species. To investigate whether Par3 exerts its effect on invasion and stemness alone or together with the Par3/Par6/aPKC complex, and the importance of mitochondrial ROS on invasiveness and stemness, invasion assays and sphere assays were performed in different GBM cell lines (control or Par3 knock down) treated or not with specific chemicals: a PKC inhibitor, MitoPQ that blocks the production of mitochondrial ROS or MitoTempo, which increases the production of mitochondrial ROS. The present findings support the notion that Par3 exerts homeostatic redox control, which could limit the tumor cell-derived pool of oxygen radicals. These functions of Par3 go beyond its contribution to junctional contacts between cells and establish roles in controlling invasive escape and tumorigenic survival in non-epithelial tumors such as GBM. In this paper we demonstrate that Par3 is as a regulator of glioblastoma invasiveness and stemness via the regulation of mitochondrial ROS production.

8 Future perspectives

8.1 Paper I: TGFβ-induced down-regulation of *LINC00707* promotes signaling and migration

We performed a whole-genome transcriptomic analysis to identify lncRNA genes regulated by TGFβ. We found that TGFβ signaling downregulates *LINC00707*. Furthermore, we observed that LINC00707 suppressed cancer cell invasion, as well as pro-mesenchymal responses to TGFβ by suppressing SMAD-dependent signaling. This is achieved by the association of *LINC00707* with SMAD proteins that retain them in the cytoplasm, necessitating *LINC00707* elimination in order to allow SMAD translocation into the nucleus. We completed this study by investigating *LINC00707* expression *in vivo* and observed that there was negative correlation with SMAD2 phosphorylation in tumor tissues. We conclude that TGFβ signaling decreases *LINC00707* expression and induces cancer cell invasion via SMAD signaling.

Although significant progress has been made in the understanding of TGFβ signaling by *LINC00707*, there are several questions that remain open. First, we show that TGFβ regulates *LINC00707* in an indirect manner, via the intermediate action of the transcription factor KLF6. KLF6 binds to the *LINC00707* promoter in order to maintain gene expression and is dissociated from the promoter by TGFβ signaling, which induces the *LINC00707* repression. It is known that KLF6 receives phosphorylation from the TGFβ-induced MAP kinases (Dionyssiou et al., 2013), which could explain its dissociation from the *LINC00707* promoter upon TGFβ stimulation. However, chromatin immunoprecipitation using a MEK inhibitor did not show any effect of the binding of KLF6 on the *LINC00707* promoter (data not shown), failing to support this hypothesis. Another possibility would be that TGFβ implicates another transcription factor that actively represses the *LINC00707* expression. This potential transcription factor possibly binds to the *LINC00707* promoter nearby the KLF6 binding site, resulting in the dislocation of KLF6. Mechanistically, we show that *LINC00707* associates with the SMAD complex in order to sequester it in the cytoplasm. The mechanism by which *LINC00707* dissociates from the SMAD complex at an early timepoint of TGFβ signaling remains unclear. One possibility would be that the TGFβ-induced phosphorylation of SMAD2 and SMAD3 changes their con-

formation and therefore release *LINC00707* from their bound complex. Another possibility would be that TGFβ stimulation increases the expression of another *LINC00707*-binding protein with higher affinity in order to relocate *LINC00707* and release the SMAD complex. This hypothesis could be tested by mass spectrometric analysis followed by RNA immunoprecipitation (RIP) and chromatin-RNA immunoprecipitation (chiRP). Moreover, a complete screen of the *LINC00707*-binding proteins would identify new *LINC00707* partners possibly involved in biological processes other than TGFβ signaling and cell invasion. For instance, transcriptomic analysis showed that *LINC00707* positively regulates the expression of interferon-γ-related genes, suggesting an implication of *LINC00707* in immune responses. It would be of interest to validate and understand this observation. Finally, we showed *in vivo* that *LINC00707* expression is negatively correlated with the translocation of SMAD2 to the nucleus. The technic we have used does not allow us to determine whether *LINC00707* expression and its effect on SMAD2 translocation are specific to a certain cell type within the tumor. Finally, it would be interesting to know whether this effect of *LINC00707* on TGFβ signaling and cell invasion is a general mechanism observed in every type of tumor. If so, *LINC00707* expression might be used as a prognostic tool for tumor invasion.

8.2 PAPER II: Glutamine deprivation alters TGFβ signaling in hepatocellular carcinoma

We studied the effect of long-term glutamine deprivation, that mimics pathophysiological processes occuring in tumor cells located in the core of solid tumors, by creating resistant cell lines to glutamine deprivation. Using a hepatocellular carcinoma model, we performed transcriptomic analysis to compare gene expression in cells repleted with or deprived of glutamine. We found a number of up-regulated genes involved in cell differentiation and down-regulated genes involved in cell adhesion and matrix reorganization. TGFβ being a master regulator of these biological processes, led us hypothesize that TGFβ signaling might be affected by the lack of glutamine. Indeed, transcriptomic analysis showed similar affected pathways as in the absence of TGFβ and a decreased number of genes affected by TGFβ in the absence of glutamine compared with the control, suggesting that TGFβ signaling requires glutamine metabolism to be fully efficient. Using 2D and 3D culture experiments and consistently with transcriptomic analysis, the lack of glutamine leads to a decrease of sphere formation capacity, cell adhesion and invasion, which can be explained by a decrease of cell adhesion-related genes as well as a decrease of mesenchymal markers, especially in mesenchymal cell lines.

The implication of glutamine metabolism in TGFβ responses reveals a new aspect of TGFβ biology but still needs further investigations. It is reported that TGFβ affects glucose and glutamine metabolism but the mechanism by which glutamine metabolism directly affects the activation of TGFβ-activated pathways is established here, but the mechanism remains unknown. One possible intermediate between glutamine metabolism and TGFβ signaling could be the mTOR kinase, which is regulated by the concentration of nutrients and is required for TGFβ signaling. Second, we need to understand the reason why mesenchymal cells show a greater dependency on glutamine than epithelial cells. Indeed, the lack of glutamine strongly decreased the cell invasion capacity and the stemness frequency of mesenchymal cells. Consistently with these observations, mesenchymal cells are mostly found on the periphery of the tumor *in vivo*, therefore receiving adequate levels of glutamine and thus becoming able to invade surrounding tissues. We aim to extend these findings in two more aggressive types of tumor, the pancreatic ductal adenocarcnima (PDAC) and glioblastoma. Preliminary data also show a decrease of stemness frequency after glutamine deprivation in PDAC and GBM, accompanied with a decrease of the expression of the mesenchymal gene SNAI1 in PDAC (not yet tested in GBM). Finally, another aspect of glutamine metabolism is its indirect involvement in the regulation of epigenetic changes via its regulation of histone methylation. It would be of interest to study the possible epigenetic changes regulated by the lack of glutamine and their implication in cancer aggressiveness, and even the TGFβ- induced epigenetic changes and how they could be altered by the lack or presence of glutamine.

8.3 PAPER III: Par3 promotes glioblastoma stem cell self-renewal while inhibiting cell invasion

In this paper, we explored the role of polarity machinery in glioblastoma aggressiveness and unravelled new roles of the polarity protein Par3, often completely or partially lost in glioblastoma patients and associated with poor prognosis. Beyond its role on cell polarity, we describe Par3 as a regulator of glioblastoma invasion and stem-cell renewal. Although Par3 does not directly regulate gene expression, transcriptomic analysis showed that loss of Par3, concomitantly with experiments in cell culture, contributes to down-regulation of self-renewal inducing factors and stem cell transcription factors (SOX2, NESTIN). On the other hand, Par3 silencing increases cell invasion. We explained the effect of Par3 of stem-cell renewal and invasiveness by the alteration of mitochondrial enzymes resulting in an increase of reactive oxygen species production. The increase of ROS production decreases the stem cell self-renewal capacity of the cells while increasing cell invasion.

Mechanistically, we show that Par3 co-localizes with mitochondria and affects mitochondrial ROS generating activity. However, the mode of interaction between Par3 and the mitochondria remains unknown. It would be of interest to investigate whether this interaction is direct, via proteins of the mitochondrial outer membrane, or if it involves additional partners, possibly proteins of the Par3/Par6/aPKC complex. In addition, it would be interesting to understand how the co-localization of Par3 with the mitochondria affects the activity of the electron transport chain and ATP production. Once located on the mitochondrial membrane, Par3 could possibly disturb the activity of the enzymes of the electron transport chain (NADH dehydrogenase, succinate dehydrogenase, ATP synthase). Another possibility is the disruption of the conformation or location of one of the five complexes involved in the electron transport chain by Par3. In addition, one can wonder about the fate of the excess of ROS in the cells. It is known that intracellular ROS regulate a large number of signaling pathways involved in various cellular processes and a variation of ROS concentration leads to an alteration of these pathways. In this study, the specific pathways affected by the loss of Par3-induced ROS production remain unknown. Finally, we conducted this study in glioblastoma cell lines derived from patients. It would be interesting to investigate whether the implication of Par3 occurs in an early or late stage of tumorigenesis, for instance by investigating the effect of Par3 silencing in neural progenitors.

The *LINC00707*, Par3 and glutamine deprivation projects cover different aspect of cancer cell biology including cell signaling, non-coding RNA and metabolism. In the future, it would be interesting to use transcriptomic data that we generated to develop a new project that connects these different fields. More and more non-coding RNAs are described as regulators of cell metabolism in cancer, diabetes or cardiovascular diseases (Mongelli et al., 2019). One example of a metabolic pathway regulated by non-coding RNAs in cancer would be the regulation of the glycolytic pathway of HCC in hypoxic conditions by the lncRNAs *RAET1K* (Zhou et al., 2020) and *Ftx* (Li et al., 2018b).

9 Acknowledgements

First, I express all my gratitude to my first supervisor, **Aristidis Moustakas**. Infinite thanks for giving me the chance to be part of your group and for your guidance along my PhD studies. Your enthusiasm and sense of humor create this friendly and warm environment in our group that has been extremely important for me. **Cark-Henrik Heldin**, thank you for your precious advice and kindness! I truly admire your devotion to science and your ability to remember every details of a paper you read 20 years ago. **Laia Caja Puigsubira**, in addition to your scientific guidance, your spontaneity and honesty pushed me to do always better. I´m looking forward to meet you again in the future with a good crème brûlée.
It´s been a privilege to work with the three of you.

Thanks to all the other group leaders for the stimulating discussions and everyone in the B11:3 corridor for being so helpful and contributing in the nice atmosphere in our corridor. In addition, I would like to acknowledge some of our distant collaborators that made one of my research projects possible: **Patricia**, **Beatriz**, **Sabrina**, **Andrea**, thank you for your input! Speaking about distant connections, I also would like to thank all my previous supervisors back in Bordeaux; **Fred**, **Karine**, **Martin** for bringing me into your lab and giving me the taste of research.

My dear friends and colleagues were also essential for me during the past 5 years. First, thanks to all the past members of Aris group, **Panos**, **Kallia**, **Yutaro** and **Mahsa** for introducing me to the field and teaching me important technics. **Anita**, I see you as my future me and that´s pretty nice. I´ll never thank you enough for our nice discussions, your precious help in the lab and for the kanelbulle receipt. **Irene**, **Yae** and **Mohamad**, I have learnt a lot from you, great postdoc team! **Dorival**, massa cara! I also respect your scientific skills but I´ll mostly remember the crazy parties we had together and your performances at karaoke. **Chrysa**, **Yu** and **Melanie**, you girls are the first real friends I made when I arrived here in Uppsala. At this time, I was seriously questioning my choice of leaving France for so many years but you made me feel home, infinite thanks for that. We´ve been through this together along with all the life changes that go with it and I know (I hope) our friendship will remain! In this, I include **Jonathan**, **Lonneke**, **Emma**

and **Kalyani**, my fellow soldiers and friends. I feel so grateful for having all of you in my life. Thanks for all the fun, support and – sometimes – the scientific discussions!

Niki, **Zi Xin**, **Ria, Cen** and **Özge**, best office-mates! I enjoyed your company a lot. **Kehuan**, you just defended your thesis as I am writing this text. Congratulations, I wish you all the best. A special thank for **Anders**, **Maryia** and **Ihor**, always pleasant talking to you!

I also would like to mention my others PhD-mates, teaching-mates and party-mates; **Xinran, Chen Li**, **Axel**, **Anish**, **Viktor, Anishia**, **Filip**, **Philipp**, **Michelle**, **Ophélie**, **Lewis**, **Axel**: cheers and may the force be with us in our future career! There are also some more distant people that are probably never going to read this book but were part of my journey: **Felix** and all the amazing people I met in this crazy Spetses summer school. Unforgettable!

Tussen tack to my cat **Floki** for being on my side every single day and enlightening my life with her cuteness and kindness. Best cat ever.

Last but not least, I want to thank all my old friends, family and especially my parents **Anne-Marie** and **Guy**. I can only be thankful for your complete trust in my choices and for giving me full freedom in everything since I am a kid. Your unconditional support brought me here today so good job, I guess!

10 References

Aigner, K., B. Dampier, L. Descovich, M. Mikula, A. Sultan, M. Schreiber, W. Mikulits, T. Brabletz, D. Strand, P. Obrist, W. Sommergruber, N. Schweifer, A. Wernitznig, H. Beug, R. Foisner, and A. Eger. 2007. The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. *Oncogene*. 26:6979-6988.

Akinc, A., A. Zumbuehl, M. Goldberg, E.S. Leshchiner, V. Busini, N. Hossain, S.A. Bacallado, D.N. Nguyen, J. Fuller, R. Alvarez, A. Borodovsky, T. Borland, R. Constien, A. de Fougerolles, J.R. Dorkin, K. Narayanannair Jayaprakash, M. Jayaraman, M. John, V. Koteliansky, M. Manoharan, L. Nechev, J. Qin, T. Racie, D. Raitcheva, K.G. Rajeev, D.W. Sah, J. Soutschek, I. Toudjarska, H.P. Vornlocher, T.S. Zimmermann, R. Langer, and D.G. Anderson. 2008. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat Biotechnol*. 26:561-569.

Alexandrova, A.Y., P.B. Kopnin, J.M. Vasiliev, and B.P. Kopnin. 2006. ROS up-regulation mediates Ras-induced changes of cell morphology and motility. *Exp Cell Res*. 312:2066-2073.

Alexopoulou, L., A.C. Holt, R. Medzhitov, and R.A. Flavell. 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*. 413:732-738.

Ali, N.J.A., M. Dias Gomes, R. Bauer, S. Brodesser, C. Niemann, and S. Iden. 2016. Essential Role of Polarity Protein Par3 for Epidermal Homeostasis through Regulation of Barrier Function, Keratinocyte Differentiation, and Stem Cell Maintenance. *J Invest Dermatol*. 136:2406-2416.

Anderson, K.J., A.C. Tan, J. Parkinson, M. Back, M. Kastelan, A. Newey, J. Brewer, H. Wheeler, A.L. Hudson, S.B. Amin, K.C. Johnson, F.P. Barthel, R.G.W. Verhaak, and M. Khasraw. 2020. Molecular and clonal evolution in recurrent metastatic gliosarcoma. *Cold Spring Harb Mol Case Stud*. 6.

Annes, J.P., Y. Chen, J.S. Munger, and D.B. Rifkin. 2004. Integrin alphaVbeta6-mediated activation of latent TGF-beta requires the latent TGF-beta binding protein-1. *J Cell Biol*. 165:723-734.

Arase, M., K. Horiguchi, S. Ehata, M. Morikawa, S. Tsutsumi, H. Aburatani, K. Miyazono, and D. Koinuma. 2014. Transforming growth factor-beta-induced lncRNA-Smad7 inhibits apoptosis of mouse breast cancer JygMC(A) cells. *Cancer Sci*. 105:974-982.

Azam, S., S. Hou, B. Zhu, W. Wang, T. Hao, X. Bu, M. Khan, and H. Lei. 2019. Nuclear retention element recruits U1 snRNP components to restrain spliced lncRNAs in the nucleus. *RNA Biol*. 16:1001-1009.

Barnett, P., R.S. Arnold, R. Mezencev, L.W. Chung, M. Zayzafoon, and V. Odero-Marah. 2011. Snail-mediated regulation of reactive oxygen species in

ARCaP human prostate cancer cells. *Biochem Biophys Res Commun*. 404:34-39.

Basuroy, S., M. Dunagan, P. Sheth, A. Seth, and R.K. Rao. 2010. Hydrogen peroxide activates focal adhesion kinase and c-Src by a phosphatidylinositol 3 kinase-dependent mechanism and promotes cell migration in Caco-2 cell monolayers. *Am J Physiol Gastrointest Liver Physiol*. 299:G186-195.

Batlle, R., L. Alba-Castellon, J. Loubat-Casanovas, E. Armenteros, C. Franci, J. Stanisavljevic, R. Banderas, J. Martin-Caballero, F. Bonilla, J. Baulida, J.I. Casal, T. Gridley, and A. Garcia de Herreros. 2013. Snail1 controls TGF-beta responsiveness and differentiation of mesenchymal stem cells. *Oncogene*. 32:3381-3389.

Bedi, U., V.K. Mishra, D. Wasilewski, C. Scheel, and S.A. Johnsen. 2014. Epigenetic plasticity: a central regulator of epithelial-to-mesenchymal transition in cancer. *Oncotarget*. 5:2016-2029.

Betschinger, J., K. Mechtler, and J.A. Knoblich. 2003. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature*. 422:326-330.

Bharathy, S., W. Xie, J.M. Yingling, and M. Reiss. 2008. Cancer-associated transforming growth factor beta type II receptor gene mutant causes activation of bone morphogenic protein-Smads and invasive phenotype. *Cancer Res*. 68:1656-1666.

Bompard, G., J. van Dijk, J. Cau, Y. Lannay, G. Marcellin, A. Lawera, S. van der Laan, and K. Rogowski. 2018. CSAP Acts as a Regulator of TTLL-Mediated Microtubule Glutamylation. *Cell Rep*. 25:2866-2877 e2865.

Boque-Sastre, R., M. Soler, C. Oliveira-Mateos, A. Portela, C. Moutinho, S. Sayols, A. Villanueva, M. Esteller, and S. Guil. 2015. Head-to-head antisense transcription and R-loop formation promotes transcriptional activation. *Proc Natl Acad Sci U S A*. 112:5785-5790.

Boroughs, L.K., and R.J. DeBerardinis. 2015. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol*. 17:351-359.

Bray, F., J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, and A. Jemal. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 68:394-424.

Carroll, A.G., H.J. Voeller, L. Sugars, and E.P. Gelmann. 1993. p53 oncogene mutations in three human prostate cancer cell lines. *Prostate*. 23:123-134.

Chao, Y.L., C.R. Shepard, and A. Wells. 2010. Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. *Mol Cancer*. 9:179.

Chen, I.T., P.H. Hsu, W.C. Hsu, N.J. Chen, and P.H. Tseng. 2015. Polyubiquitination of Transforming Growth Factor beta-activated Kinase 1 (TAK1) at Lysine 562 Residue Regulates TLR4-mediated JNK and p38 MAPK Activation. *Sci Rep*. 5:12300.

Chen, J., and M. Zhang. 2013. The Par3/Par6/aPKC complex and epithelial cell polarity. *Exp Cell Res*. 319:1357-1364.

Cheng, X., P. Holenya, S. Can, H. Alborzinia, R. Rubbiani, I. Ott, and S. Wolfl. 2014. A TrxR inhibiting gold(I) NHC complex induces apoptosis through

ASK1-p38-MAPK signaling in pancreatic cancer cells. *Mol Cancer*. 13:221.

Chillon, I., and M. Marcia. 2020. The molecular structure of long non-coding RNAs: emerging patterns and functional implications. *Crit Rev Biochem Mol Biol*. 55:662-690.

Choi, J., S.Y. Park, and C.K. Joo. 2007. Transforming growth factor-beta1 represses E-cadherin production via slug expression in lens epithelial cells. *Invest Ophthalmol Vis Sci*. 48:2708-2718.

Chow, L.M., R. Endersby, X. Zhu, S. Rankin, C. Qu, J. Zhang, A. Broniscer, D.W. Ellison, and S.J. Baker. 2011. Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain. *Cancer Cell*. 19:305-316.

Dadras, M.S., L. Caja, A. Mezheyeuski, S. Liu, C. Gélabert, M.C. Gomez-Puerto, R. Gallini, C.J. Rubin, P. ten Dijke, C.-H. Heldin, and A. Moustakas. 2021. The polarity protein Par3 coordinates positively self-renewal and negatively invasiveness in glioblastoma. *Cell Death Dis.* 12:932.

Datto, M.B., Y. Li, J.F. Panus, D.J. Howe, Y. Xiong, and X.F. Wang. 1995. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc Natl Acad Sci U S A*. 92:5545-5549.

David, C.J., and J. Massague. 2018. Contextual determinants of TGFbeta action in development, immunity and cancer. *Nat Rev Mol Cell Biol*. 19:419-435.

Ding, Z., C.J. Wu, G.C. Chu, Y. Xiao, D. Ho, J. Zhang, S.R. Perry, E.S. Labrot, X. Wu, R. Lis, Y. Hoshida, D. Hiller, B. Hu, S. Jiang, H. Zheng, A.H. Stegh, K.L. Scott, S. Signoretti, N. Bardeesy, Y.A. Wang, D.E. Hill, T.R. Golub, M.J. Stampfer, W.H. Wong, M. Loda, L. Mucci, L. Chin, and R.A. DePinho. 2011. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. *Nature*. 470:269-273.

Dionyssiou, M.G., J. Salma, M. Bevzyuk, S. Wales, L. Zakharyan, and J.C. McDermott. 2013. Kruppel-like factor 6 (KLF6) promotes cell proliferation in skeletal myoblasts in response to TGFbeta/Smad3 signaling. *Skelet Muscle*. 3:7.

Dueva, R., K. Akopyan, C. Pederiva, D. Trevisan, S. Dhanjal, A. Lindqvist, and M. Farnebo. 2019. Neutralization of the Positive Charges on Histone Tails by RNA Promotes an Open Chromatin Structure. *Cell Chem Biol*. 26:1436-1449 e1435.

Duran, R.V., W. Oppliger, A.M. Robitaille, L. Heiserich, R. Skendaj, E. Gottlieb, and M.N. Hall. 2012. Glutaminolysis activates Rag-mTORC1 signaling. *Mol Cell*. 47:349-358.

Fagerberg, L., B.M. Hallstrom, P. Oksvold, C. Kampf, D. Djureinovic, J. Odeberg, M. Habuka, S. Tahmasebpoor, A. Danielsson, K. Edlund, A. Asplund, E. Sjostedt, E. Lundberg, C.A. Szigyarto, M. Skogs, J.O. Takanen, H. Berling, H. Tegel, J. Mulder, P. Nilsson, J.M. Schwenk, C. Lindskog, F. Danielsson, A. Mardinoglu, A. Sivertsson, K. von Feilitzen, M. Forsberg, M. Zwahlen, I. Olsson, S. Navani, M. Huss, J. Nielsen, F. Ponten, and M. Uhlen. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics*. 13:397-406.

Fan, P.C., Y. Zhang, Y. Wang, W. Wei, Y.X. Zhou, Y. Xie, X. Wang, Y.Z. Qi, L. Chang, Z.P. Jia, Z. Zhou, H. Guan, H. Zhang, P. Xu, and P.K. Zhou. 2019. Quantitative proteomics reveals mitochondrial respiratory chain as a dominant target for carbon ion radiation: Delayed reactive oxygen species generation caused DNA damage. *Free Radic Biol Med*. 130:436-445.

Fang, F., C. Guo, W. Zheng, and Q. Li. 2022. LINC00707 Promotes Cell Proliferation in Cervical Cancer via the miR-374c-5p/SDC4 Axis. *Biomed Res Int*. 2022:5793912.

Fraser, M., H. Zhao, K.R. Luoto, C. Lundin, C. Coackley, N. Chan, A.M. Joshua, T.A. Bismar, A. Evans, T. Helleday, and R.G. Bristow. 2012. PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: implications for radiotherapy and chemotherapy. *Clin Cancer Res*. 18:1015-1027.

Gameiro, P.A., and K. Struhl. 2018. Nutrient Deprivation Elicits a Transcriptional and Translational Inflammatory Response Coupled to Decreased Protein Synthesis. *Cell Rep*. 24:1415-1424.

Goldman, E.H., L. Chen, and H. Fu. 2004. Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14-3-3 dissociation. *J Biol Chem*. 279:10442-10449.

Gorelik, L., S. Constant, and R.A. Flavell. 2002. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med*. 195:1499-1505.

Gorrini, C., I.S. Harris, and T.W. Mak. 2013. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov*. 12:931-947.

Guo, H., J. Li, F. Fan, and P. Zhou. 2021. LINC00707 Regulates miR-382-5p/VEGFA Pathway to Enhance Cervical Cancer Progression. *J Immunol Res*. 2021:5524632.

Hamidi, A., J. Song, N. Thakur, S. Itoh, A. Marcusson, A. Bergh, C.H. Heldin, and M. Landstrom. 2017. TGF-beta promotes PI3K-AKT signaling and prostate cancer cell migration through the TRAF6-mediated ubiquitylation of p85alpha. *Sci Signal*. 10.

Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell*. 144:646-674.

Hapak, S.M., C.V. Rothlin, and S. Ghosh. 2018. PAR3-PAR6-atypical PKC polarity complex proteins in neuronal polarization. *Cell Mol Life Sci*. 75:2735-2761.

Hashmi, M.Z., A. Hasnain, J.H. Syed, M. Tariq, X. Su, H. Mubarak, W. Nasim, and C. Shen. 2018. PCB118-Induced Cell Proliferation Mediated by Oxidative Stress and MAPK Signaling Pathway in HELF Cells. *Dose Response*. 16:1559325817751525.

Hayashida, T., M. Decaestecker, and H.W. Schnaper. 2003. Cross-talk between ERK MAP kinase and Smad signaling pathways enhances TGF-beta-dependent responses in human mesangial cells. *FASEB J*. 17:1576-1578.

Heldin, C.H., and A. Moustakas. 2016. Signaling Receptors for TGF-beta Family Members. *Cold Spring Harb Perspect Biol*. 8.

Holdt, L.M., S. Hoffmann, K. Sass, D. Langenberger, M. Scholz, K. Krohn, K. Finstermeier, A. Stahringer, W. Wilfert, F. Beutner, S. Gielen, G. Schuler, G. Gabel, H. Bergert, I. Bechmann, P.F. Stadler, J. Thiery, and D. Teupser. 2013. Alu elements in ANRIL non-coding RNA at chromosome 9p21

modulate atherogenic cell functions through trans-regulation of gene networks. *PLoS Genet*. 9:e1003588.

Hombach, S., and M. Kretz. 2016. Non-coding RNAs: Classification, Biology and Functioning. *Adv Exp Med Biol*. 937:3-17.

Horikoshi, Y., A. Suzuki, T. Yamanaka, K. Sasaki, K. Mizuno, H. Sawada, S. Yonemura, and S. Ohno. 2009. Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *J Cell Sci*. 122:1595-1606.

Hsiao, J., T.Y. Yuan, M.S. Tsai, C.Y. Lu, Y.C. Lin, M.L. Lee, S.W. Lin, F.C. Chang, H. Liu Pimentel, C. Olive, C. Coito, G. Shen, M. Young, T. Thorne, M. Lawrence, M. Magistri, M.A. Faghihi, O. Khorkova, and C. Wahlestedt. 2016. Upregulation of Haploinsufficient Gene Expression in the Brain by Targeting a Long Non-coding RNA Improves Seizure Phenotype in a Model of Dravet Syndrome. *EBioMedicine*. 9:257-277.

Hsu, I.C., T. Tokiwa, W. Bennett, R.A. Metcalf, J.A. Welsh, T. Sun, and C.C. Harris. 1993. p53 gene mutation and integrated hepatitis B viral DNA sequences in human liver cancer cell lines. *Carcinogenesis*. 14:987-992.

Huang, T., L. David, V. Mendoza, Y. Yang, M. Villarreal, K. De, L. Sun, X. Fang, F. Lopez-Casillas, J.L. Wrana, and A.P. Hinck. 2011. TGF-beta signalling is mediated by two autonomously functioning TbetaRI:TbetaRII pairs. *EMBO J*. 30:1263-1276.

Huse, M., Y.G. Chen, J. Massague, and J. Kuriyan. 1999. Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell*. 96:425-436.

Jacques, T.S., A. Swales, M.J. Brzozowski, N.V. Henriquez, J.M. Linehan, Z. Mirzadeh, O.M. C, H. Naumann, A. Alvarez-Buylla, and S. Brandner. 2010. Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes. *EMBO J*. 29:222-235.

Jain, A.K., Y. Xi, R. McCarthy, K. Allton, K.C. Akdemir, L.R. Patel, B. Aronow, C. Lin, W. Li, L. Yang, and M.C. Barton. 2016. LncPRESS1 Is a p53-Regulated LncRNA that Safeguards Pluripotency by Disrupting SIRT6-Mediated De-acetylation of Histone H3K56. *Mol Cell*. 64:967-981.

Jang, C.W., C.H. Chen, C.C. Chen, J.Y. Chen, Y.H. Su, and R.H. Chen. 2002. TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. *Nat Cell Biol*. 4:51-58.

Jariyal, H., C. Gupta, S. Andhale, S. Gadge, and A. Srivastava. 2021. Comparative stemness and differentiation of luminal and basal breast cancer stem cell type under glutamine-deprivation. *J Cell Commun Signal*. 15:207-222.

Jia, B., Z. Wang, X. Sun, J. Chen, J. Zhao, and X. Qiu. 2019. Long noncoding RNA LINC00707 sponges miR-370-3p to promote osteogenesis of human bone marrow-derived mesenchymal stem cells through upregulating WNT2B. *Stem Cell Res Ther*. 10:67.

Jiang, B., J. Zhang, G. Zhao, M. Liu, J. Hu, F. Lin, J. Wang, W. Zhao, H. Ma, C. Zhang, C. Wu, L. Yao, Q. Liu, X. Chen, Y. Cao, Y. Zheng, C. Zhang, A. Han, D. Lin, and Q. Li. 2022. Filamentous GLS1 promotes ROS-induced apoptosis upon glutamine deprivation via insufficient asparagine synthesis. *Mol Cell*. 82:1821-1835 e1826.

Jin, H.O., S.E. Hong, J.Y. Kim, S.K. Jang, and I.C. Park. 2021. Amino acid deprivation induces AKT activation by inducing GCN2/ATF4/REDD1 axis. *Cell Death Dis*. 12:1127.

Jin, H.Y., A. Gonzalez-Martin, A.V. Miletic, M. Lai, S. Knight, M. Sabouri-Ghomi, S.R. Head, M.S. Macauley, R.C. Rickert, and C. Xiao. 2015. Transfection of microRNA Mimics Should Be Used with Caution. *Front Genet*. 6:340.

Joberty, G., C. Petersen, L. Gao, and I.G. Macara. 2000. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat Cell Biol*. 2:531-539.

Jung, H.Y., L. Fattet, J.H. Tsai, T. Kajimoto, Q. Chang, A.C. Newton, and J. Yang. 2019. Apical-basal polarity inhibits epithelial-mesenchymal transition and tumour metastasis by PAR-complex-mediated SNAI1 degradation. *Nat Cell Biol*. 21:359-371.

Jung, S.-N., W.K. Yang, J. Kim, H.S. Kim, E.J. Kim, H. Yun, H. Park, S.S. Kim, W. Choe, I. Kang, and J. Ha. 2008. Reactive oxygen species stabilize hypoxia-inducible factor-1 alpha protein and stimulate transcriptional activity via AMP-activated protein kinase in DU145 human prostate cancer cells. *Carcinogenesis*. 29:713-721.

Kamphorst, J.J., J.R. Cross, J. Fan, E. de Stanchina, R. Mathew, E.P. White, C.B. Thompson, and J.D. Rabinowitz. 2013. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. *Proc Natl Acad Sci U S A*. 110:8882-8887.

Kang, M.S., H.J. Lee, J.H. Lee, J.L. Ku, K.P. Lee, M.J. Kelley, Y.J. Won, S.T. Kim, and J.G. Park. 1996. Mutation of p53 gene in hepatocellular carcinoma cell lines with HBX DNA. *Int J Cancer*. 67:898-902.

Kawasaki, N., T. Miwa, S. Hokari, T. Sakurai, K. Ohmori, K. Miyauchi, K. Miyazono, and D. Koinuma. 2018. Long noncoding RNA NORAD regulates transforming growth factor-beta signaling and epithelial-to-mesenchymal transition-like phenotype. *Cancer Sci*. 109:2211-2220.

Kim, H.I., H. Lim, and A. Moon. 2018. Sex Differences in Cancer: Epidemiology, Genetics and Therapy. *Biomol Ther (Seoul)*. 26:335-342.

Kitagawa, T., A. Masumi, and Y. Akamatsu. 1991. Transforming growth factor-beta 1 stimulates glucose uptake and the expression of glucose transporter mRNA in quiescent Swiss mouse 3T3 cells. *Journal of Biological Chemistry*. 266:18066-18071.

Klepinin, A., S. Zhang, L. Klepinina, E. Rebane-Klemm, A. Terzic, T. Kaambre, and P. Dzeja. 2020. Adenylate Kinase and Metabolic Signaling in Cancer Cells. *Front Oncol*. 10:660.

Komura, K., C.J. Sweeney, T. Inamoto, N. Ibuki, H. Azuma, and P.W. Kantoff. 2018. Current treatment strategies for advanced prostate cancer. *Int J Urol*. 25:220-231.

Kretz, M., Z. Siprashvili, C. Chu, D.E. Webster, A. Zehnder, K. Qu, C.S. Lee, R.J. Flockhart, A.F. Groff, J. Chow, D. Johnston, G.E. Kim, R.C. Spitale, R.A. Flynn, G.X. Zheng, S. Aiyer, A. Raj, J.L. Rinn, H.Y. Chang, and P.A. Khavari. 2013. Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature*. 493:231-235.

Larisch, S., Y. Yi, R. Lotan, H. Kerner, S. Eimerl, W. Tony Parks, Y. Gottfried, S. Birkey Reffey, M.P. de Caestecker, D. Danielpour, N. Book-Melamed, R. Timberg, C.S. Duckett, R.J. Lechleider, H. Steller, J. Orly, S.J. Kim, and

A.B. Roberts. 2000. A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. *Nat Cell Biol*. 2:915-921.
Lee, M.K., C. Pardoux, M.C. Hall, P.S. Lee, D. Warburton, J. Qing, S.M. Smith, and R. Derynck. 2007. TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO J*. 26:3957-3967.
Lee, S., F. Kopp, T.C. Chang, A. Sataluri, B. Chen, S. Sivakumar, H. Yu, Y. Xie, and J.T. Mendell. 2016. Noncoding RNA NORAD Regulates Genomic Stability by Sequestering PUMILIO Proteins. *Cell*. 164:69-80.
Lehman, T.A., R. Modali, P. Boukamp, J. Stanek, W.P. Bennett, J.A. Welsh, R.A. Metcalf, M.R. Stampfer, N. Fusenig, E.M. Rogan, and et al. 1993. p53 mutations in human immortalized epithelial cell lines. *Carcinogenesis*. 14:833-839.
Leslie, N.R., D. Bennett, Y.E. Lindsay, H. Stewart, A. Gray, and C.P. Downes. 2003. Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *EMBO J*. 22:5501-5510.
Li, Q., A.P. Hutchins, Y. Chen, S. Li, Y. Shan, B. Liao, D. Zheng, X. Shi, Y. Li, W.Y. Chan, G. Pan, S. Wei, X. Shu, and D. Pei. 2017. A sequential EMT-MET mechanism drives the differentiation of human embryonic stem cells towards hepatocytes. *Nat Commun*. 8:15166.
Li, Q., W. Yue, M. Li, Z. Jiang, Z. Hou, W. Liu, N. Ma, W. Gan, Y. Li, T. Zhou, W. Yue, and S. Chen. 2021. Downregulating Long Non-coding RNAs CTBP1-AS2 Inhibits Colorectal Cancer Development by Modulating the miR-93-5p/TGF-beta/SMAD2/3 Pathway. *Front Oncol*. 11:626620.
Li, R.H., M. Chen, J. Liu, C.C. Shao, C.P. Guo, X.L. Wei, Y.C. Li, W.H. Huang, and G.J. Zhang. 2018a. Long noncoding RNA ATB promotes the epithelial-mesenchymal transition by upregulating the miR-200c/Twist1 axe and predicts poor prognosis in breast cancer. *Cell Death Dis*. 9:1171.
Li, X., Q. Zhao, J. Qi, W. Wang, D. Zhang, Z. Li, and C. Qin. 2018b. lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPARgamma pathway in hepatocellular carcinoma. *Int J Oncol*. 53:551-566.
Li, Z., M. Li, P. Xia, L. Wang, and Z. Lu. 2022. Targeting long non-coding RNA PVT1/TGF-beta/Smad by p53 prevents glioma progression. *Cancer Biol Ther*. 23:225-233.
Lin, L.H., K.W. Chang, H.W. Cheng, and C.J. Liu. 2019. SMAD4 Somatic Mutations in Head and Neck Carcinoma Are Associated With Tumor Progression. *Front Oncol*. 9:1379.
Liu, H., and Y.G. Chen. 2022. The Interplay Between TGF-beta Signaling and Cell Metabolism. *Front Cell Dev Biol*. 10:846723.
Lopez-Casillas, F., H.M. Payne, J.L. Andres, and J. Massague. 1994. Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. *J Cell Biol*. 124:557-568.
Luo, K., J. Geng, Q. Zhang, Y. Xu, X. Zhou, Z. Huang, K.Q. Shi, C. Pan, and J. Wu. 2019. LncRNA CASC9 interacts with CPSF3 to regulate TGF-beta signaling in colorectal cancer. *J Exp Clin Cancer Res*. 38:249.
Ma, G., Y. Liang, Y. Chen, L. Wang, D. Li, Z. Liang, X. Wang, D. Tian, X. Yang, and H. Niu. 2020. Glutamine Deprivation Induces PD-L1 Expression via Activation of EGFR/ERK/c-Jun Signaling in Renal Cancer. *Mol Cancer Res*. 18:324-339.

Macias-Silva, M., S. Abdollah, P.A. Hoodless, R. Pirone, L. Attisano, and J.L. Wrana. 1996. MADR2 is a substrate of the TGFbeta receptor and its phosphorylation is required for nuclear accumulation and signaling. *Cell*. 87:1215-1224.

Madan, E., R. Gogna, P. Kuppusamy, M. Bhatt, A.A. Mahdi, and U. Pati. 2013. SCO2 induces p53-mediated apoptosis by Thr845 phosphorylation of ASK-1 and dissociation of the ASK-1-Trx complex. *Mol Cell Biol*. 33:1285-1302.

Massague, J. 2008. TGFbeta in Cancer. *Cell*. 134:215-230.

Mazat, J.P., A. Devin, and S. Ransac. 2020. Modelling mitochondrial ROS production by the respiratory chain. *Cell Mol Life Sci*. 77:455-465.

McCaffrey, L.M., J. Montalbano, C. Mihai, and I.G. Macara. 2012. Loss of the Par3 polarity protein promotes breast tumorigenesis and metastasis. *Cancer Cell*. 22:601-614.

McKarns, S.C., R.H. Schwartz, and N.E. Kaminski. 2004. Smad3 is essential for TGF-beta 1 to suppress IL-2 production and TCR-induced proliferation, but not IL-2-induced proliferation. *J Immunol*. 172:4275-4284.

Mele, M., K. Mattioli, W. Mallard, D.M. Shechner, C. Gerhardinger, and J.L. Rinn. 2017. Chromatin environment, transcriptional regulation, and splicing distinguish lincRNAs and mRNAs. *Genome Res*. 27:27-37.

Mestre-Farrera, A., M. Bruch-Oms, R. Pena, J. Rodriguez-Morato, L. Alba-Castellon, L. Comerma, M. Quintela-Fandino, M. Dunach, J. Baulida, O.J. Pozo, and A. Garcia de Herreros. 2021. Glutamine-Directed Migration of Cancer-Activated Fibroblasts Facilitates Epithelial Tumor Invasion. *Cancer Res*. 81:438-451.

Miller, D.S.J., B. Schmierer, and C.S. Hill. 2019. TGF-beta family ligands exhibit distinct signalling dynamics that are driven by receptor localisation. *J Cell Sci*. 132.

Miyazono, K., and C.H. Heldin. 1989. Role for carbohydrate structures in TGF-beta 1 latency. *Nature*. 338:158-160.

Modarresi, F., M.A. Faghihi, M.A. Lopez-Toledano, R.P. Fatemi, M. Magistri, S.P. Brothers, M.P. van der Brug, and C. Wahlestedt. 2012. Inhibition of natural antisense transcripts in vivo results in gene-specific transcriptional upregulation. *Nat Biotechnol*. 30:453-459.

Mongelli, A., F. Martelli, A. Farsetti, and C. Gaetano. 2019. The Dark That Matters: Long Non-coding RNAs as Master Regulators of Cellular Metabolism in Non-communicable Diseases. *Front Physiol*. 10:369.

Moustakas, A., and C.H. Heldin. 2007. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci*. 98:1512-1520.

Murayama, K., M. Kato-Murayama, Y. Itoh, K. Miyazono, K. Miyazawa, and M. Shirouzu. 2020. Structural basis for inhibitory effects of Smad7 on TGF-beta family signaling. *J Struct Biol*. 212:107661.

Nagel, Z.D., G.J. Kitange, S.K. Gupta, B.A. Joughin, I.A. Chaim, P. Mazzucato, D.A. Lauffenburger, J.N. Sarkaria, and L.D. Samson. 2017. DNA Repair Capacity in Multiple Pathways Predicts Chemoresistance in Glioblastoma Multiforme. *Cancer Res*. 77:198-206.

Nakasuka, F., S. Tabata, T. Sakamoto, A. Hirayama, H. Ebi, T. Yamada, K. Umetsu, M. Ohishi, A. Ueno, H. Goto, M. Sugimoto, Y. Nishioka, Y. Yamada, M.

Tomita, A.T. Sasaki, S. Yano, and T. Soga. 2021. TGF-beta-dependent reprogramming of amino acid metabolism induces epithelial-mesenchymal transition in non-small cell lung cancers. *Commun Biol*. 4:782.

Natsuizaka, M., T. Omura, T. Akaike, Y. Kuwata, K. Yamazaki, T. Sato, Y. Karino, J. Toyota, T. Suga, and M. Asaka. 2005. Clinical features of hepatocellular carcinoma with extrahepatic metastases. *J Gastroenterol Hepatol*. 20:1781-1787.

Nilchian, A., N. Giotopoulou, W. Sun, and J. Fuxe. 2020. Different Regulation of Glut1 Expression and Glucose Uptake during the Induction and Chronic Stages of TGFbeta1-Induced EMT in Breast Cancer Cells. *Biomolecules*. 10.

Nowak, D.G., H. Cho, T. Herzka, K. Watrud, D.V. DeMarco, V.M. Wang, S. Senturk, C. Fellmann, D. Ding, T. Beinortas, D. Kleinman, M. Chen, R. Sordella, J.E. Wilkinson, M. Castillo-Martin, C. Cordon-Cardo, B.D. Robinson, and L.C. Trotman. 2015. MYC Drives Pten/Trp53-Deficient Proliferation and Metastasis due to IL6 Secretion and AKT Suppression via PHLPP2. *Cancer Discov*. 5:636-651.

Padmakumar, S., G. Jones, G. Pawar, O. Khorkova, J. Hsiao, J. Kim, M.M. Amiji, and B.S. Bleier. 2021. Minimally Invasive Nasal Depot (MIND) technique for direct BDNF AntagoNAT delivery to the brain. *J Control Release*. 331:176-186.

Pan, M., M.A. Reid, X.H. Lowman, R.P. Kulkarni, T.Q. Tran, X. Liu, Y. Yang, J.E. Hernandez-Davies, K.K. Rosales, H. Li, W. Hugo, C. Song, X. Xu, D.E. Schones, D.K. Ann, V. Gradinaru, R.S. Lo, J.W. Locasale, and M. Kong. 2016. Regional glutamine deficiency in tumours promotes dedifferentiation through inhibition of histone demethylation. *Nat Cell Biol*. 18:1090-1101.

Park, I.K., J.J. Letterio, and J.D. Gorham. 2007. TGF-beta 1 inhibition of IFN-gamma-induced signaling and Th1 gene expression in CD4+ T cells is Smad3 independent but MAP kinase dependent. *Mol Immunol*. 44:3283-3290.

Park, S.Y., W.J. Jang, E.Y. Yi, J.Y. Jang, Y. Jung, J.W. Jeong, and Y.J. Kim. 2010. Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. *J Pineal Res*. 48:178-184.

Perlman, R., W.P. Schiemann, M.W. Brooks, H.F. Lodish, and R.A. Weinberg. 2001. TGF-beta-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation. *Nat Cell Biol*. 3:708-714.

Postepska-Igielska, A., A. Giwojna, L. Gasri-Plotnitsky, N. Schmitt, A. Dold, D. Ginsberg, and I. Grummt. 2015. LncRNA Khps1 Regulates Expression of the Proto-oncogene SPHK1 via Triplex-Mediated Changes in Chromatin Structure. *Mol Cell*. 60:626-636.

Prasad, P., and S.S. Roy. 2021. Glutamine regulates ovarian cancer cell migration and invasion through ETS1. *Heliyon*. 7:e07064.

Qin, C.F., and F.L. Zhao. 2017. Long non-coding RNA TUG1 can promote proliferation and migration of pancreatic cancer via EMT pathway. *Eur Rev Med Pharmacol Sci*. 21:2377-2384.

Qiu, Z., H. Li, Z. Zhang, Z. Zhu, S. He, X. Wang, P. Wang, J. Qin, L. Zhuang, W. Wang, F. Xie, Y. Gu, K. Zou, C. Li, C. Li, C. Wang, J. Cen, X. Chen, Y. Shu, Z. Zhang, L. Sun, L. Min, Y. Fu, X. Huang, H. Lv, H. Zhou, Y. Ji, Z. Zhang, Z. Meng, X. Shi, H. Zhang, Y. Li, and L. Hui. 2019. A

Pharmacogenomic Landscape in Human Liver Cancers. *Cancer Cell*. 36:179-193 e111.

Rackham, O., A.M. Shearwood, T.R. Mercer, S.M. Davies, J.S. Mattick, and A. Filipovska. 2011. Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. *RNA*. 17:2085-2093.

Ramesh, S., X.J. Qi, G.M. Wildey, J. Robinson, J. Molkentin, J. Letterio, and P.H. Howe. 2008. TGF beta-mediated BIM expression and apoptosis are regulated through SMAD3-dependent expression of the MAPK phosphatase MKP2. *EMBO Rep*. 9:990-997.

Ramjaun, A.R., S. Tomlinson, A. Eddaoudi, and J. Downward. 2007. Upregulation of two BH3-only proteins, Bmf and Bim, during TGF beta-induced apoptosis. *Oncogene*. 26:970-981.

Raza, A., and G.K. Sood. 2014. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. *World J Gastroenterol*. 20:4115-4127.

Rebetz, J., D. Tian, A. Persson, B. Widegren, L.G. Salford, E. Englund, D. Gisselsson, and X. Fan. 2008. Glial progenitor-like phenotype in low-grade glioma and enhanced CD133-expression and neuronal lineage differentiation potential in high-grade glioma. *PLoS One*. 3:e1936.

Recouvreux, M.V., M.R. Moldenhauer, K.M.O. Galenkamp, M. Jung, B. James, Y. Zhang, A. Lowy, A. Bagchi, and C. Commisso. 2020. Glutamine depletion regulates Slug to promote EMT and metastasis in pancreatic cancer. *J Exp Med*. 217.

Rich, P.R., and A. Marechal. 2010. The mitochondrial respiratory chain. *Essays Biochem*. 47:1-23.

Rodriguez-Boulan, E., and I.G. Macara. 2014. Organization and execution of the epithelial polarity programme. *Nat Rev Mol Cell Biol*. 15:225-242.

Rogowski, K., J. van Dijk, M.M. Magiera, C. Bosc, J.C. Deloulme, A. Bosson, L. Peris, N.D. Gold, B. Lacroix, M. Bosch Grau, N. Bec, C. Larroque, S. Desagher, M. Holzer, A. Andrieux, M.J. Moutin, and C. Janke. 2010. A family of protein-deglutamylating enzymes associated with neurodegeneration. *Cell*. 143:564-578.

Roll-Mecak, A. 2020. The Tubulin Code in Microtubule Dynamics and Information Encoding. *Dev Cell*. 54:7-20.

Rossi, M., G. Bucci, D. Rizzotto, D. Bordo, M.J. Marzi, M. Puppo, A. Flinois, D. Spadaro, S. Citi, L. Emionite, M. Cilli, F. Nicassio, A. Inga, P. Briata, and R. Gherzi. 2019. LncRNA EPR controls epithelial proliferation by coordinating Cdkn1a transcription and mRNA decay response to TGF-beta. *Nat Commun*. 10:1969.

Rothenberg, S.M., G. Mohapatra, M.N. Rivera, D. Winokur, P. Greninger, M. Nitta, P.M. Sadow, G. Sooriyakumar, B.W. Brannigan, M.J. Ulman, R.M. Perera, R. Wang, A. Tam, X.J. Ma, M. Erlander, D.C. Sgroi, J.W. Rocco, M.W. Lingen, E.E. Cohen, D.N. Louis, J. Settleman, and D.A. Haber. 2010. A genome-wide screen for microdeletions reveals disruption of polarity complex genes in diverse human cancers. *Cancer Res*. 70:2158-2164.

Saito, N., N. Hirai, K. Aoki, S. Sato, R. Suzuki, Y. Hiramoto, S. Fujita, H. Nakayama, M. Hayashi, T. Sakurai, and S. Iwabuchi. 2019. Genetic and Lineage Classification of Glioma-Initiating Cells Identifies a Clinically Relevant Glioblastoma Model. *Cancers (Basel)*. 11.

Sakai, S., T. Ohhata, K. Kitagawa, C. Uchida, T. Aoshima, H. Niida, T. Suzuki, Y. Inoue, K. Miyazawa, and M. Kitagawa. 2019. Long Noncoding RNA ELIT-1 Acts as a Smad3 Cofactor to Facilitate TGFbeta/Smad Signaling and Promote Epithelial-Mesenchymal Transition. *Cancer Res*. 79:2821-2838.

Sakakibara, A., and Y. Hatanaka. 2015. Neuronal polarization in the developing cerebral cortex. *Front Neurosci*. 9:116.

Salmena, L., L. Poliseno, Y. Tay, L. Kats, and P.P. Pandolfi. 2011. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell*. 146:353-358.

Savary, K., D. Caglayan, L. Caja, K. Tzavlaki, S. Bin Nayeem, T. Bergstrom, Y. Jiang, L. Uhrbom, K. Forsberg-Nilsson, B. Westermark, C.H. Heldin, M. Ferletta, and A. Moustakas. 2013. Snail depletes the tumorigenic potential of glioblastoma. *Oncogene*. 32:5409-5420.

Schmidt, J.M., E. Panzilius, H.S. Bartsch, M. Irmler, J. Beckers, V. Kari, J.R. Linnemann, D. Dragoi, B. Hirschi, U.J. Kloos, S. Sass, F. Theis, S. Kahlert, S.A. Johnsen, K. Sotlar, and C.H. Scheel. 2015. Stem-cell-like properties and epithelial plasticity arise as stable traits after transient Twist1 activation. *Cell Rep*. 10:131-139.

Sheng, Y., I.A. Abreu, D.E. Cabelli, M.J. Maroney, A.F. Miller, M. Teixeira, and J.S. Valentine. 2014. Superoxide dismutases and superoxide reductases. *Chem Rev*. 114:3854-3918.

Shi, M., J. Zhu, R. Wang, X. Chen, L. Mi, T. Walz, and T.A. Springer. 2011. Latent TGF-beta structure and activation. *Nature*. 474:343-349.

Sledz, C.A., M. Holko, M.J. de Veer, R.H. Silverman, and B.R. Williams. 2003. Activation of the interferon system by short-interfering RNAs. *Nat Cell Biol*. 5:834-839.

Smith, E.R., and T.D. Hewitson. 2020. TGF-beta1 is a regulator of the pyruvate dehydrogenase complex in fibroblasts. *Sci Rep*. 10:17914.

Soukupova, J., A. Malfettone, E. Bertran, M.I. Hernandez-Alvarez, I. Penuelas-Haro, F. Dituri, G. Giannelli, A. Zorzano, and I. Fabregat. 2021. Epithelial-Mesenchymal Transition (EMT) Induced by TGF-beta in Hepatocellular Carcinoma Cells Reprograms Lipid Metabolism. *Int J Mol Sci*. 22.

Soukupova, J., A. Malfettone, P. Hyrossova, M.I. Hernandez-Alvarez, I. Penuelas-Haro, E. Bertran, A. Junza, J. Capellades, G. Giannelli, O. Yanes, A. Zorzano, J.C. Perales, and I. Fabregat. 2017. Role of the Transforming Growth Factor-beta in regulating hepatocellular carcinoma oxidative metabolism. *Sci Rep*. 7:12486.

Statello, L., M. Maugeri, E. Garre, M. Nawaz, J. Wahlgren, A. Papadimitriou, C. Lundqvist, L. Lindfors, A. Collen, P. Sunnerhagen, M. Ragusa, M. Purrello, C. Di Pietro, N. Tigue, and H. Valadi. 2018. Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. *PLoS One*. 13:e0195969.

Sundqvist, A., E. Vasilaki, O. Voytyuk, Y. Bai, M. Morikawa, A. Moustakas, K. Miyazono, C.H. Heldin, P. Ten Dijke, and H. van Dam. 2020. TGFbeta and EGF signaling orchestrates the AP-1- and p63 transcriptional regulation of breast cancer invasiveness. *Oncogene*. 39:4436-4449.

Tai, W.T., P.Y. Chu, C.W. Shiau, Y.L. Chen, Y.S. Li, M.H. Hung, L.J. Chen, P.L. Chen, J.C. Su, P.Y. Lin, H.C. Yu, and K.F. Chen. 2014. STAT3 mediates regorafenib-induced apoptosis in hepatocellular carcinoma. *Clin Cancer Res*. 20:5768-5776.

Tan, B., S. Yatim, S. Peng, J. Gunaratne, W. Hunziker, and A. Ludwig. 2020. The Mammalian Crumbs Complex Defines a Distinct Polarity Domain Apical of Epithelial Tight Junctions. *Curr Biol*. 30:2791-2804 e2796.

Taylor, B.S., N. Schultz, H. Hieronymus, A. Gopalan, Y. Xiao, B.S. Carver, V.K. Arora, P. Kaushik, E. Cerami, B. Reva, Y. Antipin, N. Mitsiades, T. Landers, I. Dolgalev, J.E. Major, M. Wilson, N.D. Socci, A.E. Lash, A. Heguy, J.A. Eastham, H.I. Scher, V.E. Reuter, P.T. Scardino, C. Sander, C.L. Sawyers, and W.L. Gerald. 2010. Integrative genomic profiling of human prostate cancer. *Cancer Cell*. 18:11-22.

Thakur, N., A. Sorrentino, C.H. Heldin, and M. Landstrom. 2009. TGF-beta uses the E3-ligase TRAF6 to turn on the kinase TAK1 to kill prostate cancer cells. *Future Oncol*. 5:1-3.

Torre, L.A., R.L. Siegel, E.M. Ward, and A. Jemal. 2016. Global Cancer Incidence and Mortality Rates and Trends--An Update. *Cancer Epidemiol Biomarkers Prev*. 25:16-27.

Torrino, S., E.M. Grasset, S. Audebert, I. Belhadj, C. Lacoux, M. Haynes, S. Pisano, S. Abelanet, F. Brau, S.Y. Chan, B. Mari, W.M. Oldham, A.J. Ewald, and T. Bertero. 2021. Mechano-induced cell metabolism promotes microtubule glutamylation to force metastasis. *Cell Metab*. 33:1342-1357 e1310.

Troyanovsky, R.B., I. Indra, R. Kato, B.J. Mitchell, and S.M. Troyanovsky. 2021. Basolateral protein Scribble binds phosphatase PP1 to establish a signaling network maintaining apicobasal polarity. *J Biol Chem*. 297:101289.

Tsai, J.H., J.L. Donaher, D.A. Murphy, S. Chau, and J. Yang. 2012. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell*. 22:725-736.

Tsirigoti, C., M.M. Ali, V. Maturi, C.H. Heldin, and A. Moustakas. 2022. Loss of SNAI1 induces cellular plasticity in invasive triple-negative breast cancer cells. *Cell Death Dis*. 13:832.

Tsubakihara, Y., Y. Ohata, Y. Okita, S. Younis, J. Eriksson, M.E. Sellin, J. Ren, P. Ten Dijke, K. Miyazono, A. Hikita, T. Imamura, M. Kato, C.H. Heldin, and A. Moustakas. 2022. TGFbeta selects for pro-stemness over pro-invasive phenotypes during cancer cell epithelial-mesenchymal transition. *Mol Oncol*. 16:2330-2354.

Tsukada, Y., J. Fang, H. Erdjument-Bromage, M.E. Warren, C.H. Borchers, P. Tempst, and Y. Zhang. 2006. Histone demethylation by a family of JmjC domain-containing proteins. *Nature*. 439:811-816.

Tummala, K.S., M. Brandt, A. Teijeiro, O. Grana, R.F. Schwabe, C. Perna, and N. Djouder. 2017. Hepatocellular Carcinomas Originate Predominantly from Hepatocytes and Benign Lesions from Hepatic Progenitor Cells. *Cell Rep*. 19:584-600.

Tzavlaki, K., and A. Moustakas. 2020. TGF-beta Signaling. *Biomolecules*. 10.

van der Reest, J., S. Lilla, L. Zheng, S. Zanivan, and E. Gottlieb. 2018. Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress. *Nat Commun*. 9:1581.

Vance, K.W., and C.P. Ponting. 2014. Transcriptional regulatory functions of nuclear long noncoding RNAs. *Trends Genet*. 30:348-355.

Vervoort, S.J., A.R. Lourenco, R. van Boxtel, and P.J. Coffer. 2013. SOX4 mediates TGF-beta-induced expression of mesenchymal markers during mammary cell epithelial to mesenchymal transition. *PLoS One*. 8:e53238.

Vo, B.T., D. Morton, Jr., S. Komaragiri, A.C. Millena, C. Leath, and S.A. Khan. 2013. TGF-beta effects on prostate cancer cell migration and invasion are mediated by PGE2 through activation of PI3K/AKT/mTOR pathway. *Endocrinology*. 154:1768-1779.

Wang, H., H. Luan, T. Zhan, X. Liu, J. Song, and H. Dai. 2020. Long non-coding RNA LINC00707 acts as a competing endogenous RNA to enhance cell proliferation in colorectal cancer. *Exp Ther Med*. 19:1439-1447.

Wang, P., M.L. Luo, E. Song, Z. Zhou, T. Ma, J. Wang, N. Jia, G. Wang, S. Nie, Y. Liu, and F. Hou. 2018. Long noncoding RNA lnc-TSI inhibits renal fibrogenesis by negatively regulating the TGF-beta/Smad3 pathway. *Sci Transl Med*. 10.

Wang, X., M. Kruithof-de Julio, K.D. Economides, D. Walker, H. Yu, M.V. Halili, Y.P. Hu, S.M. Price, C. Abate-Shen, and M.M. Shen. 2009. A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature*. 461:495-500.

Wang, X., Z. Lan, J. He, Q. Lai, X. Yao, Q. Li, Y. Liu, H. Lai, C. Gu, Q. Yan, Y. Fang, Y. Zhang, A. Li, and S. Liu. 2019. LncRNA SNHG6 promotes chemoresistance through ULK1-induced autophagy by sponging miR-26a-5p in colorectal cancer cells. *Cancer Cell Int*. 19:234.

Wang, X., J.Z. Liu, J.X. Hu, H. Wu, Y.L. Li, H.L. Chen, H. Bai, and C.X. Hai. 2011. ROS-activated p38 MAPK/ERK-Akt cascade plays a central role in palmitic acid-stimulated hepatocyte proliferation. *Free Radic Biol Med*. 51:539-551.

Wang, Z.A., A. Mitrofanova, S.K. Bergren, C. Abate-Shen, R.D. Cardiff, A. Califano, and M.M. Shen. 2013. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. *Nat Cell Biol*. 15:274-283.

Wei, L., T. Wu, P. He, J.L. Zhang, and W. Wu. 2018. LncRNA ATB promotes the proliferation and metastasis of lung cancer via activation of the p38 signaling pathway. *Oncol Lett*. 16:3907-3912.

Whiteman, E.L., C.J. Liu, E.R. Fearon, and B. Margolis. 2008. The transcription factor snail represses Crumbs3 expression and disrupts apico-basal polarity complexes. *Oncogene*. 27:3875-3879.

Winkle, M., S.M. El-Daly, M. Fabbri, and G.A. Calin. 2021. Noncoding RNA therapeutics - challenges and potential solutions. *Nat Rev Drug Discov*. 20:629-651.

Wolfraim, L.A., T.M. Walz, Z. James, T. Fernandez, and J.J. Letterio. 2004. p21Cip1 and p27Kip1 act in synergy to alter the sensitivity of naive T cells to TGF-beta-mediated G1 arrest through modulation of IL-2 responsiveness. *J Immunol*. 173:3093-3102.

Wong-Ekkabut, J., Z. Xu, W. Triampo, I.M. Tang, D.P. Tieleman, and L. Monticelli. 2007. Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study. *Biophys J*. 93:4225-4236.

Wu, J.W., M. Hu, J. Chai, J. Seoane, M. Huse, C. Li, D.J. Rigotti, S. Kyin, T.W. Muir, R. Fairman, J. Massague, and Y. Shi. 2001. Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-beta signaling. *Mol Cell*. 8:1277-1289.

Xie, D., X. Wu, L. Lan, F. Shangguan, X. Lin, F. Chen, S. Xu, Y. Zhang, Z. Chen, K. Huang, R. Wang, L. Wang, X. Song, Y. Liu, and B. Lu. 2016. Downregulation of TFAM inhibits the tumorigenesis of non-small cell lung cancer by activating ROS-mediated JNK/p38MAPK signaling and reducing cellular bioenergetics. *Oncotarget*. 7:11609-11624.

Xie, M., T. Ma, J. Xue, H. Ma, M. Sun, Z. Zhang, M. Liu, Y. Liu, S. Ju, Z. Wang, and W. De. 2019. The long intergenic non-protein coding RNA 707 promotes proliferation and metastasis of gastric cancer by interacting with mRNA stabilizing protein HuR. *Cancer Lett*. 443:67-79.

Xu, J., S. Lamouille, and R. Derynck. 2009. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res*. 19:156-172.

Xu, L., W. Liu, T. Li, Y. Hu, Y. Wang, L. Huang, Y. Wang, S. Shao, X. Liu, and Q. Zhan. 2021. Long non-coding RNA SMASR inhibits the EMT by negatively regulating TGF-beta/Smad signaling pathway in lung cancer. *Oncogene*. 40:3578-3592.

Xue, B., K. Krishnamurthy, D.C. Allred, and S.K. Muthuswamy. 2013. Loss of Par3 promotes breast cancer metastasis by compromising cell-cell cohesion. *Nat Cell Biol*. 15:189-200.

Yamamoto, Y., J. Matsui, T. Matsushima, H. Obaishi, K. Miyazaki, K. Nakamura, O. Tohyama, T. Semba, A. Yamaguchi, S.S. Hoshi, F. Mimura, T. Haneda, Y. Fukuda, J.I. Kamata, K. Takahashi, M. Matsukura, T. Wakabayashi, M. Asada, K.I. Nomoto, T. Watanabe, Z. Dezso, K. Yoshimatsu, Y. Funahashi, and A. Tsuruoka. 2014. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc Cell*. 6:18.

Yamashita, M., K. Fatyol, C. Jin, X. Wang, Z. Liu, and Y.E. Zhang. 2008. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Mol Cell*. 31:918-924.

Yap, K., S. Mukhina, G. Zhang, J.S.C. Tan, H.S. Ong, and E.V. Makeyev. 2018. A Short Tandem Repeat-Enriched RNA Assembles a Nuclear Compartment to Control Alternative Splicing and Promote Cell Survival. *Mol Cell*. 72:525-540 e513.

Yap, K.L., S. Li, A.M. Munoz-Cabello, S. Raguz, L. Zeng, S. Mujtaba, J. Gil, M.J. Walsh, and M.M. Zhou. 2010. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell*. 38:662-674.

Yin, Q.F., L. Yang, Y. Zhang, J.F. Xiang, Y.W. Wu, G.G. Carmichael, and L.L. Chen. 2012. Long noncoding RNAs with snoRNA ends. *Mol Cell*. 48:219-230.

Yoo, H.C., Y.C. Yu, Y. Sung, and J.M. Han. 2020. Glutamine reliance in cell metabolism. *Exp Mol Med*. 52:1496-1516.

Yu, K., B. Chen, D. Aran, J. Charalel, C. Yau, D.M. Wolf, L.J. van 't Veer, A.J. Butte, T. Goldstein, and M. Sirota. 2019. Comprehensive transcriptomic

analysis of cell lines as models of primary tumors across 22 tumor types. *Nat Commun*. 10:3574.

Yuan, J.H., F. Yang, F. Wang, J.Z. Ma, Y.J. Guo, Q.F. Tao, F. Liu, W. Pan, T.T. Wang, C.C. Zhou, S.B. Wang, Y.Z. Wang, Y. Yang, N. Yang, W.P. Zhou, G.S. Yang, and S.H. Sun. 2014. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell*. 25:666-681.

Zhang, F., M. Monkkonen, S. Roth, and M. Laiho. 2002. TGF-beta induced G(1) cell cycle arrest requires the activity of the proteasome pathway. Transforming growth factor. *Exp Cell Res*. 281:190-196.

Zhang, G., J. He, X. Ye, J. Zhu, X. Hu, M. Shen, Y. Ma, Z. Mao, H. Song, and F. Chen. 2019. beta-Thujaplicin induces autophagic cell death, apoptosis, and cell cycle arrest through ROS-mediated Akt and p38/ERK MAPK signaling in human hepatocellular carcinoma. *Cell Death Dis*. 10:255.

Zhang, K., X. Han, Z. Zhang, L. Zheng, Z. Hu, Q. Yao, H. Cui, G. Shu, M. Si, C. Li, Z. Shi, T. Chen, Y. Han, Y. Chang, Z. Yao, T. Han, and W. Hong. 2017. The liver-enriched lnc-LFAR1 promotes liver fibrosis by activating TGFbeta and Notch pathways. *Nat Commun*. 8:144.

Zhang, L., F. Zhou, A. Garcia de Vinuesa, E.M. de Kruijf, W.E. Mesker, L. Hui, Y. Drabsch, Y. Li, A. Bauer, A. Rousseau, K.A. Sheppard, C. Mickanin, P.J. Kuppen, C.X. Lu, and P. Ten Dijke. 2013. TRAF4 promotes TGF-beta receptor signaling and drives breast cancer metastasis. *Mol Cell*. 51:559-572.

Zhang, X., W. Feng, J. Zhang, L. Ge, Y. Zhang, X. Jiang, W. Peng, D. Wang, A. Gong, and M. Xu. 2018. Long non-coding RNA PVT1 promotes epithelial-mesenchymal transition via the TGF-beta/Smad pathway in pancreatic cancer cells. *Oncol Rep*. 40:1093-1102.

Zhang, Y., X.H. Feng, and R. Derynck. 1998. Smad3 and Smad4 cooperate with c-Jun/c-Fos to mediate TGF-beta-induced transcription. *Nature*. 394:909-913.

Zhao, Y., Y. Liu, L. Lin, Q. Huang, W. He, S. Zhang, S. Dong, Z. Wen, J. Rao, W. Liao, and M. Shi. 2018. The lncRNA MACC1-AS1 promotes gastric cancer cell metabolic plasticity via AMPK/Lin28 mediated mRNA stability of MACC1. *Mol Cancer*. 17:69.

Zhou, Y., Y. Huang, K. Hu, Z. Zhang, J. Yang, and Z. Wang. 2020. HIF1A activates the transcription of lncRNA RAET1K to modulate hypoxia-induced glycolysis in hepatocellular carcinoma cells via miR-100-5p. *Cell Death Dis*. 11:176.

Zorov, D.B., M. Juhaszova, and S.J. Sollott. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev*. 94:909-950.

Zuckerman, B., M. Ron, M. Mikl, E. Segal, and I. Ulitsky. 2020. Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex. *Mol Cell*. 79:251-267 e256.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1943

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-500362

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2023