UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1768

Role of TGFβ-induced hyaluronan-CD44 signaling in cancer progression

CONSTANTINOS KOLLIOPOULOS

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2021

ISSN 1651-6206
ISBN 978-91-513-1279-8
URN urn:nbn:se:uu:diva-451907

Dissertation presented at Uppsala University to be publicly examined in C8:301, BMC, Husargatan 3, Uppsala, Tuesday, 19 October 2021 at 10:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor Jonathan Sleeman (Institute of Toxicology and Genetics, Karlsruhe Institute of Technology (KIT), Campus Nord, Karlsruhe, Germany.).

Abstract

Kolliopoulos, C. 2021. Role of TGFβ-induced hyaluronan-CD44 signaling in cancer progression. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1768. 60 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1279-8.

Hyaluronan, a prevalent glycosaminoglycan of the extracellular space often accumulates in pathological conditions, such as chronic inflammation, infection, and cancer. Hyaluronan synthase HAS2 has been responsible for the synthesis and deposition of hyaluronan in a variety of tumors. We have shown that HAS2 was required for efficient transforming growth factor β (TGFβ)-induced epithelial to mesenchymal transition (EMT), a developmental program which is commandeered by cancer cells to increase their migratory and invasive capacity. In study I, our findings show that long non-coding RNA Has2as has a key role in TGFβ- and Has2-induced breast cancer EMT, migration and acquisition of stemness.

Hyaluronan conveys its signaling properties via binding to its cell surface receptor CD44, a well-established stem cell marker in a plethora of tumors. CD44 exerts its signaling properties by interacting with components of the actin cytoskeleton machinery, and by acting as a co-receptor for other receptor tyrosine or threonine kinases impacting their signaling properties. Furthermore, CD44 is subjected to proteolytic cleavage, which eventually liberates the cytoplasmic tail (CD44-ICD). CD44-ICD translocates to the nucleus and alters gene expression. In study II, our findings support that TRAF4/6 mediates pro-tumorigenic effects of CD44, and suggests that inhibitors of CD44 signaling via TRAF4/6 and RAC1 may be beneficial in the treatment of tumor patients.

Glioblastoma (GBM) multiforme remains one of the most aggressive and lethal types of brain tumors worldwide with a poor prognosis. In study III, we have initiated studies to elucidate the CD44-dependent molecular mechanisms in GBM progression by knocking out (KO) CD44 by employing CRISPR/Cas9 gene editing in glioma U251MG cells.

Aberrant hyaluronan levels are also found during infectious diseases. In study V, we show that in a cohort study of dengue patients, high levels of circulating Dengue Nonstructural Protein 1 (NS1) correlate with high levels of serum hyaluronan. Moreover, we propose that hyaluronan can serve as a prognostic marker for the onset of warning signs during the course of dengue viral infection. Mechanistically, NS1 treatment-induced hyaluronan production contributing to increased vascular permeability.

In study IV, we have identified a bifurcating loop during TGFβ signaling, whereby transcriptional induction of NUAK1 serves as a negative checkpoint and NUAK2 induction positively contributes to signaling and terminal differentiation responses to TGFβ activity.

In summary, the current thesis provides mechanistic insights into the roles of TGFβ-induced hyaluronan-CD44 interactions in cancer progression.

Keywords: hyaluronan, CD44, TGFβ, cancer, EMT, signal transduction, transcriptional regulation

Constantinos Kolliopoulos, Department of Medical Biochemistry and Microbiology, Box 582, Uppsala University, SE-75123 Uppsala, Sweden. Science for Life Laboratory, SciLifeLab, Box 256, Uppsala University, SE-75105 Uppsala, Sweden.

© Constantinos Kolliopoulos 2021

ISSN 1651-6206
ISBN 978-91-513-1279-8
URN urn:nbn:se:uu:diva-451907 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-451907)

To my family

List of Papers

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

I. Kolliopoulos, C., Lin, C.-Y., Heldin, C.-H., Moustakas, A., Heldin, P. (2019) Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFβ-induced EMT in breast cancer. *Matrix Biology*, 80:29-45.

II. Kolliopoulos, C., Chatzopoulos, A., Skandalis, S.S., Heldin, C.-H., Heldin, P. (2021) TRAF4/6 is needed for CD44 cleavage and migration via Rac1 activation. *Cancers (Basel)*, 13(5):1021.

III. Kolliopoulos, C., Ali, M., Castillejo-Lopez, C., Heldin, C.-H., Heldin, P. Effect of CD44 on glioma cell progression, invasion and senescence. *Manuscript*.

IV. Kolliopoulos, C., Raja, E., Razmara, M., Heldin, P., Heldin C.H., Moustakas, A., van der Heide, L.P. (2019) Transforming growth factor β (TGFβ) induces NUAK kinase expression to fine-tune its signaling output. *The Journal of Biological Chemistry*, 294(11):4119-4136.

V. Lin, C.Y., Kolliopoulos, C., Huang, C.-H., Tenhunen, J., Heldin C.H., Chen, Y.-H., Heldin, P. (2019) High levels of serum hyaluronan is an early predictor of dengue warning signs and perturbs vascular integrity. *EBioMedicine*, 48:425-441.

Reprints were made with permission from the respective publishers.

Contents

Abbreviations

4-MU	4-methylumbelliferone
ADAM	A disintegrin and metalloproteinase protein
AMPK	Adenosine monophosphate-activated protein kinase
AP-1	Activator-protein 1
BMP	Bone morphogenetic protein
CAF	Cancer-associated fibroblast
CBP	CREB binding protein
ceRNA	competing endogenous RNA
CEMIP	cell migration inducing hyaluronidase
CD	Cluster of differentiation
CREB	Cyclic AMP-responsive element-binding protein
CRISPR	Clustered regularly interspaced short palindromic repeats
CSC	Cancer stem cell
CTC	Circulating tumor cell
ECD	Extracellular domain
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-to-mesenchymal transition
ERK	Extracellular signal–regulated kinase
ERM	Ezrin-radixin-moesin
ESRP1	Epithelial splicing regulatory protein 1
FGF	Fibroblast growth factor
GAG	Glycosaminoglycan
GBM	Glioblastoma multiforme
GF	Growth factor
HAS	Hyaluronan synthase
HAS2-AS1	HAS2 antisense RNA 1
HBP	Hexosamine biosynthetic pathway
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible factor
HMGA	High mobility group AT-hook
HMW	High molecular weight
HNRNPM	Heterogeneous nuclear ribonucleoprotein m
HYAL	Hyaluronidase
ICD	Intracellular domain

IL	interleukin
LMW	Low molecular weight
lncRNA	long non-coding RNA
LOX	Lysyl oxidase
MAPK	Mitogen-activated protein kinase
MET	Mesenchymal-to-epithelial transition
MMP	Matrix metalloproteinase
MT1-MMP	Membrane-type-1 matrix metalloproteinase
NET	Neutrophil extracellular trap
NMR	Naked-mole rat
NS1	Nonstructural protein 1
NUAK	Novel (nua) kinase
PDAC	Pancreatic ductal adenocarcinoma
PDGF	Platelet-derived growth factor
PDGFRβ	Platelet-derived growth factor β-receptor
PG	Proteoglycan
PI3K	Phosphatidylinositol 3′-kinase
PKC	Protein kinase c
RA	Rheumatoid arthritis
RAC1	Rac family small GTPase 1
ROS	Reactive oxygen species
RTK	Receptor Tyrosine kinase
TACE	Tumor Necrosis Factor alpha converting enzyme
TF	Transcription factor
TNBC	Triple-negative breast cancer
TNFα	Tumor necrosis factor alpha
TPA	12-*O*-tetradecanoylphorbol 13-acetate
TRAF	Tumor necrosis factor α receptor associated factor
SMAD	Small mothers against decapentaplegic
TGFβ	Transforming growth factor β
TβR	Transforming growth factor β-receptor
TME	Tumor microenvironment
UDP-GlcA	Uridine diphosphate glucuronic acid
UDP-GlcNAc	Uridine diphosphate *N*-acetylglucosamine
VEGF	Vascular endothelial growth factor
vHMW	exceptionally high molecular weight
xCT	Solute carrier family 7 member 11

Introduction

Tumor microenvironment

The tumor microenvironment (TME), or niche, is of pivotal importance throughout cancer progression and metastasis. In addition to tumor cells, it is composed of neighboring stroma cells, such as fibroblasts, immune and endothelial cells and extracellular matrix (ECM) (Hanahan and Coussens, 2012). ECM is a highly heterogeneous supramolecular entity produced by the adjacent cells with distinct biochemical, biomechanical and architectural properties. It mainly consists of collagens, glycoproteins, highly charged proteoglycans (PGs) and glycosaminoglycans (GAGs), whose differential synthesis and/or degradation lead to diverse cellular responses. In addition, ECM acts as a reservoir for inorganic molecules and signaling molecules, such as growth factors (GFs), chemokines and cytokines, including transforming growth factor β (TGFβ), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and others, which are deposited in the extracellular milieu; ECM cannot be defined exclusively by its composition; post-translational modifications of its constituents, such as hydroxylation, transglutamination, sulfation, glycosylation and proteolytic cleavage add to the complexity and to the three-dimensional characteristics of the matrix (Winkler et al., 2020).

The matrix components are used as building blocks for structures like basement membrane, which separates epithelia or endothelia from the surrounding interstitial tissue, which additionally forms another kind of matrix, less compact and with higher porosity, referred to as interstitial matrix. These ECM structures with their unique hydrodynamic features act as scaffolds, supporting the integrity of the aforementioned tissues. Because of this, ECM was for a long time considered to serve by providing mechanical support for tissue organization; however, apart from this inert space-filling role, a large body of evidence has accumulated over the last decades, which suggests that the ECM directly regulates cellular fates, such as proliferation, differentiation and migration. Thus, a reciprocal interaction between adjacent cells and matrix exists, since cells are responsible for its deposition and turnover, nonetheless, matrix itself affects cell behavior, a phenomenon annotated as dynamic reciprocity (Cox, 2021).

Matrix and cancer

During cancer development, mimicking embryonic progression, the ECM turnover is deregulated having a more profound role in proliferative, migratory and invasive capacities of tumor cells, than previously anticipated (Lu et al., 2012). Aberrant protein matrix degradation and turnover is mediated by a plethora of calcium- or zing-dependent peptidases belonging to a vast superfamily, including matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase proteins (ADAMs) and ADAMs with thrombospondin motifs (ADAMTSs). Their expression is often deregulated at both transcriptional and post-translational level in cancer, altering their temporal and spatial distribution, eventually generating 'tumor matrices' (Karamanos et al., 2019).

Increased matrix density is frequently identified in tumors, a phenomenon called desmoplasia. Desmoplasia is caused by excessive deposition of ECM molecules. Cancer-associated fibroblasts (CAFs) are considered to be the predominant source of aberrant ECM production and secretion in solid tumors. Although the precise origin of CAFs remains elusive, it is considered that part of them stem from adjacent endothelial or epithelial cells, which have undergone endothelial to mesenchymal transition (endoMT) or epithelial to mesenchymal transition (EMT) respectively. Alternatively, they may derive from mesenchymal stem cells of the bone marrow, which may be recruited in the TME. Furthermore, they may represent a subpopulation of fibroblasts, which have been 'educated' by the tumor parenchyma, exit their quiescent state and become activated into myofibroblasts. Apart from their dominant secretory role in ECM synthesis, CAFs are characterized by enhanced contractility properties, elevated metabolic activity and their contribution to rapid ECM remodeling (Kalluri, 2016). Tumor niche stiffening induces switch towards glycolysis not only in tumor cells, but also in CAFs, which results in a reciprocal amino acid exchange between CAFs and tumor cells, sustaining tumor growth and facilitating metastasis (Becker et al., 2020). Moreover, it has been proposed that they comprise key components at the leading edge of collectively moving tumor cells, facilitating their invasion and intravasation processes (Winkler et al., 2020), even in a 'non-invasive' way (Glentis et al., 2017). These pleiotropic actions of CAFs render them as major contributors in the TME.

Extensive collagen cross-linking, mediated by lysyl oxidase family members (LOX) for instance, significantly contributes to desmoplastic tumors, elevating intratumoral fluid pressure, perturbing drug penetration and efficacy. In parallel, total tension and matrix stiffness are also increased, leading to the transmission of mechanosensing cues. This process, known as mechanotransduction, elicits signals affecting cell-cell and cell-matrix adhesion (Cox, 2021). Integrins are major mediators of such signals intracellularly, by binding to their ECM ligands such as fibronectin (Johansson et al., 1997) and their

expression is dysregulated in both tumor and stromal cells (Hamidi and Ivaska, 2018).

Apart from the primary tumor, matrix remodeling occurs also at the metastatic niche. Alterations at the ECM of distant organs are evident, even before the stage of colonization, probably due to the secretion of extracellular vesicles stemming from the primary tumor or from circulating tumor cells (CTCs). Eventually, such alterations in organ-specific pre-metastatic niches foster a hospitable environment for the attachment and the colonization of CTCs (Kai et al., 2019). Interestingly, proteomic profiling of the ECM in metastatic sites at different tissues, revealed distinct matrisome composition (Hebert et al., 2020), exemplifying the 'seed and soil' hypothesis (Paget, 1889). For instance, myeloid cell-derived versican was found to mediate mesenchymal to epithelial transition (MET) of breast CTCs at lung metastatic niches upon their arrival, promoting their proliferation, eventually giving rise to overt metastases (Gao et al., 2012). Finally, modulation of ECM has been implicated also in the context of dormancy. Upon their arrival at the metastatic sites, disseminated CTCs may enter a quiescent state of dormancy, preserving them without being able to proliferate for several years, even decades. Sustained lung inflammation caused awakening of dormant CTCs via neutrophil extracellular traps (NETs) (Albrengues et al., 2018). NETs, which consist of externalized chromatin and secreted proteases, have been implicated in cancer-related organ failures (Olsson and Cedervall, 2016). NET formation at metastatic sites leads to cleavage of ECM glycoprotein laminin, revealing an epitope, which activates integrin signaling and proliferation in previously dormant cancer cells (Albrengues et al., 2018).

In summary, ECM deposition and remodeling is prevalent in solid tumors and tightly connected to multiple steps during cancer progression, providing opportunities for novel therapeutic targets or markers with diagnostic or prognostic value.

Hyaluronan-CD44 axis in cancer progression

Karl Meyer first isolated a polysaccharide, which was abundant in the vitreous body of the eye in 1934 and named it hyaluronic acid, later renamed hyaluronan, stemming from the greek word ὑαλος, which means glass (Meyer and Palmer, 1934). Apart from the vitreous body, hyaluronan constitutes a prevalent component of the ECM in the cartilage, skin, brain, umbilical cord and synovial fluid and plays a major role in cellular behavior both at physiological and pathological conditions, such as chronic inflammation and cancer (Toole, 2004). Hyaluronan, which falls into the molecular class of GAGs, is a large linear unbranched molecule, which consists of repeating dissacharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine linked by alternating β1-3 and β1-4 linkages (Fig. 1). As a result, it bears carboxyl groups, that at neutral pH are negatively charged, which renders it ideal for retaining large volumes of water and thus acts as lubricant, supporting tissue architecture (Laurent et al., 1995). Hyaluronan possesses signaling properties mainly executed after interacting with its cell surface receptors, such as cluster of differentiation 44 (CD44), a known homing receptor for lymphocytes (Aruffo et al., 1990; Goldstein et al., 1989; Stamenkovic et al., 1989) and hyaluronan mediated motility receptor (RHAMM) (Cheung et al., 1999).

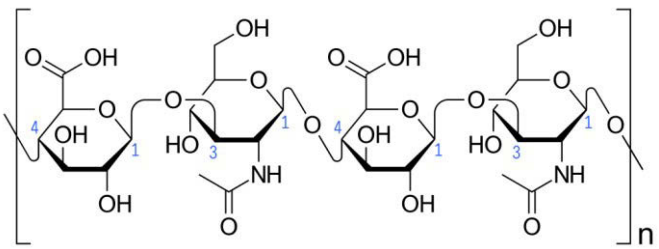

Figure 1. Hayworth representation of tetrasaccharide hyaluronic acid unit.

CD44 has been adequately characterized and physiologically has been involved in hematopoiesis and lymphocyte homing. CD44 is ubiquitous, being expressed in most cells and is encoded by a single and well-conserved gene containing twenty exons; exons 1-5 and 16-20 are always transcribed and are present in all isoforms, generating the standard isoform of CD44 (CD44s) (Fig. 2). CD44s is often annotated also as CD44H, since it was first purified from hematopoietic cells (Goldstein et al., 1989). The core protein comprises a 37 kDa polypeptide, nonetheless, CD44 is extensively subjected to both *O*- and *N*-glycosylation, eventually generating a 85-90 kDa glycoprotein (Lokeshwar and Bourguignon, 1991). Exons 6-15 can be alternatively spliced resulting in multiple variants of CD44, usually referred to as CD44v (variant1-variant10) (Goldstein et al., 1989) (Fig. 2). The first five exons of the standard isoform encode the amino-terminal globular protein domain, part of which share homology with the cartilage proteoglycan core and link proteins. This

part is referred to as 'Link' domain, which is stabilized by three disulfide bonds and is responsible for binding to hyaluronan (Naor et al., 1997). Nevertheless, a second site for binding of hyaluronan was identified outside the link region (Teriete et al., 2004). The extracellular domain (ECD) of CD44 is completed by exons 16 and 17 and together with part of exon 5 form the 'stem' region, which lies at the membrane proximal region, the juxtamembrane part of the ECD. The stem region comprises the inclusion site for the incorporation of variant exons, generating CD44v isoforms. Furthermore, the stem region contains putative sites for proteolytic cleavage performed by members of the MMP and ADAM family. Exon 18 encodes the hydrophobic transmembrane part of the glycoprotein, whereas exons 19 and 20 encode the cytoplasmic tail of CD44 (CD44-ICD) (Fig. 2). Exon 19 is spliced out, since its incorporation gives rise to a short cytoplasmic tail (Goldstein et al., 1989). CD44s is mostly expressed in cells of mesenchymal origin, or hematopoietic cells, whereas CD44v is widely expressed in cells of epithelial origin. For example, CD44v8-10 is also referred to CD44E representing the epithelial isoform, while the longest CD44 isoform CD44v2-10 is expressed in keratinocytes (Ponta et al., 2003).

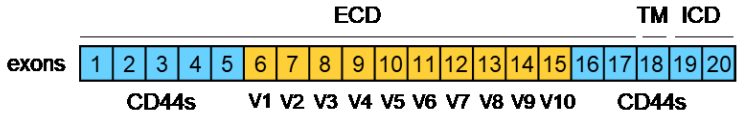

Figure 2: Genomic structure of *CD44* and corresponding coding regions. Exons depicted with blue color comprise the constant isoform CD44s. Inclusion of variant exons generate the different CD44v isoforms. ECD, extracellular domain; TM, Transmembrane domain; ICD, Intracellular domain (Heldin et al., 2020).

CD44 acts as a cell adhesion molecule binding to a variety of ECM components. Apart from being the primary receptor for hyaluronan as already described, CD44 can also bind to collagen, fibronectin, laminin, serglycin and osteopontin (Orian-Rousseau and Sleeman, 2014) (Fig. 3). Inclusion of exons v6 and v7 expands the repertoire for CD44 binding to other GAGs, such as chondroitin sulfate and heparin (Sleeman et al., 1997). Interestingly, CD44 does not possess any intrinsic kinase activity, nonetheless, is able to exert signaling via interactions of intracellular domain of CD44 and actin cytoskeleton-related proteins, mainly ankyrin (Lokeshwar et al., 1994), IQGAP1 (Bourguignon et al., 2005; Kozlova et al., 2012; Skandalis et al., 2010) and ezrin-radixin-moesin family (ERM) (Tsukita et al., 1994). Interestingly, the tumor suppressor protein merlin, encoded by the gene *NF2* keeps at bay these interactions and this partially accounts for its well-established tumor-suppressing role (Hartmann et al., 2015a). Related to the aforementioned tumor-

suppressing axis of hyaluronan/CD44/merlin, it was demonstrated, that hyaluronan synthase 2 (HAS2)-mediated exceptionally high molecular weight (vHMW) hyaluronan promoted contact inhibition, even in sparse cultures of naked-mole rat (NMR) skin fibroblasts. This effect was abrogated when HAS2 was knocked down, or upon treatment with hyaluronidase or an antibody blocking binding of CD44 to hyaluronan. NMRs are well known for their longevity and cancer resistance and noteworthy, when xenograft experiments with NMR cells were carried out in mice, those which were knocked down for HAS2 or overexpressing hyaluronidase-2 (HYAL2), readily formed tumors compared to the control cells (Tian et al., 2013). In contrast to HMW hyaluronan, vHMW hyaluronan protects not only NMR, but also mouse and human cells from stress-induced cell cycle arrest and cell death. vHMW hyaluronan binds to CD44, perturbing its protein-protein interactions and altering gene expression, eventually conferring these cytoprotective properties (Takasugi et al., 2020). These results clearly pinpoint the significance of hyaluronan size in tumor progression.

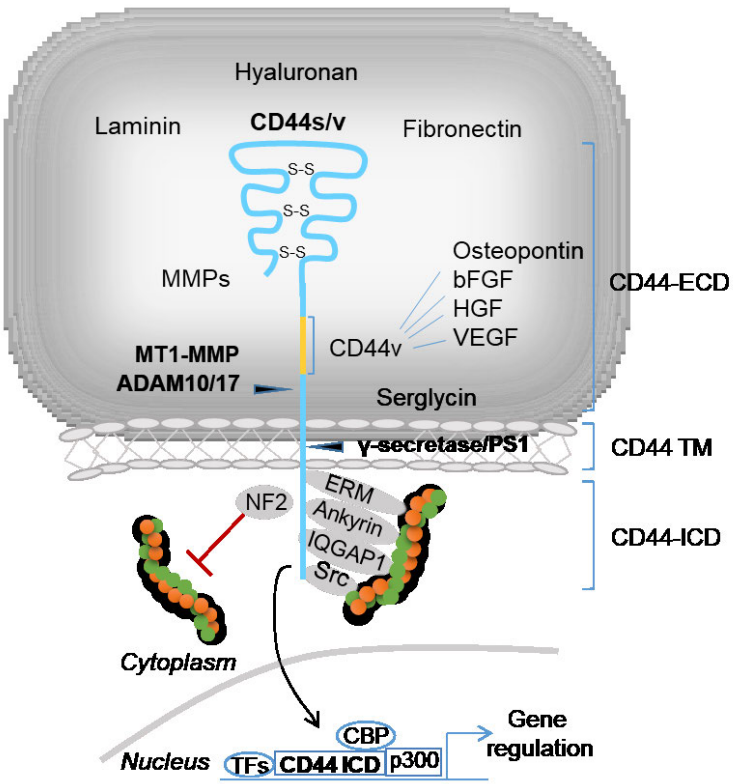

Figure 3: Protein structure of CD44. CD44s (blue) or CD44v with the optional inclusion of coding variants at the stem region (yellow) is shown. Three disulfide bonds stabilize the globular region. Various ligands, such as ECM glycoproteins, growth factors and proteases are also

demonstrated. In a context-dependent manner, CD44-ICD binds to tumor suppressor merlin or actin filaments-linking proteins such as members of the ERM family and ankyrin. Succeeding proteolytic cleavage events are catalyzed by members of the MMP and ADAM families and intramembranously by γ-secretase. The released CD44-ICD translocates to the nucleus, where it affects gene transcription (Heldin et al., 2020).

Except for its role in anchoring ECM constituents, CD44 has been characterized as a signaling hub, acting as a co-receptor for receptor tyrosine kinases (RTKs) or serine/threonine kinase receptors, modulating their signaling outcomes (Fig. 4). In particular, it has been reported that CD44 binds to hepatocyte growth factor (HGF) receptor Met (Orian-Rousseau et al., 2002) and vascular endothelial growth factor (VEGF) receptor VEGFR-2 (Tremmel et al., 2009), enhancing their signaling capacity upon their cognate ligand binding. The CD44v3 isoform is required for heparin sulfate binding, acting as a recruitment platform for MMP-7; MMP-7 cleaves heparin-binding epidermal growth factor (HB-EGF) and subsequently activates downstream receptor ErbB4 (Yu et al., 2002). The CD44v3 isoform is also required for HB-EGF-induced EGFR signaling and CD44v6 acts as a co-receptor for EGF-induced ErbB1 and NRG1-induced ERbB3 and ErbB4 activation in breast cancer cells (Morath et al., 2018). Noteworthy in glioma cells, it was reported that internalized CD44s in the endosomal compartment inhibited GTPase Rab7A-mediated EGFR trafficking to lysosomes and subsequent degradation (Wang et al., 2017). This exemplifies how CD44 can augment RTK signaling, without necessarily binding them directly. Interestingly, in a hyaluronan-dependent manner, CD44 acts as a co-receptor for CXCR4 signaling affecting angiogenesis (Fuchs et al., 2013). On the contrary, in a hyaluronan-independent manner, CD44 positively regulated WNT signaling by forming a complex with LRP6 (Schmitt et al., 2015). Moreover, CD44 potentiates TGFβ signaling in breast cancer cells, by directly binding to transforming growth factor β receptor type I (TβRI) (Bourguignon et al., 2002). Interestingly, in a yeast two-hybrid screen, CD44-ICD was found to interact with bone morphogenetic protein (BMP) downstream mediator Smad1, promoting Smad1 nuclear translocation and transcriptional activation, which was suppressed upon CD44 silencing (Peterson et al., 2004). Moreover, it has been illustrated that CD44 is able to immobilize and activate matrix MMP-9 at the cell surface, an event which leads to proteolytic cleavage and activation of latent TGFβ (Yu and Stamenkovic, 2000). On the contrary, in a hyaluronan-dependent manner, CD44 can act by attenuating PDGF β-receptor (PDGFRβ) signaling in human dermal fibroblasts (Li et al., 2006). In the same cells, CD44 interacted with both PDGFRβ and TβRI, and silencing of CD44 led to stabilization of both receptors and sustainment of their signals (Porsch et al., 2014).

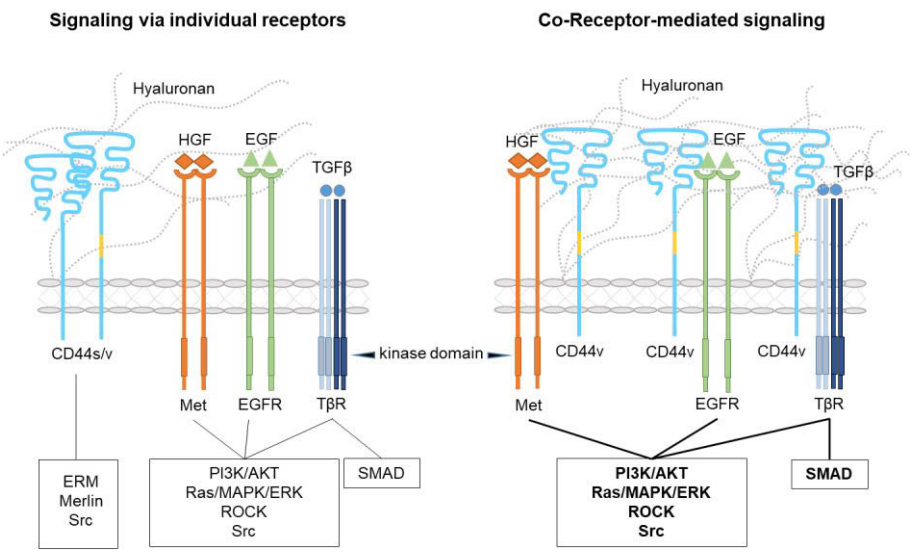

Figure 4: CD44 acts as a signaling hub, modulating RTK and Ser/Thr kinase receptor signaling outputs as in the case of HGF/Met and TGFβ/TβRI respectively. CD44s acts as a co-receptor by interacting with ligands and/or their cognate receptors. These interactions become more evident in the context of tumors, where signal transduction is sustained (Heldin et al., 2020).

Finally, it was recently reported that hyaluronan acts as a fence, forming a pericellular coat by binding to CD44, which act as a picket, tethering actin cytoskeleton to the membrane. This leads to perturbation of diffusion of phagocytic receptors in macrophages, obstructing access to their targets. Force application was sufficient to overcome this physical barrier, so that the receptors could diffuse efficiently to engage their targets (Freeman et al., 2018). All these studies outline the importance of CD44 in both hyaluronan-dependent and –independent signal transduction.

CD44 cleavage in cancer biology

CD44 cleavage occurs with high incidence in cancer. CD44 can be subjected to two sequential proteolytic cleavage events. Members of the MMP and ADAM family and in particular the membrane-bound MT1-MMP, also known as MMP-14, ADAM10 and ADAM17, also known as TACE (Nakamura et al., 2004) mediate the ectodomain (ECD) shedding of CD44 by recognizing putative sites at the stem region at the membrane-proximal region of the extracellular part of CD44. The resulting cleavage product, which consists of a short remnant of the ECD, the transmembrane region and the cytoplasmic tail is further processed by the catalytic subunit of protease γ-secretase

presenilin-1, at the intramembranous domain, eventually liberating the cytoplasmic tail CD44-ICD (Lammich et al., 2002; Murakami et al., 2003). From functional perspective, CD44 cleavage contributes to cell migration on a hyaluronan substratum (Okamoto et al., 1999). Noteworthy, following its release, CD44-ICD translocates to the nucleus, where it can modulate gene expression by activating the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-responsive element. Nuclear CD44-ICD forms a complex with the transcriptional activator CBP/p300, regulating the transcription of target genes, with *CD44* being a target gene itself. Thus, a positive feedback loop mechanism is established, in order to replenish CD44 levels constantly at the plasma membrane (Okamoto et al., 2001).

A plethora of external stimuli triggers CD44 cleavage, such as EGF (Murai et al., 2006), PDGF, HGF (Hartmann et al., 2015a) and TGFβ (Kuo et al., 2009), resulting in ectodomain shedding of CD44. As far the mechanism is concerned, signaling transduced by Ras oncoprotein (Kawano et al., 2000), Rho family of small GTPases members, mainly Rac1, protein kinase C (PKC), or influx of extracellular Ca^{2+} drives CD44 ectodomain shedding (Okamoto et al., 1999). Notably, both cleavage events are promoted also by hyaluronan oligosaccharides in various tumors (Sugahara et al., 2003, 2006). Interestingly, oligomerization of CD44 was a prerequisite for induced cleavage (Hartmann et al., 2015b). In addition, post-translational modifications of CD44-ICD affect its transcriptional activity, since dephosporylation at serine 291 was reported to be indispensable for its TPA-mediated cleavage (Parra et al., 2015). Interestingly, binding partners also exhibit a crucial role in the process, since interaction of CD44-ICD with merlin suppresses, whereas binding to ERM family members promotes its ectodomain cleavage, respectively (Hartmann et al., 2015a).

The proliferation of thyroid cancer cells is sustained by CD44 cleavage. CD44-ICD interacted with TF CREB, enhancing its activity. Subsequently CREB bound to the cyclin D1 promoter, inducing its expression and hence driving cell proliferation (Falco et al., 2012). More recently, CD44 cleavage was demonstrated to drive glioblastoma mutiforme (GBM) progression and was correlated with poor survival, by endowing the cancer cells with stem cell properties and rendering them radiation-resistant (Pietras et al., 2014). In particular, in perinecrotic and perivascular areas in gliomas, the ubiquitously expressed CD44 ligand osteopontin induces proteolytic cleavage of CD44, contributing to stabilization of HIF-2α, facilitating the prevalence of hypoxia-induced gene signature (Johansson et al., 2017). In line with these studies, it was shown that upon its translocation to the nucleus, CD44-ICD interacted with stem cells factors such as Nanog, Oct4 and Sox2, potentiating their transcriptional activity enhancing stemness capacity in breast cancer cells (Cho et al., 2015). Moreover, a CD44-ICD consensus binding element was identified at the promoter of the *PD-L1* gene, inducing PD-L1 expression, conferring immunosuppressive properties in breast and lung cancer cells (Kong et al., 2020).

Intriguingly, in a recent study, soluble CD44 extracellular domain derived from breast cancer cells promoted tumor progression, by triggering expression and secretion of IL1β in macrophages, outlining that soluble CD44 also possesses functional properties (Jang et al., 2020).

From a clinical perspective, the shed N-terminal CD44 circulated in the serum of patients with malignant tumors (Guo et al., 1994) or in arthritic synovial fluid (Haynes et al., 1991). In conjuction with these observations, CD44 cleavage products have been detected in extracts from human tumor tissues of brain, breast, lung and ovarian origin (Okamoto et al., 2002), and were also seen in articular chondrocytes of Rheumatoid arthritis (RA) patients (Takahashi et al., 2010).

In summary, it is plausible, that suppressing one of the two, or both, CD44 cleavage events would counteract aggressive phenotypes, and thereby invasion and aberrant gene regulation. In line with this notion, γ-secretase inhibitors significantly obstructed the pro-tumorigenic effects, which were manifested in the aforementioned studies in this section, suggesting that inhibition of CD44 cleavage is a possible therapeutic approach.

CD44 in EMT and stemness

A wide range of studies have established the impact of EMT in inflammation, cancer invasiveness and chemoresistance. During this process, mammary epithelial cells remodel their ECM, shed their junctions with the ECM and neighboring cells, undergo polarity and cytoskeletal reorganization and acquire a fibroblastic migratory phenotype (Derynck and Weinberg, 2019; Nieto et al., 2016; Thiery, 2002). CD44 has also been studied in this context. Dynamic formation of CD44-high expressing cells at the leading edge, assists collective invasion in luminal breast cancer cells (Yang et al., 2019). Notably, CD44 is expressed at much higher levels in invasive breast cancer cells compared to non-invasive ones (Heldin et al., 1996) and is a marker for cancer stem cells (CSCs) (Al-Hajj et al., 2003). CSCs represent a minor population of the tumor exhibiting self-renewing capacity, as well as multidrug resistance.

In the EMT-related context, it has been shown that, when mammary epithelial cells undergo EMT, a switch from variant isoforms (CD44v) to standard isoforms (CD44s) is evident, and is a prerequisite for this process. From a mechanistic perspective, the splicing factor epithelial splicing regulatory protein 1 (ESRP1) mediates the CD44 isoform switch and therefore is instrumental for regulating the EMT phenotype. The effect of this switch was activation of PI3K/AKT signaling, which is known to mediate EMT (Brown et al., 2011). Interestingly, nuclear ribonucleoprotein M (hnRNPM) was identified as a pivotal splicing regulator in CD44-dependent TGFβ-mediated EMT, since inhibition of ESRP1 led to hnRNPM-mediated CD44-exon skipping. This event

was found to be essential for breast cancer metastasis (Xu et al., 2014). Another study consolidated the aforementioned findings by identifying a gain of function mutation in the chromatin modulator MORC2, which enhanced its binding to hnRNPM driving CD44v to CD44s splicing switch and EMT in triple negative breast cancer (TNBC) (Zhang et al., 2018). Moreover, it was reported that CD44s was positively associated with the CSC gene signature and activated PDGFRβ/Stat3 cascade to promote stemness (Zhang et al., 2019). However, in another study the opposite trend was reported, with the switch from CD44s to CD44v, being required for efficient colonization of TNBC cells 4T1 to the lung. In the same study, it was shown that ESRP1 silencing led to abrogation of lung metastasis due to diminished levels of cysteine transporter xCT at the plasma membrane (Yae et al., 2012). CD44v was shown to be required for stabilizing xCT at the plasma membrane in gastrointestinal cancer cells, positively regulating redox homeostasis and protecting the cells from reactive oxygen species (ROS)-induced cell death (Ishimoto et al., 2011). Therefore, more studies are required to understand the mechanistic implications of CD44 isoform switching for the metastatic process. The observed ambiguities may be partially explained by cell-context dependence and the plasticity of EMT states in acquisition of stemness (Pastushenko and Blanpain, 2019).

Interestingly, interaction between mesenchymal stem cells (MSCs) and breast cancer cells led to CD44 translocation to the nucleus in a hyaluronan-dependent manner, increased transcription of LOX and subsequent induction of Twist leading to enhanced EMT in the cancer cells (El-Haibi et al., 2012). Noteworthy, hyaluronan induced, in a CD44v3-dependent manner, formation of complexes between Oct4, Nanog and Sox2, which are well established master transcription factors (TFs) of stemness, in head and neck squamus cell carcinoma (HNSCC) cells leading to enhanced self-renewal and clonal formation (Bourguignon et al., 2012). Additionally, CD44 interaction with Nanog in a hyaluronan-dependent context, resulted in increased expression of pluripotency-inducing TFs Rex1 and Sox2 in breast and ovarian tumor cells (Bourguignon et al., 2008).

Finally, a novel role of CD44 in modulating the epigenome was unveiled; it was reported that CD44 mediates iron endocytosis in a hyaluronan-dependent manner in several tumor cells. This resulted in enhanced activity of iron-dependent demethylases, removal of repressive histone marks at the promoters of mesenchymal genes, and thus promotion of EMT (Müller et al., 2020).

Regulation and function of HAS2 in cancer

There are three members of hyaluronan synthases, i.e. HAS1, HAS2, HAS3, which are multi-transmembrane proteins and catalyze hyaluronan synthesis in

their cytoplasmic parts. Hyaluronan synthesis takes place in almost all vertebrate cells and is stimulated by GFs and cytokines, including PDGF-BB, TGFβ (Heldin et al., 2009; Li et al., 2007a) and tumor necrosis factor alpha (TNFα) (Vigetti et al., 2010). HAS activity is dependent on many factors, including the availability of the substrates UDP-GlcNAc and UDP-GlcA (Jokela et al., 2011), and post-translational modifications, such as ubiquitination and phosphorylation. It has been reported that mono-ubiquitination of HAS2 and its dimerization are required for hyaluronan synthesizing capacity (Karousou et al., 2010). Interestingly, the deubiquitinating enzyme USP4 preferentially removes HAS2 mono-ubiquitination, whereas USP17 removes HAS2 polyubiquitination stabilizing its levels (Mehić et al., 2017). Hyaluronan biosynthesis and deposition are elevated in pathological conditions (Heldin et al., 2019). Obviously, anabolic reactions require energy and therefore activity of enzymes participating in these processes need to be tightly regulated. To this end, another study demonstrated that adenosine monophosphate-activated protein kinase (AMPK), which acts as an energy sensor of the cells, since it is activated when the ratio of AMP/ATP is high, was able to phosphorylate HAS2, an event which led to inhibition of hyaluronan synthesis in human aortic smooth muscle cells (Vigetti et al., 2011).

As far as cancer progression is concerned, HAS2 is crucial for the invasion of breast cancer cells (Li et al., 2007b; Udabage et al., 2005), and interestingly it exerts this function by negatively regulating tissue inhibitor of metalloproteinases-1 (TIMP-1) expression (Bernert et al., 2011). Notably, CD44s promoted activation of PI3K/AKT inducing HAS2 expression, with the elevated hyaluronan sustaining AKT activation, cementing a positive feedback circuit in breast cancer cells (Liu and Cheng, 2017). Importantly, the interaction between HAS2-synthesized hyaluronan and its cell surface receptor CD44, accounts for the adhesion of these cells to microvascular endothelium (Olofsson et al., 2014). HAS2 was found to be overexpressed in highly metastatic breast CSCs and was indispensable for crosstalk of these cells with tumor associated macrophages (TAMs). The consequence of this interaction was the secretion of PDGF-BB from TAMs which led to activation of the surrounding stroma and secretion of GFs and cytokines, like FGF7, FGF9, alongside BMP7, which enhanced the self-renewal properties of the metastatic cells (Okuda et al., 2012). In coherence with this study, FGF receptor activation in mammary tumor cells induced accumulation of HAS2-synthesized hyaluronan within the ECM and abrogation of its synthesis led to decreased proliferation, migration and therapeutic resistance in vivo (Bohrer et al., 2014). Similarly, sustained p38/MAPK signaling, driven by *KRAS* mutation in lung cancer, created a CAF-mediated hyaluronan-enriched TME, driving lung tumorigenesis (Brichkina et al., 2016).

HAS2 has been shown to play an indispensable physiological role in EMT; mice deficient in HAS2 are embryonically lethal, because of failure of executing EMT in the developing heart, which leads to abrogation of endocardial

cushion formation (Camenisch et al., 2000). Further highlighting the role of HAS2 in heart development, it was found that zebrafish *dicer* mutant embryos, which are depleted from their mature miRNAs, form excessive endocardial cushions. miR-23 was identified as a crucial factor in restricting the number of endocardial cells, which differentiate into endocardial cushion cells. HAS2 was identified as one of the primary targets of miR-23 (Lagendijk et al., 2011). Another study performed in zebrafish also, illustrated that knockdown of HAS2 leads to loss of hyaluronan coating and severe defects in migratory capacity of ventrolateral cells during gastrulation. As a result, these cells fail to develop lamellipodia and migrate dorsally. Mechanistically, this event is mediated by hyaluronan-induced activation of Rac1 (Bakkers et al., 2004). It is worth mentioning, that a study performed by utilizing conditional transgenic mice overexpressing HAS2 in spontaneous mammary tumors, revealed increased intratumoral stroma enriched in hyaluronan-versican aggregates and enhanced neovascularization. Concurrently, tumor cells exhibited mesenchymal traits, low E-cadherin expression and increased nuclear β-catenin localization, both hallmarks of EMT (Koyama et al., 2007). Importantly, expression of HAS2 switches the epithelial phenotype of mesothelioma cells to a mesenchymal migratory one (Li and Heldin, 2001) and plays an indispensable role in TGFβ-mediated EMT (Porsch et al., 2012).

Cells which have undergone EMT are endowed with stem cell (SC) properties (Mani et al., 2008), which prompts a deeper investigation of the role of HAS2/hyaluronan in the maintenance and expansion of CSCs (Skandalis et al., 2019). Under physiological conditions in normal stem cell niches, MSCs produced elevated amounts of hyaluronan coats, which are important for maintaining SC properties (Qu et al., 2014). In a recent study, it was demonstrated that cancer mammary epithelial cells overproducing hyaluronan, mediated by HAS2, underwent EMT in vivo and acquired SC traits through upregulation of TGFβ and subsequent activation of specific TFs, such as Snail and Twist, which are known to act as master inducers of the EMT process (Chanmee et al., 2014). To sum up, the HAS2/hyaluronan/CD44 axis acts on many different levels, which converge to the propagation and maintenance of CSCs, shedding more light on the molecular events driving this process.

From early on, it was widely considered that hyaluronan-CD44 interactions are implicated in the induced glycolytic phenotype in cancer cells, a phenomenon known as the Warburg effect. CD44 was shown to form complexes with the multifunctional glycoprotein EMMPRIN and monocarboxylate transporters at the plasma membrane, stabilizing their levels and supporting efflux of endogenously produced lactate due to enhanced glycolysis in breast carcinoma cells (Slomiany et al., 2009). Metabolic rewiring is an established hallmark of cancer and correlates with the metastatic process (Bergers and Fendt, 2021). Importantly, elevated HAS2-mediated synthesis of hyaluronan resulted in accelerated metabolic flux in the hexosamine biosynthetic pathway (HBP), a biosynthetic branch derived from the glycolytic pathway. Concomitantly a

shift to glycolysis resulted in accumulation of the TF HIF-1α and maintenance of a stem-like state (Chanmee et al., 2016). UDP-GlcNAc is synthesized via the HBP and acts as a donor substrate for protein *O*-GlcNacylation. Increased overall protein *O*-GlcNAcyaltion and hyaluronan synthesis are highly correlated with poor prognosis in advanced breast cancer (Chokchaitaweesuk et al., 2019). These studies highlight metabolic reprogramming in hyaluronan-overproducing breast cancer cells, as a novel potential mechanism for maintaining cancer stem-like properties. Furthermore, inhibition of fatty acid synthase (FASN) diminished CD44 protein levels, attenuating RTK signaling and reducing metastatic potential in colorectal cancer (Zaytseva et al., 2012). Thus, a reciprocal dependence of hyaluronan-CD44 signaling and metabolic reprogramming drive cancer progression.

Hyaluronan catabolism is aberrantly regulated in cancer development

Apart from its synthesis, hyaluronan catabolism is of equal importance for homeostasis. Thus, it is plausible that hyaluronan metabolism is deregulated during chronic inflammatory conditions and in several carcinomas (Heldin et al., 2014). In particular, hyaluronan size seems to be the crucial cue, eventually leading to distinct biological activities. For instance, there is a wide line of evidence, that HMW hyaluronan suppresses inflammatory responses. On the contrary, when hyaluronan is degraded, generating low molecular weight constructs (LMW) HA, its lubricant abilities are abolished, e.g. in synovial fluid of rheumatoid arthritis (RA) or osteoarthritis (OA) patients, leading to chronic inflammation (Vuorio et al., 1982). Hyaluronan catabolism is mediated by ROS, in a non-enzymatic manner, and therefore it has a protective role by acting as a scavenger (Zhao et al., 2008), or enzymatically by hyaluronidases encoded by six genes in total. They are separated into two clusters at distant chromosomal loci, i.e. 3p21.3 (*HYAL1, HYAL2* and *HYAL3*) and 7Q31.3 (*HYAL4, SPAM1* and *PHYAL1). PHYAL1* is a pseudogene and therefore it doesn't give rise to protein, whereas HYAL4 and PH-20/SPAM1 are tissue specific, and HYAL3 acts mainly as a chondroitinase; thus HYAL1 and HYAL2 are the most well characterized enzymes in hyaluronan catabolism (Stern and Jedrzejas, 2006). Intriguingly, HYAL2 translocates to the nucleus, where it regulates splicing of *CD44* pre-mRNA, mediating inclusion of variant exons 10 and 11, generating the antifibrotic CD44v7/8 isoform (Midgley et al., 2017).

Interestingly, recently two novel hyaluronidases have been identified. Silencing of HYAL1, HYAL2 and CD44 failed to account for the loss of hyaluronan in human skin fibroblasts, which led to the discovery of a new enzyme

possessing hyaluronan degrading capacity termed as hyaluronan binding protein involved in hyaluronan depolymerization (HYBID) or CEMIP, encoded by *KIAA1199* (Yoshida et al., 2013). Since then, CEMIP has been implicated in breast and colorectal cancer progression (Fink et al., 2015; Zhang et al., 2014) and cytokines and GFs, like TGFβ, PDGF-BB, EGF and bFGF, can modulate its expression levels (Nagaoka et al., 2015). Interestingly, in breast cancer cells, CEMIP was elevated in exosomes from brain but not lung or bone metastatic tumors, highlighting its role in connecting metastasis to organ tropism (Rodrigues et al., 2019). In addition, CEMIP-homologous protein transmembrane protein 2 (TMEM2) or CEMIP2 was also found to degrade hyaluronan at the cell surface (Yamamoto et al., 2017). Via using a whole-genome CRISPR knockout screen, TMEM2 was identified as a prevalent modulator of ER-stress resistance. Mechanistically, TMEM2-mediated hyaluronan fragments activate ERK- and p38-MAPK pro-survival signaling downstream of CD44, providing viability under stress (Schinzel et al., 2019). These studies outline the importance of hyaluronan catabolism in homeostasis and cancer.

Targeting hyaluronan-CD44 axis as therapeutic approach

It becomes apparent from the aforementioned sections, that targeting hyaluronan-CD44 signaling could pose a prudent therapeutic approach, worth interrogating further. Thus, targeting of hyaluronan synthesis and deposition, and interference of hyaluronan-CD44 interactions or interactions between CD44 and co-receptors and ligands have been investigated. Hyaluronan-mediated drug delivery or application of anti-CD44 antibodies have been proposed and tested, however, despite encouraging early results, many of these treatments exerted severe off-target effects and had to be discontinued (Platt and Francis C. Szoka, 2008).

Since its discovery as a potent inhibitor of hyaluronan synthesis a coumarin derivative, 4-methylumbelliferone (4-MU), has been used to abrogate hyaluronan synthesis in several tumors both *in vitro* and *in vivo* (Nagy et al., 2015). Mechanistically, 4-MU suppresses hyaluronan synthesis by depleting the substrate donor UDP-GlcA (Kakizaki et al., 2004). For example, 4-MU suppressed migration, adhesion and invasion in ERα-negative breast cancer cells (Karalis et al., 2019), highlighting the impact of hyaluronan inhibition in breast cancer progression. Nonetheless, due to its non-specific nature, reservations have risen, regarding its use for specifically targeting hyaluronan in solid tumors.

Chemotherapy treatments and surgical resection of tumors often leads to the formation of a pro-fibrotic environment, yielding desmoplasia. Tumor

stiffness affects response to chemotherapy; as exemplified in the case of breast cancer, softer tumors are more sensitive to therapeutic agents compared to stiffer tumors (Falou et al., 2013). Hyaluronan and chondroitin sulfate, as large anionic GAGs, retain high levels of water; augmented hydration leads to an increase in hydrostatic pressure, potentially perturbing perfusion and diffusion of therapeutic agents. To this end, hyaluronan degradation has been proposed to compromise this effect and allow chemotherapeutic agents to reach their targets, by decreasing interstitial fluid pressure. Intratumoral injections of pegylated hyaluronidase PEGPH20 in combination with chemotherapies have been used in clinical trials against pancreatic tumors, with mixed outcomes (Cutsem et al., 2020; Hingorani et al., 2017; Ramanathan et al., 2019).

TGFβ acts as a potent pro-fibrotic factor, highly contributing to desmoplasia (Caja et al., 2018). To this end, pirfenidone, which was originally developed as pulmonary anti-fibrotic agent, has been shown to act by impairing TGFβ-mediated increased synthesis and deposition of ECM components collagen type I and hyaluronan by CAFs, decreasing desmoplasia. Noteworthy, pirfenidone has been reported to suppress the growth of demoplastic breast tumors and metastasis to the lung (Takai et al., 2016). Similar effects were observed in highly fibrotic pancreatic tumors (Kozono et al., 2013). Therefore, targeting hyaluronan in desmoplastic PDAC would be a promising therapeutic approach (Koltai et al., 2021). In parallel, perturbation of CD44v6 interactions with its ECM ligands, such as hyaluronan and osteopontin, or RTK receptors Met and VEGFR-2 by the use of anti-CD44v6 soluble peptide or monoclonal antibody has been proposed in PDAC and colorectal cancer (Ma et al., 2019).

Targeting cancer-specific glycoconjugates also gain momentum, due to recent advancements in studying glycomics (Pinho and Reis, 2015). Interestingly, CD44 has been identified to be highly sialylated in hepatocellular carcinoma (HCC). Treatment with the sialidase NEU4 cleaved the sialic acid chains in CD44, increasing its adhesion to hyaluronan, possibly to an extent inhibiting motility of HCC cells (Zhang et al., 2021). It will be interested to examine the cancer-specific sialylated form of CD44 further as potential therapeutic target.

To sum up, the role of both hyaluronan and CD44 in cancer biology has been increasingly appreciated during the recent decades. Thus, further investigation of targeting hyaluronan, CD44 or both in a combinational treatment, may prove fruitful, mainly in desmoplastic tumors with high hyaluronan content, in order to combat cancer progression and metastasis.

TGFβ family and signal transduction

The TGFβ family encompasses pleiotropic cytokines encoded by 33 genes in mammals, giving rise to three TGFβ isoforms TGFβ1, TGFβ2 and TGFβ3, as well as several bone morphogenic proteins (BMPs), growth and differentiation factors (GDFs) and inhibins, and the anti-Müllerian factor (AMF). TGFβ is secreted as a latent polypeptide sequestered in the ECM, having limiting access to its cognate receptors. Upon activation by proteolysis or conformational changes, the mature dimeric polypeptide ligand binds to a heterocomplex of TGFβ receptors formed by two type I (TβRI) and two type II (TβRII) receptor proteins, which possess dual specificity Ser/Thr and Tyr kinase activity, albeit their Tyr kinase activity is weak (Tzavlaki and Moustakas, 2020). Seven TβRI and five TβRII receptors have been identified in mammals. Upon ligand binding, TβRII trans-phosphorylates and activates TβRI which consequently phosphorylates the receptor-activated mothers against decapentaplegic homolog (R-SMADs) in their C-terminals (SMAD2 and SMAD3 in the case of TGFβ, and SMAD1, SMAD5 and SMAD8 in the case of BMPs). When R-SMADs are phosphorylated they assemble heterocomplexes with a common mediator SMAD4 (co-SMAD) which translocate into the nucleus, where they regulate gene expression, by acting as TFs (Heldin and Moustakas, 2016).

In addition, apart from the SMAD pathway, TGFβRI can recruit and activate the ubiquitin ligase tumor necrosis factor α receptor associated factor 4 (TRAF4) and 6 (TRAF6) which play a key role in mediating the non-SMAD pathways. TRAF4 and TRAF6 are able to ubiquitinate and activate TGFβ-activated kinase 1 (TAK1) which subsequently phosphorylates and activates mitogen-associated protein (MAP) kinase p38 and c-Jun N-terminal kinase (JNK). The Phosphatidylinositol 3′-kinase (PI3K)/ Protein kinase B (PKB)/AKT pathway is also activated. The tyrosine-protein kinase Src and extracellular signal–regulated kinases 1/2 (ERK1/2) have been reported to be activated as well downstream of the TGFβ receptors (Mu et al., 2011; Zhang et al., 2013). Moreover, TRAF6 promotes activation of proteases, like TACE and Presenilin 1 (PS1), which sequentially cleave the TβRI first at the transmembrane domain and thereafter at the intracellular part generating a cytosolic fragment (TβRI-ICD). Upon release, the TβRI-ICD translocates to the nucleus altering transcription of various target genes by acting as a TF (Gudey et al., 2014). Furthermore, TβRII can phosphorylate the polarity complex protein PAR6, which recruits another ubiquitin ligase, called SMAD Specific E3 Ubiquitin Protein Ligase 1 SMURF1, which polyubiquitinates and signals degradation of the small GTPase Ras homolog gene family, member A (RhoA). As a consequence, actin microfilaments depolymerize leading to the collapse of tight junctions, which is a necessary event for the EMT process (Moustakas and Heldin, 2016).

Dual role of TGFβ in cancer progression

The action of TGFβ is well studied in epithelial cells, and arguably exerts dual roles in carcinoma development acting, as tumor suppressor at the onset of the disease and as a tumor promoter at later stages, a phenomenon known as the TGFβ paradox. Its tumor suppressive role is predominantly attributed to its potent growth arresting effect on pre-malignant cells primarily at the G1 phase of the cell cycle, abrogating their ability to proliferate, which protects from uncontrolled tumor growth. In particular, TGFβ induces cyclin-dependent kinase inhibitor (CDKi genes) like *CDKN2B* and *CDK1A*, encoding p15 and p21, respectively, whereas at the same time it directly represses the transcription of established genes for mitogenic factors, like *c-Myc* and inhibitor of differentiation (ID) family members (*ID1, ID2, ID3*). Apoptosis is also induced by TGFβ in a panel of epithelial cells and is mediated by transcriptional activation of TGFβ Inducible Early Gene-1 (TIEG1). Death-associated protein kinase 1 (DAPK) and Signaling Inositol Polyphosphate Phosphatase (SHIP) are also direct target genes of TGFβ in hepatoma and haematopoietic cells, respectively, driving apoptosis (Pardali and Moustakas, 2007).

Nevertheless, when epithelial tumor cells succeed in overriding the anti-mitogenic effects of TGFβ, by undergoing EMT, they acquire migratory and invasive properties important for intravasation to the bloodstream (Tsubakihara and Moustakas, 2018). Moreover, it was reported that SMADs collaborate with other TFs, including members of the AP-1 family, to promote breast cancer invasiveness (Sundqvist et al., 2012). Interestingly, RAS and TGFβ signaling co-operate, by activating ΔNp63 transcriptional activity, to enhance cancer progression (Vasilaki et al., 2016). Furthermore, TGFβ converge with other signaling pathways, such as EGF signaling, in the case of HER2+ and/or EGFR+ breast cancer cells, to promote invasiveness (Sundqvist et al., 2020). TGFβ also exerts tumor promoting effects, by acting on non-tumor cells, e.g., by inhibiting growth and activation of T lymphocytes. Additionally, TGFβ promotes angiogenesis, by driving the transcription of VEGF and connective tissue growth factor (CTGF) in both epithelial and endothelial cells. Last but not least, TGFβ also participates actively in metastatic niches to create an environment fostering tumor growth. It has been reported for instance that upregulation of CTGF and the osteoclastic differentiation factor interleukin-11 (IL11) at the transcriptional level, as well as sustaining increased protein levels of another osteoclast-differentation factor, parathyroid-hormone-related peptide (PTHrP), are indispensable events for bone colonization of breast cancer cells. Noteworthy, consequent osteolysis leads to increased release of ECM-deposited TGFβ from the remodeled bone, generating a vicious cycle, which eventually results in formation of macrometastases (Siegel and Massagué, 2003).

Gene regulation by chromatin modifying factors

Transcription of the genome requires a concerted action of TFs and co-factors, which eventually recruit and activate the RNA polymerase II. Chromatin is highly organized and tightly regulated and can be divided into two categories based on its status, i.e. hetero- and euchromatin. Heterochromatin represents a highly condensed form of chromatin, being inaccessible to TFs and other required components of the transcriptional machinery and hence, transcription is repressed. On the other hand, when chromatin adopts a more loosened conformation, a state referred to as euchromatin, TFs gain access to their respective DNA binding elements and thus transcription can be initiated. Accordingly, chromatin architectural regulators mediate the tight balance between the two states, which is deregulated during cancer development. Non-histone high mobility group (HMG) proteins fall into this category of modulators.

HMGA family members HMGA1 and HMGA2 directly bind to DNA and alter its structure, leading to either activation or repression of gene expression. They utilize three DNA binding regions known as 'AT hooks', recognizing AT-rich sequences in the minor groove of DNA (Fusco and Fedele, 2007). HMGA protein levels are induced during embryogenesis, however, they are barely expressed in adult tissues. Particularly, HMGA2 is predominantly expressed in the mesenchyme, but is also upregulated in tumors of epithelial origin (Fedele and Fusco, 2010). HMGA2 has been found to play a crucial role in heart development, since knockdown of HMGA2 abrogated cardiomyocyte differentitation, both in vitro and in vivo (Monzen et al., 2008). TGFβ induces HMGA2, which is required to elicit EMT in mammary epithelial cells, by upregulating the master EMT-TFs Snail and Twist (Tan et al., 2012; Thuault et al., 2008). Of notice, HMGA2 exerts also an epigenetic transcriptionally repressing role in the promoter of *CDH1* gene which encodes for E-cadherin. The outcome of this event is silencing of *CDH1*, loss of E-cadherin expression and increase in cell invasion (Tan et al., 2015). In vivo, HMGA2 was identified at the invasive front of human and mouse tumors. In the same study, overexpression of HMGA2 in non-metastatic breast cancer cells converted them to metastatic ones which created lesions in the liver, highlighting the role of HMGA2 in metastasis (Morishita et al., 2013).

Another chromatin remodeling mechanism involves a relatively novel class of biomolecules in this category, the long non-coding RNAs (lncRNAs). LncRNAs are defined as the non-coding RNAs with size longer than 200 nucleotides, whereas microRNAs (miRNAs) are shorter than 20 nucleotides. LncRNAs also serve as precursor to miRNAs. LncRNAs are transcribed by RNA polymerase II and can be capped, spliced and poly-adenylated like mRNAs. However, they are devoid of long or functional open reading frame, and therefore they cannot give rise to proteins (Statello et al., 2020). Some years ago, lncRNAs were deemed as 'transcriptional noise', since they are

poorly conserved and their transcriptional levels are low. It was only very recently that the functional roles of lncRNAs have started to be appreciated in physiological conditions and in the context of cancer and inflammation (Ling et al., 2015).

One group of lncRNAs is transcribed from the opposite strand of the transcriptional units and are annotated as antisense lncRNAs. Antisense lncRNAs may act either in *cis* or *trans* manner, relative to the gene locus that encodes them affecting gene transcription. If they act on genes in the close vicinity of their own gene, then they belong to the first category (*cis*); the latter (*trans*) includes the antisense lncRNAs which exert their actions on genes at distant loci (Villegas and Zaphiropoulos, 2015). Usually the lncRNAs, which act in *cis* fashion and interact with the chromatin, tend to act as enhancer-associated RNAs (eRNAs) by forming chromatin loops, or recruiting protein factors, which change the histone code or the DNA methylation status locally. In the context of cancer, a wide body of evidence implicates lncRNAs in both TGFβ signaling and the EMT process (Papoutsoglou and Moustakas, 2020). Following transcriptomic analysis, it was reported that TGFβ-induced lncRNAs TGFB2-AS1 (Papoutsoglou et al., 2019) and MIR100HG (Papoutsoglou et al., 2021), which in feedback circuits, affect TGFβ signaling in a negative and positive way respectively. TGFβ also induced the expression of a specific lncRNA, called Hotair, which triggered the EMT program. Interestingly, ablation of Hotair expression prevented the EMT program and also the colony-forming capacity of colon and breast cancer cells (Alves et al., 2013). Another lncRNA, MALAT1, was found to be overexpressed in oral squamous cell carcinoma tissues and was shown to be required for sustaining EMT, migration and invasion (Zhou et al., 2015).

Interestingly, from the HAS family, HAS2 is the only member, whose gene locus encodes a natural antisense RNA (*HAS2-AS1*), which is transcribed at the opposite strand of the DNA relative to the protein coding mRNA. Importantly, among promoters of the *HAS* family genes, it has been shown that the *HAS2* promoter is the least active under normal conditions, nonetheless, it becomes readily inducible upon external stimuli by e.g. GFs and cytokines. The *HAS2* promoter contains conserved binding elements for multiple TFs, including CREB (Makkonen et al., 2009), NF-kB (Saavalainen et al., 2007), STAT3, RAR (Saavalainen et al., 2005), YY1 (Jokela et al., 2011), ZEB1 (Preca et al., 2017), E2F-myc (Monslow et al., 2003), and SP1/3 (Monslow et al., 2006) (Fig. 5). It has been proposed that due to complementarity between exon 2 of *HAS2-AS1* with exon 1 of *HAS2* mRNA, the *HAS2:HAS2-AS1* RNA duplex formation leads to enhanced stability of the coding mRNA of *HAS2* (Michael et al., 2011). Moreover, upon *N*-acetyl-D-glucosamine treatment, p65, one of the subunits of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) was *O*-GlcNAcylated and bound to the *HAS2-AS1* promoter. This event drove transcription of *HAS2-AS1*, which altered the accessibility for the *HAS2* gene, by acting in cis manner, inducing *HAS2* expression

(Vigetti et al., 2014). Apart from its role in the nucleus, *HAS2-AS1* exerts its functions in the cytoplasmic compartment also, by acting as a competing endogenous RNA (ceRNA), sponging *miR-137* and promoting invasion and proliferation of glioma cells (Lu et al., 2021; Wang et al., 2020). Noteworthy, *HAS2-AS1* has been identified as a hypoxia-inducible lncRNA in oral squamous cell carcinoma (OSCC), bearing a hypoxia-related element (HRE) at its promoter (Fig. 5). In a HIF1α-dependent induction, *HAS2-AS1* promoted EMT of OSCC cells, by stabilizing HAS2 (Zhu et al., 2017). Finally, *HAS2-AS1* has been correlated with poor prognosis in papillary thyroid cancer (Ma et al., 2016). Contradictory to these findings, inducible overexpression of *HAS2-AS1* in osteosarcoma cells decreased *HAS2* mRNA levels and secretion of hyaluronan was diminished, leading to a reduction in their proliferation rate (Chao and Spicer, 2005) and indicating cell context-dependent regulation of HAS2 by *HAS2-AS1*.

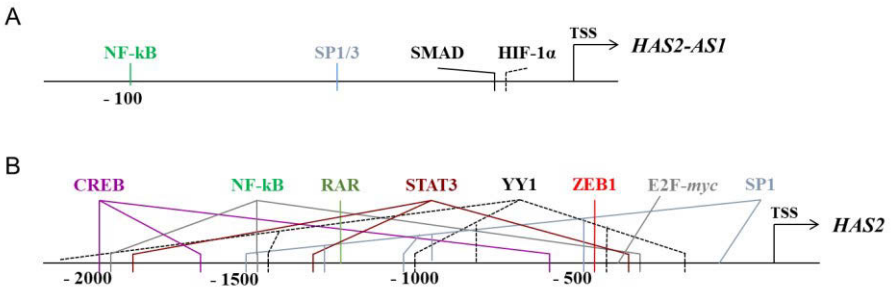

Figure 5. Transcription factor binding elements in the promoters of *HAS2-AS1* (**A**) and *HAS2* (**B**), which have been characterized (Heldin et al., 2019).

Hyaluronan and vascular integrity in viral infections

Hyaluronan comprises a prevalent component of the glycocalyx, a glycoconjugate-based ensemble covering the cell surface. Hyaluronan-CD44 interactions have been highlighted in leukocyte homing during inflammatory processes (DeGrendele et al., 1997). Both hyaluronan and CD44 have been implicated in the pathogenesis of viral infections (Heldin et al., 2020). In vascular tissues, hyaluronan has a major role in supporting vascular integrity, and its removal from the glycocalyx perturbs the well-organized endothelial cell-cell junctions (Jackson, 2019). Intruding pathogens, such as viruses, often dysregulate the glycocalyx, impairing endothelial barrier function and causing leakage. For instance, flavivirus-derived protein component nonstructural protein 1 (NS1) impairs vasculature, by disrupting endothelial glycocalyx components causing hyperpermeability in tissue-specific manner (Puerta-Guardo et al., 2016, 2019). Increased serum hyaluronan levels have been reported in

the case of Dengue virus infection, which associated with disease severity (Tang et al., 2017), indicating that circulating hyaluronan may be exploited as prognostic marker. Interestingly, hyaluronan-CD44 interactions have been implicated in the pandemic severe acute respiratory syndrome (SARS) coronavirus disease (COVID-19), being caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Recently, it was shown that circulating hyaluronan was elevated in plasma of COVID-19 patients, and treatment of endothelial cells with these isolated hyaluronan fragments induced vascular hyperpermeability, in a ROCK- and CD44-dependent manner, compared to treatment with plasma from healthy donors (Queisser et al., 2021). These studies outline the important role of hyaluronan-CD44 biology in supporting vascular integrity upon virus-triggered challenges.

AMPK-related protein kinases superfamily

The AMPK-related kinase family (ARKs) consists of 13 serine/threonine protein kinases (BRSK1, BRSK2, Nuak1, Nuak2, SNRK, QIK, QSK, SIK, MARK1, MARK2, MARK3, MARK4 and MELK), which share sequence similarity with the catalytic subunit of the AMP-activated protein kinase (AMPK). AMPK is mainly activated by AMP, which accumulates upon low ATP levels, and is related to whole body energy homeostasis. All ARKs can be activated by the master upstream kinases, like LKB1, TAK1 and CaMKKβ, which phosphorylate Thr172 in the activation loop of their catalytic domain (Bright et al., 2009). Although the role of AMPK in homeostasis and cancer progression has been studied, the involvement of the ARKs in these processes is poorly understood.

The novel (nua) kinase (Nuak) subfamily consists of two members, Nuak1 (also called AMPK-related kinase 5;ARK5) and Nuak2 (also called sucrose non-fermenting AMPK-related kinase; SNARK). Nuak2 was initially discovered as an ultraviolet-induced gene in human keratinocytes (Lefebvre et al., 2001) and can be activated under various types of stresses, DNA damage, oxidative stress, glucose or glutamine deprivation and low AMP:ATP ratio, to name just a few (Lefebvre and Rosen, 2005). It was also reported that Nuak2 levels were increased with muscle cell differentiation protecting myocytes from undergoing apoptosis (Lessard et al., 2016). Stress fibers play an important role in cellular contractility, regulating processes, such as cell adhesion and motility. Nuak2 has been found to modulate the function of the myosin regulatory light chain (MLC) phosphatase, by physically interacting with the myosin-phosphatase Rho-interacting protein (MRIP). This event leads to suppression of MYPT1, the regulatory subunit of the MLC phosphatase. Following this inhibition, formation of actin stress fibers is prolonged, controlling contraction of smooth muscle cells (Vallenius et al., 2011). Notably, MYPT1 is the only known substrate of Nuak2 to date (Yamamoto et al., 2008). In another interesting note, Nuak2 was found to be important in WIPI4-mediated autophagy (Bakula et al., 2017).

In pathological conditions, Nuak2 was found to regulate hepatitis C virus replication via enhancing TGFβ signaling eventually leading to hepatic fibrosis (Goto et al., 2013). Noteworthy, salt-inducible kinase (SIK) is a direct TGFβ target gene (Lönn et al., 2012) and was shown to negatively affect TGFβ signaling by downregulating the protein levels of TβRI (Kowanetz et al., 2008). These studies clearly establish a link between ARK family and TGFβ signaling. Nuak2 has also been implicated in cancer progression, being extensively studied in melanomas, where it affects cell cycle progression and cell migration (Namiki et al., 2011). The combination of genetically amplified *SNARK* and deletion of *PTEN* led to increased phospho-AKT levels, increased CDK2 expression and elevated growth of melanoma tumors (Namiki et al., 2015). Importantly, Nuak2 was identified as a critical mediator of YAP-driven

tumorigenesis in liver cancer (Yuan et al., 2018). In a feed forward mechanism, Nuak2 activates the Hippo pathway, by inhibiting LATS-mediated phosphorylation of YAP/TAZ in several cancer cells and in mammary tumors *in vivo* (Gill et al., 2018).

Nuak1 was demonstrated to interact with various myosin phosphatases and as in the case of Nuak2, it can physically interact with and phosphorylate MYPT1, and thus inhibit phosphatase activity, enhancing phosphorylation of MLC2 (Zagórska et al., 2010). Nuak1 contains a putative AKT phosphorylation motif which, when it is phosphorylated, activates AKT, resulting in elevated phosphorylation of ataxia-telangiectasia protein (ATM) and p53, mediating survival signaling (Suzuki et al., 2003a). In coherence with these findings, Nuak1 was shown to suppress cell death induced by nutrient starvation and death receptors in human hepatoma cells (Suzuki et al., 2003b).

Of interest, Nuak1 was found to be required for modulating AMPK activity and therefore ATP levels, in Myc-driven tumors by limiting protein synthesis mediated by mTORC1 protein kinase. Nuak1 depletion led to an induction of a plethora of pro-apoptotic signals both in vitro and in vivo in hepatocellular carcinoma, establishing it as a key factor for survival of tumor cells (Liu et al., 2012). Recently, Nuak1 was reported to be indispensable in mediating spliceosome activity in Myc-driven transcription, by inhibiting protein phosphatase 1 (PP1) (Cossa et al., 2020). Furthermore, Nuak1 was demonstrated to activate the polo kinase-1, indirectly via inhibition of the protein phosphatase 1β (PP1β), stimulating progression through the S phase (Banerjee et al., 2014). Moreover, elevated Nuak1 levels drove MMP-dependent invasion of cancer pancreatic cells (Suzuki et al., 2004), exerted tumor promoting effects in breast cancer cells (Chang et al., 2011), and Nuak1 levels were also upregulated in a panel of colorectal cancer cells (Kusakai et al., 2004). Opposite to the conclusions of these studies, Nuak1 was found to exert anti-tumorigenic actions as well, since it directly binds and phosphorylates p53 in a LKB1 activation-dependent manner leading to cell cycle arrest at the G1/S phase, by inducing expression of the CKi p21 (Hou et al., 2011). Loss of function screening in normal human diploid fibroblasts revealed Nuak1 as an inducible kinase mediating senescence upon ageing (Humbert et al., 2010), further supporting a tumor suppressing role for Nuak1.

Present Investigation

Aim and main findings of the studies

Hyaluronan synthesis is deregulated during cancer progression in various tumors. HAS2 has been responsible for the synthesis and deposition of hyaluronan in a plethora of tumors and adjacent stroma. The primary goal of study I was to delineate the molecular events, regulating the induction of *HAS2* in TGFβ-induced EMT, migration and stemness in breast cancer.

Hyaluronan conveys its signaling properties via binding to its cell surface hyaladherin receptors, such as CD44. Aberrant expression of CD44 isoforms occurs during cancer progression. CD44 doesn't possess any intrinsic kinase activity, therefore the way it exerts its signaling properties mainly depends on interacting molecules. As already discussed, full length CD44 can act as a co-receptor, while its tail associates with actin cytoskeleton components. Apart from that, upon CD44 cleavage, the released product CD44-ICD can translocate to the nucleus, altering transcription of target genes. The aim of study II was to elucidate the mechanism by which TGFβ-induced CD44 cleavage occurs in tumor progression.

Glioblastoma (GBM) multiforme remains one of the most aggressive and lethal types of brain tumors worldwide with a poor prognosis. The main attributes of GBM include high heterogeneity, increased invasive and proliferative capacity, chemoresistance, and a high rate of recurrence (Putavet and Keizer, 2021). In study III, we sought to investigate deeper the role of CD44 in GBM progression.

Aberrant hyaluronan levels are also found during infectious diseases. Hyaluronan has been shown to play a pivotal role in sustaining vascular integrity. Dengue is a vector-borne virus and infected patients during critical phase show severe bleeding. High levels of the Dengue Nonstructural Protein 1 (NS1) are found in the circulation and correlated to excessive cytokine production leading to vascular leakage. In study V, we examined the role of hyaluronan in dengue viral infection and how it may be involved in vascular leakage.

TGFβ signaling, via the downstream effectors of the SMAD family and protein kinase pathways, up- or down-regulates the expression of many genes, and thus affects physiological processes, such as differentiation, migration, cell cycle arrest, and apoptosis during developmental or adult tissue homeostasis. During cancer progression, TGFβ signaling is differentially modulated

to enhance its pro-tumorigenic properties. Earlier microarray screening in human breast cancer cells identified salt-inducible kinase (SIK), a member of the AMP-activated protein kinase (AMPK) superfamily, as a gene that was transcriptionally induced in response to TGFβ. SIK was found to negatively regulate TGFβ receptor signaling, by promoting TGFβRI turnover. Influenced by the impact of SIK on TGFβ signaling, in study IV, we performed a screen of all AMPK family members in established cell models, to examin their roles as regulators of TGFβ signaling.

Main findings:

Paper I: Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFβ-induced EMT in breast cancer
In paper I, we report a negative correlation between the expression of HAS2, its natural antisense transcript HAS2-AS, the chromatin architectural tool HMGA2 and TGFβ, and survival of patients with invasive breast carcinomas. In mammary epithelial cells, TGFβ induced transcription of *Has2*, *Has2as*, and *Hmga2*, as well as EMT-TFs, such as Snail. Importantly, *Has2as* silencing inhibited the TGFβ induction of EMT markers and the mesenchymal phenotype. TGFβ mediated hyaluronan synthesis was accompanied by activation of Akt and Erk1/2 MAP-kinases, which were necessary for breast cancer cell motility. Noteworthy, Cd44 facilitated TGFβ-mediated EMT. Interestingly, *Has2as* was found to contribute to the expression of stem cell factors, when breast cancer cells were grown in stem cell-like mammospheres. Our findings show that Has2as has a key role in TGFβ- and HAS2-induced breast cancer EMT, migration and acquisition of stemness.

Paper II: TRAF4/6 is needed for CD44 cleavage and migration via RAC1 activation
In study II, we report that CD44 cleavage is promoted by TGFβ in lung cancer cells in a manner dependent on the ubiquitin ligases TRAF4- and/or TRAF6. Lung cancer cells grown in stem cell-like spheres showed elevated TRAF4-dependent expression of CD44 variant isoforms, CD44 cleavage, and hyaluronan synthesis. Mechanistically, TRAF4 activated the small GTPase RAC1. CD44-dependent motility of lung cancer cells was suppressed upon TRAF4 silencing, which was rescued by the transfection of a constitutively active RAC1 mutant. We propose that TRAF4/6 mediates pro-tumorigenic effects of CD44, and that inhibition of CD44 signaling via TRAF4/6 and RAC1 may be beneficial in the treatment of tumor patients.

Study III: Effect of CD44 on glioma cell progression, invasion and senescence
In study III, we sought to elucidate the CD44-dependent molecular mechanisms in GBM progression by knocking out (KO) *CD44* in glioma cells. Proliferation rate was decreased and cell cycle inhibitor p16 was increased in

CD44-depleted cells. Importantly, the CD44 KO cells acquired a pro-senescence state. Interestingly, CD44 KO cells showed enhanced migratory and invasive properties, which may be partially attributed to the acquisition of a senescence-associated secretory phenotype (SASP). Transcriptomic analysis of stem-like U251MG cells, grown in spheres, unveiled a CD44-dependency for expression of molecules involved in hyaluronan synthesis and degradation, and of members of the PDGF and PDGF receptor families. These observations highlight the importance of CD44 on GBM progression.

Study IV: Transforming growth factor β (TGFβ) induces NUAK kinase expression to fine-tune its signaling output

In study IV, we identified AMPK family members NUAK1 and NUAK2 as two TGFβ target genes. We found that TGFβ-mediated transcriptional induction of NUAK1 and NUAK2 was dependent on both SMADs and MAPK pathways. Importantly, NUAK2 associated with SMAD3 and the TGFβRI. From a functional perspective, NUAK1 inhibited and NUAK2 enhanced TGFβ-mediated epithelial cell cycle arrest, mesenchymal differentiation, and myofibroblast contractility. In summary, we have identified a bifurcating loop during TGFβ signaling. Transcriptional induction of NUAK1 serves as a negative modulator, whereas NUAK2 induction positively contributes to TGFβ signaling and mediated responses.

Study V: High levels of serum hyaluronan is an early predictor of dengue warning signs and perturbs vascular integrity

In study V, we show that in a large cohort group of dengue patients, high levels of circulating viral NS1 protein correlate with high levels of serum hyaluronan, which can be used as a prognostic marker for the onset of warning signs, during the course of dengue viral infection. Importantly, treatment with NS1 decreased the expression of CD44 in differentiating endothelial cells, perturbing the organization of vessel-like structures. Mechanistically, NS1 stimulated the synthesis of hyaluronan in dermal fibroblasts and endothelial cells and intercellular hualuronan deposition recruited CD44-expressing macrophage-like cells, potentially contributing to inflammation. In endothelial cells, impaired hyaluronan-CD44 interactions led to increased permeability, via aberrant endothelial intercellular junctions.

Future Perspectives

The primary aim of the current thesis was to delineate the molecular events driving hyaluronan-CD44 tumorigenic signaling and thus, identifying potential targets for therapeutic interventions (papers I-III). In parallel, hyaluronan-CD44 interactions were explored in the context of Dengue virus-mediated impairment of vascular integrity (paper V). Finally, the role of two novel TGFβ-inducible kinases NUAK1 and NUAK2 was characterized in TGFβ-dependent outputs.

In study I, we explored and characterized the role of Has2as in TGFβ-induced Has2 transcription. We demonstrated that Has2 acts in a concerted way with other TFs and chromatin modulators, such as Hmga2 and Smads to promote Has2 expression, by acting locally, *in cis*. We hypothesized that *Has2as* acts in close proximity to the *Has2* gene locus, specifically affecting Has2 expression. Nonetheless, whether Has2as also acts *in trans*, affecting transcription of other genes, remains to be elucidated. An interesting approach to investigate this further, would be to perform transcriptomic analysis in mammary epithelial cells, upon silencing of either *Has2as* or Has2 after TGFβ treatment and examine the non-overlapping genes that are differentially regulated by *Has2as* alone. Interestingly though, lncRNAs exert pleiotropic functions in the cytoplasmic compartment as well (Statello et al., 2020). Intriguingly, we found that a significant proportion of Has2as was localized in the cytoplasm. According to recent reports, *Has2as* acts as a ceRNA, titrating away well-established tumor-suppressor miRNAs (Lu et al., 2021; Wang et al., 2020). It would be of interest to interrogate the interactome of *Has2as*, by performing RNA pull-down assay. This approach could uncover important roles of the *Has2as* in the cytosol.

In study II, we identified the ubiquitin ligases TRAF4/6 to be important for CD44 cleavage, by activating RAC1 in lung cancer cells, highly responsive to TGFβ. However, whether CD44 can be a substrate for TRAF4/6 remains an open question. In would be of importance to perform co-immunoprecipitation or proximity-ligation assay to explore the possibility of a direct association between CD44 and TRAF4 or TRAF6. In case of direct binding, interrogation of ubiquitination status of CD44 upon loss and gain of function studies of either TRAF4 or TRAF6, or both, in the absence and presence of TGFβ, would be of interest. It has been reported previously, that the released CD44-ICD upon its translocation to the nucleus binds to the *CD44* promoter, driving its

own transcription and replenishing CD44 levels at the plasma membrane (Okamoto et al., 2001). Whether CD44 can regulate TRAF4 or TRAF6 levels is not known and would be worth examining further. Noteworthy, CD44 cleavage occurs with high incidence in many solid tumors (Okamoto et al., 2002). It would be of interest to investigate whether this mechanism is universal and reproducible in other types of cancers as well.

In study III, we have initiated experiments to characterize in depth the role of CD44 in GBM. Transcriptomic analysis revealed that CD44 depletion led to diminished *HAS2* mRNA levels and decreased hyaluronan synthesis, when U251MG cells grew in stem-cell spheres. The molecular mechanism driving these events has not been characterized yet. It is of importance to seek whether this is a direct effect of CD44 on hyaluronan family members or an indirect one. Intriguingly, HAS2-AS1 levels were also down-regulated in CD44 KO cells. It would be worth exploring, whether CD44-ICD directly binds to the *HAS2* gene locus, affecting its transcription. One of the main findings of the study was that CD44 loss led to acquisition of a senescence state, which was manifested readily in conditioned medium with decreased serum concentration. Intriguingly, CD44 KO cells displayed increased motility and invasion, probably due to SASP. This needs to be investigated further to understand better the impact of CD44 on GBM progression.

In study V, we provided evidence that hyaluronan-CD44 interactions were required for vascular integrity, and perturbations provoked by soluble Dengue fever viral protein NS1 impaired them. Notably, we showed that CD44 levels were down-regulated in differentiating endothelial cells upon NS1 treatment. Shed CD44 has been identified in circulation of inflammatory diseases (Takahashi et al., 2010). It would be of interest to examine whether CD44 cleavage occurs in this context and if CD44-ICD contributes to the sustainment of the pro-inflammatory environment by modulating transcription. Moreover, it would be of importance to expand this study by using secreted NS1 derived from other flaviviruses, including Zika, West Nile, Japanese Enchephalitis and yellow fever viruses, which have also been reported to induce hyperpermeability in a tissue-specific manner (Puerta-Guardo et al., 2016).

In study IV, we have identified AMPK-related kinases Nuak1 and Nuak2 to be induced by TGFβ and to exert opposite roles in regulating TGFβ signaling and its functional outputs. We provided evidence that Nuak2 directly associates with TβRI and Smad3. Nonetheless, the exact outcome of these interactions remains to be elucidated. The fact that Nuak2 forms a complex with both the receptor and its immediate effector, implies that Nuak2 may recruit Smad3 to its cognate receptor, in order to be phosphorylated and activated. Thus, it would be plausible to speculate that Nuak2 kinase activity would be important for this event. We failed to show direct phosphorylation of TβRI or Smad3 by Nuak2 (data not shown), suggesting that Nuak2 exerts its actions on other molecules that are recruited in the same complex, tightly fine-tuning

TGFβ signaling. Therefore, the significance of these interactions remain an open question, worth exploring further. In contrast, we were not able to observe any association between Nuak1 and either TβRI or Smad3, Suggesting that Nuak1 affects TGFβ signaling in another level than Nuak2, which needs to be characterized more adequately.

Acknowledgements

I would like to express my sincere gratitude to all the people who have significantly contributed to the current thesis and to my well-being all these years, since I moved to Uppsala.

First of all, I would like to thank my supervisors for their excellence in science. My main supervisor Professor Evi Heldin for the all the effort you have put and constant confidence you have been showing to me along the way. Your unscheduled willingness to help and perseverance in paying attention to details have highly motivated me to continue and have been key to me, becoming a better scientist and better person. I feel honored that I have been your student and member of your group.

I would like to thank also my co-supervisor Professor Carl-Henrik Heldin, for your unparalleled devotion to science, acting as a role model for all the people surrounding you and especially young scientists. I highly appreciate the fact you would always share time with us in order to proceed with our studies.

Professor Aristidis Moustakas, I feel privileged you have been my co-supervisor. You have been a very inspirational and supportive figure and always fostered a very warm atmosphere in your group, providing extra incentive to strive to deliver the best we can. I am really glad I have experienced and been part of it.

Furthermore, I would like to thank my previous supervisors Professors Demitrios Vynios, Nikos Karamanos, Achilleas Theocharis and collaborator Professor Spyros Skandalis from the University of Patras, for giving me the chance to participate and present our research at FEBS conferences, receiving valuable feedback, developing the studies included in the thesis.

I consider myself lucky and blessed to collaborate with Helena Porsch, Berit Olofsson and Erna Raja, during my early days in Ludwig. They really helped me to adapt and start growing in our studies. I would like to express my appreciation for the past and present members of Matrix Biology group. Aino Ruusala for all your positive vibes and kind-heartedness as a pure Finn. I particularly thank Chun-Yu Lin for our fruitful collaboration and for being such a kind person and friend in the lab. Merima Mehić for being an outstanding researcher and introducing me to a healthier lifestyle. Theodoros Karalis and Thanos Chatzopoulos for being close friends and bright collaborators. Kaustuv, Inna, Vanessa, Yanshuang, Sandra for being nice colleagues.

Moreover, I feel grateful I have been part of TGFβ signaling group. Anita Morén, for always taking care of the group. Panos Papoutsoglou, you have been the best friend and colleague, one would wish for, you are an excellent scientist and I just want to reiterate our mutual willingness to collaborate at some point in the future. Kallia Tzavlaki, you deserve special praise for your scientific achievements as well, being always there for me and for your fighting spirit, when things don't go according to schedule. Laia Caja, for your scientific sharpness and the feedback I received from you, Claudia Bellomo for your spontaneous character and directness, and for being a true friend to me. George Divolis for your meticulous way of conducting research and for your friendship. Chrysa Tsirigoti for sharing your passion for science with me, I am sure you will do great. Mohamad Ali, thank you for your invaluable knowledge and our collaboration. Yutaro Tsubakihara, you have been a perfectionist and always eager to help, I appreciate it. EJ Tan and Kaoru Kahata for useful advice and tips for technically demanding experiments. Dear Dorival and Yae, thank you for learning a lot from your presentations and for the fun we have had together. Caroline, for the memorable midsommar, good luck with your studies. Jon, Panagiotis Bouris, Yukihide, Mahsa, Varun for being good friends.

Ria Vassilaki, you have been truly an exceptional researcher, an amazing person to interact with and I am really grateful for your and George's hospitality from my very first days here. Raffaelo, your dedication to science has been unprecedented, I really appreciate everything you have done for me all these years and the experiences we have shared together. Maria, thank you for all the funny stories and your unlimited positive energy you would transmit to people around you. Cristina, you have been a very good friend to me, I have missed your artistic personality. Nikki, I appreciate your kindness and the fact you have had to put up with me, next to your bench last few years.

I would like to thank all the group leaders, in Ludwig and later IMBIM, Professors Staffan Johansson, Anna Karin Olsson, Lena Kjellén, Ingvar Ferby, Johan Lennartsson, Jing-Ping Li and Andor Pivarcsi for providing feedback during presentations and for willingness to share reagents. Ihor, Anders, Natalia, Oleks, Jessica, Siamak, Masato, Tomohiko, Anahita and Noopur for giving excellent talks, I have learned a lot from you. Mariya, for optimally sharing our favorite hood, Lasse, Lotti, Ulla, Uffe and Per for the warm atmosphere from Ludwig days. It was a pleasure to interact with Marco, Yanju, Honglian, Yu Bai, Kehuan, Melanie, Bhavna, Irene, Xiaowen, Chen, Chengxi and Mahmood. I wish you best of luck with your studies. Also best of luck to Glenda, Carmen, Ana Rosa and Giulia, thank you for the time we shared together from Ludwig era.

I would like to thank all my friends from Campus 1477, for sharing all the energy and hustle during classes, it has been quite fun and really kept me going all these years.

Importantly, I would like to thank my family, my parents Ioannis and Anna and my sister Aristi, for all the support I have received from them throughout the years, for boosting my confidence and backing my decisions. Ευχαριστώ για την αμέριστη αγάπη και υποστήριξη που μου δείξατε! Furthermore, I would like to thank my brother-in-law Nikos and my nephew Constantinos for being an integral part of my life.

References

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. *100*, 3983–3988.

Albrengues, J., Shields, M.A., Ng, D., Park, C.G., Ambrico, A., Poindexter, M.E., Upadhyay, P., Uyeminami, D.L., Pommier, A., Küttner, V., et al. (2018). Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (80-.). *361*.

Alves, C.P., Fonseca, A.S., Muys, B.R., Bueno, R. de B. e L., Bürger, M.C., Souza, J.E.S. de, Valente, V., Zago, M.A., and Silva, W.A. (2013). Brief Report: The lincRNA Hotair Is Required for Epithelial-to-Mesenchymal Transition and Stemness Maintenance of Cancer Cell Lines. Stem Cells *31*, 2827–2832.

Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C.B., and Seed, B. (1990). CD44 is the principal cell surface receptor for hyaluronate. Cell *61*, 1303–1313.

Bakkers, J., Kramer, C., Pothof, J., Quaedvlieg, N.E.M., Spaink, H.P., and Hammerschmidt, M. (2004). Has2 is required upstream of Rac1 to govern dorsal migration of lateral cells during zebrafish gastrulation. Development *131*, 525–537.

Bakula, D., Müller, A.J., Zuleger, T., Takacs, Z., Franz-Wachtel, M., Thost, A.-K., Brigger, D., Tschan, M.P., Frickey, T., Robenek, H., et al. (2017). WIPI3 and WIPI4 β-propellers are scaffolds for LKB1-AMPK-TSC signalling circuits in the control of autophagy. Nat. Commun. 2017 81 *8*, 1–18.

Banerjee, S., Zagórska, A., Deak, M., Campbell, D.G., Prescott, A.R., and Alessi, D.R. (2014). Interplay between Polo kinase, LKB1-activated NUAK1 kinase, PP1βMYPT1 phosphatase complex and the SCFβTrCP E3 ubiquitin ligase. Biochem. J. *461*, 233–245.

Becker, L.M., O'Connell, J.T., Vo, A.P., Cain, M.P., Tampe, D., Bizarro, L., Sugimoto, H., McGow, A.K., Asara, J.M., Lovisa, S., et al. (2020). Epigenetic Reprogramming of Cancer-Associated Fibroblasts Deregulates Glucose Metabolism and Facilitates Progression of Breast Cancer. Cell Rep. *31*, 107701.

Bergers, G., and Fendt, S.-M. (2021). The metabolism of cancer cells during metastasis. Nat. Rev. Cancer 2021 213 *21*, 162–180.

Bernert, B., Porsch, H., and Heldin, P. (2011). Hyaluronan Synthase 2 (HAS2) Promotes Breast Cancer Cell Invasion by Suppression of Tissue Metalloproteinase Inhibitor 1 (TIMP-1). J. Biol. Chem. *286*, 42349–42359.

Bohrer, L.R., Chuntova, P., Bade, L.K., Beadnell, T.C., Leon, R.P., Brady, N.J., Ryu, Y., Goldberg, J.E., Schmechel, S.C., Koopmeiners, J.S., et al. (2014). Activation of the FGFR–STAT3 Pathway in Breast Cancer Cells Induces a Hyaluronan-Rich Microenvironment That Licenses Tumor Formation. Cancer Res. *74*, 374–386.

Bourguignon, L.Y.W., Singleton, P.A., Zhu, H., and Zhou, B. (2002). Hyaluronan Promotes Signaling Interaction between CD44 and the Transforming Growth Factor β Receptor I in Metastatic Breast Tumor Cells. J. Biol. Chem. *277*, 39703–39712.

Bourguignon, L.Y.W., Gilad, E., Rothman, K., and Peyrollier, K. (2005). Hyaluronan-CD44 Interaction with IQGAP1 Promotes Cdc42 and ERK Signaling, Leading to Actin Binding, Elk-1/Estrogen Receptor Transcriptional Activation, and Ovarian Cancer Progression. J. Biol. Chem. *280*, 11961–11972.

Bourguignon, L.Y.W., Peyrollier, K., Xia, W., and Gilad, E. (2008). Hyaluronan-CD44 Interaction Activates Stem Cell Marker Nanog, Stat-3-mediated MDR1 Gene Expression, and Ankyrin-regulated Multidrug Efflux in Breast and Ovarian Tumor Cells. J. Biol. Chem. *283*, 17635–17651.

Bourguignon, L.Y.W., Wong, G., Earle, C., and Chen, L. (2012). Hyaluronan-CD44v3 Interaction with Oct4-Sox2-Nanog Promotes miR-302 Expression Leading to Self-renewal, Clonal Formation, and Cisplatin Resistance in Cancer Stem Cells from Head and Neck Squamous Cell Carcinoma. J. Biol. Chem. *287*, 32800–32824.

Brichkina, A., Bertero, T., Loh, H.M., Nguyen, N.T.M., Emelyanov, A., Rigade, S., Ilie, M., Hofman, P., Gaggioli, C., and Bulavin, D. V. (2016). p38MAPK builds a hyaluronan cancer niche to drive lung tumorigenesis. Genes Dev. *30*, 2623–2636.

Bright, N.J., Thornton, C., and Carling, D. (2009). The regulation and function of mammalian AMPK-related kinases. Acta Physiol. *196*, 15–26.

Brown, R.L., Reinke, L.M., Damerow, M.S., Perez, D., Chodosh, L.A., Yang, J., and Cheng, C. (2011). CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. J. Clin. Invest. *121*, 1064–1074.

Caja, L., Dituri, F., Mancarella, S., Caballero-Diaz, D., Moustakas, A., Giannelli, G., and Fabregat, I. (2018). TGF-β and the Tissue Microenvironment: Relevance in Fibrosis and Cancer. Int. J. Mol. Sci. 2018, Vol. 19, Page 1294 *19*, 1294.

Camenisch, T.D., Spicer, A.P., Brehm-Gibson, T., Biesterfeldt, J., Augustine, M. Lou, Calabro, A., Kubalak, S., Klewer, S.E., and McDonald, J.A. (2000). Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. J. Clin. Invest. *106*, 349–360.

Chang, X.-Z., Yu, J., Liu, H.-Y., Dong, R.-H., and Cao, X.-C. (2011). ARK5 is associated with the invasive and metastatic potential of human breast cancer cells. J. Cancer Res. Clin. Oncol. 2011 1382 *138*, 247–254.

Chanmee, T., Ontong, P., Mochizuki, N., Kongtawelert, P., Konno, K., and Itano, N. (2014). Excessive Hyaluronan Production Promotes Acquisition of Cancer Stem Cell Signatures through the Coordinated Regulation of Twist and the Transforming Growth Factor β (TGF-β)-Snail Signaling Axis. J. Biol. Chem. *289*, 26038–26056.

Chanmee, T., Ontong, P., Izumikawa, T., Higashide, M., Mochizuki, N., Chokchaitaweesuk, C., Khansai, M., Nakajima, K., Kakizaki, I., Kongtawelert, P., et al. (2016). Hyaluronan Production Regulates Metabolic and Cancer Stem-like Properties of Breast Cancer Cells via Hexosamine Biosynthetic Pathway-coupled HIF-1 Signaling. J. Biol. Chem. *291*, 24105–24120.

Chao, H., and Spicer, A.P. (2005). Natural Antisense mRNAs to Hyaluronan Synthase 2 Inhibit Hyaluronan Biosynthesis and Cell Proliferation. J. Biol. Chem. *280*, 27513–27522.

Cheung, W.-F., Cruz, T.F., and Turley, E.A. (1999). Receptor for hyaluronan-mediated motility (RHAMM), a hyaladherin that regulates cell responses to growth factors. Biochem. Soc. Trans. *27*, 135–141.

Cho, Y., Lee, H.-W., Kang, H.-G., Kim, H.-Y., Kim, S.-J., Chun, K.-H., Cho, Y., Lee, H.-W., Kang, H.-G., Kim, H.-Y., et al. (2015). Cleaved CD44 intracellular domain supports activation of stemness factors and promotes tumorigenesis of breast cancer. Oncotarget *6*, 8709–8721.

Chokchaitaweesuk, C., Kobayashi, T., Izumikawa, T., and Itano, N. (2019). Enhanced hexosamine metabolism drives metabolic and signaling networks involving hyaluronan production and O-GlcNAcylation to exacerbate breast cancer. Cell Death Dis. 2019 1011 *10*, 1–15.

Cossa, G., Roeschert, I., Prinz, F., Baluapuri, A., Silveira Vidal, R., Schülein-Völk, C., Chang, Y.C., Ade, C.P., Mastrobuoni, G., Girard, C., et al. (2020). Localized Inhibition of Protein Phosphatase 1 by NUAK1 Promotes Spliceosome Activity and Reveals a MYC-Sensitive Feedback Control of Transcription. Mol. Cell *77*, 1322-1339.e11.

Cox, T.R. (2021). The matrix in cancer. Nat. Rev. Cancer 2021 214 *21*, 217–238.

Cutsem, E. Van, Tempero, M.A., Sigal, D., Oh, D.-Y., Fazio, N., Macarulla, T., Hitre, E., Hammel, P., Hendifar, A.E., Bates, S.E., et al. (2020). Randomized Phase III Trial of Pegvorhyaluronidase Alfa With Nab-Paclitaxel Plus Gemcitabine for Patients With Hyaluronan-High Metastatic Pancreatic Adenocarcinoma. Https://Doi.Org/10.1200/JCO.20.00590 *38*, 3185–3194.

DeGrendele, H.C., Estess, P., and Siegelman, M.H. (1997). Requirement for CD44 in activated T cell extravasation into an inflammatory site. Science (80-.). *278*, 672–675.

Derynck, R., and Weinberg, R.A. (2019). EMT and Cancer: More Than Meets the Eye. Dev. Cell *49*, 313–316.

El-Haibi, C.P., Bell, G.W., Zhang, J., Collmann, A.Y., Wood, D., Scherber, C.M., Csizmadia, E., Mariani, O., Zhu, C., Campagne, A., et al. (2012). Critical role for lysyl oxidase in mesenchymal stem cell-driven breast cancer malignancy. Proc. Natl. Acad. Sci. *109*, 17460–17465.

Falco, V. De, Tamburrino, A., Ventre, S., Castellone, M.D., Malek, M., Manié, S.N., and Santoro, M. (2012). CD44 Proteolysis Increases CREB Phosphorylation and Sustains Proliferation of Thyroid Cancer Cells. Cancer Res. *72*, 1449–1458.

Falou, O., Sadeghi-Naini, A., Prematilake, S., Sofroni, E., Papanicolau, N., Iradji, S., Jahedmotlagh, Z., Lemon-Wong, S., Pignol, J.-P., Rakovitch, E., et al. (2013). Evaluation of Neoadjuvant Chemotherapy Response in Women with Locally Advanced Breast Cancer Using Ultrasound Elastography. Transl. Oncol. *6*, 17.

Fedele, M., and Fusco, A. (2010). HMGA and Cancer. Biochim. Biophys. Acta - Gene Regul. Mech. *1799*, 48–54.

Fink, S.P., Myeroff, L.L., Kariv, R., Platzer, P., Xin, B., Mikkola, D., Lawrence, E., Morris, N., Nosrati, A., Willson, J.K. V., et al. (2015). Induction of KIAA1199/CEMIP is associated with colon cancer phenotype and poor patient survival. Oncotarget *6*, 30500–30515.

Freeman, S.A., Vega, A., Riedl, M., Collins, R.F., Ostrowski, P.P., Woods, E.C., Bertozzi, C.R., Tammi, M.I., Lidke, D.S., Johnson, P., et al. (2018). Transmembrane Pickets Connect Cyto- and Pericellular Skeletons Forming Barriers to Receptor Engagement. Cell *172*, 305-317.e10.

Fuchs, K., Hippe, A., Schmaus, A., Homey, B., Sleeman, J.P., and Orian-Rousseau, V. (2013). Opposing effects of high- and low-molecular weight hyaluronan on CXCL12-induced CXCR4 signaling depend on CD44. Cell Death Dis. 2013 410 *4*, e819–e819.

Fusco, A., and Fedele, M. (2007). Roles of HMGA proteins in cancer. Nat. Rev. Cancer 2007 712 *7*, 899–910.

Gao, D., Joshi, N., Choi, H., Ryu, S., Hahn, M., Catena, R., Sadik, H., Argani, P., Wagner, P., Vahdat, L.T., et al. (2012). Myeloid Progenitor Cells in the Premetastatic Lung Promote Metastases by Inducing Mesenchymal to Epithelial Transition. Cancer Res. *72*, 1384–1394.

Gill, M.K., Christova, T., Zhang, Y.Y., Gregorieff, A., Zhang, L., Narimatsu, M., Song, S., Xiong, S., Couzens, A.L., Tong, J., et al. (2018). A feed forward loop enforces YAP/TAZ signaling during tumorigenesis. Nat. Commun. 2018 91 *9*, 1–13.

Glentis, A., Oertle, P., Mariani, P., Chikina, A., El Marjou, F., Attieh, Y., Zaccarini, F., Lae, M., Loew, D., Dingli, F., et al. (2017). Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane. Nat. Commun. 2017 81 *8*, 1–13.

Goldstein, L.A., Zhou, D.F.H., Picker, L.J., Minty, C.N., Bargatze, R.F., Ding, J.F., and Butcher, E.C. (1989). A human lymphocyte homing receptor, the Hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell *56*, 1063–1072.

Goto, K., Lin, W., Zhang, L., Jilg, N., Shao, R.X., Schaefer, E.A.K., Zhao, H., Fusco, D.N., Peng, L.F., Kato, N., et al. (2013). The AMPK-related kinase SNARK regulates hepatitis C virus replication and pathogenesis through enhancement of TGF-β signaling. J. Hepatol. *59*, 942–948.

Gudey, S.K., Sundar, R., Mu, Y., Wallenius, A., Zang, G., Bergh, A., Heldin, C.H., and Landström, M. (2014). TRAF6 stimulates the tumor-promoting effects of TGFβ type I receptor through polyubiquitination and activation of presenilin. Sci. Signal. *7*.

Guo, Y.-J., Liu, G., Wang, X., Jin, D., Wu, M., Ma, J., and Sy, M.-S. (1994). Potential Use of Soluble CD44 in Serum as Indicator of Tumor Burden and Metastasis in Patients with Gastric or Colon Cancer. Cancer Res. *54*.

Hamidi, H., and Ivaska, J. (2018). Every step of the way: integrins in cancer progression and metastasis. Nat. Rev. Cancer 2018 189 *18*, 533–548.

Hanahan, D., and Coussens, L. (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell *21*, 309–322.

Hartmann, M., Parra, L.M., Ruschel, A., Böhme, S., Li, Y., Morrison, H., Herrlich, A., and Herrlich, P. (2015a). Tumor Suppressor NF2 Blocks Cellular Migration by Inhibiting Ectodomain Cleavage of CD44. Mol. Cancer Res. *13*, 879–890.

Hartmann, M., Parra, L.M., Ruschel, A., Lindner, C., Morrison, H., Herrlich, A., and Herrlich, P. (2015b). Inside-out Regulation of Ectodomain Cleavage of Cluster-of-Differentiation-44 (CD44) and of Neuregulin-1 Requires Substrate Dimerization. J. Biol. Chem. *290*, 17041–17054.

Haynes, B.F., Hale, L.P., Patton, K.L., Martin, M.E., and McCallum, R.M. (1991). Measurement of an adhesion molecule as an indicator of inflammatory disease activity: Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. Arthritis Rheum. *34*, 1434–1443.

Hebert, J.D., Myers, S.A., Naba, A., Abbruzzese, G., Lamar, J.M., Carr, S.A., and Hynes, R.O. (2020). Proteomic Profiling of the ECM of Xenograft Breast Cancer Metastases in Different Organs Reveals Distinct Metastatic Niches. Cancer Res. *80*, 1475–1485.

Heldin, C.-H., and Moustakas, A. (2016). Signaling Receptors for TGF-β Family Members. Cold Spring Harb. Perspect. Biol. *8*, a022053.

Heldin, P., delaTorre, M., Ytterberg, D., and Bergh, J. (1996). Differential synthesis and binding of hyaluronan by human breast cancer cell lines. Oncol. Rep. *3*, 1011–1016.

Heldin, P., Karousou, E., Bernert, B., Porsch, H., Nishitsuka, K., and Skandalis, S.S. (2009). Importance of Hyaluronan-CD44 Interactions in Inflammation and Tumorigenesis. Https://Doi.Org/10.1080/03008200802143323 *49*, 215–218.

Heldin, P., Basu, K., Kozlova, I., and Porsch, H. (2014). HAS2 and CD44 in Breast Tumorigenesis. Adv. Cancer Res. *123*, 211–229.

Heldin, P., Lin, C.-Y., Kolliopoulos, C., Chen, Y.-H., and Skandalis, S.S. (2019). Regulation of hyaluronan biosynthesis and clinical impact of excessive hyaluronan production. Matrix Biol. *78–79*.

Heldin, P., Kolliopoulos, C., Lin, C.-Y., and Heldin, C.-H. (2020). Involvement of hyaluronan and CD44 in cancer and viral infections. Cell. Signal. *65*.

Hingorani, S.R., Zheng, L., Bullock, A.J., Seery, T.E., Harris, W.P., Sigal, D.S., Braiteh, F., Ritch, P.S., Zalupski, M.M., Bahary, N., et al. (2017). HALO 202: Randomized Phase II Study of PEGPH20 Plus Nab-Paclitaxel/Gemcitabine Versus Nab-Paclitaxel/Gemcitabine in Patients With Untreated, Metastatic Pancreatic Ductal Adenocarcinoma. Https://Doi.Org/10.1200/JCO.2017.74.9564 *36*, 359–366.

Hou, X., Liu, J.-E., Liu, W., Liu, C.-Y., Liu, Z.-Y., and Sun, Z.-Y. (2011). A new role of NUAK1: directly phosphorylating p53 and regulating cell proliferation. Oncogene 2011 3026 *30*, 2933–2942.

Humbert, N., Navaratnam, N., Augert, A., Costa, M. Da, Martien, S., Wang, J., Martinez, D., Abbadie, C., Carling, D., Launoit, Y. de, et al. (2010). Regulation of ploidy and senescence by the AMPK-related kinase NUAK1. EMBO J. *29*, 376–386.

Ishimoto, T., Nagano, O., Yae, T., Tamada, M., Motohara, T., Oshima, H., Oshima, M., Ikeda, T., Asaba, R., Yagi, H., et al. (2011). CD44 Variant Regulates Redox Status in Cancer Cells by Stabilizing the xCT Subunit of System xc− and Thereby Promotes Tumor Growth. Cancer Cell *19*, 387–400.

Jackson, D.G. (2019). Hyaluronan in the lymphatics: The key role of the hyaluronan receptor LYVE-1 in leucocyte trafficking. Matrix Biol. *78–79*, 219–235.

Jang, J.-H., Kim, D.-H., Lim, J.M., Lee, J.W., Jeong, S.J., Kim, K.P., and Surh, Y.-J. (2020). Breast Cancer Cell–Derived Soluble CD44 Promotes Tumor Progression by Triggering Macrophage IL1β Production. Cancer Res. *80*, 1342–1356.

Johansson, E., Grassi, E.S., Pantazopoulou, V., Tong, B., Lindgren, D., Berg, T.J., Pietras, E.J., Axelson, H., and Pietras, A. (2017). CD44 Interacts with HIF-2α to Modulate the Hypoxic Phenotype of Perinecrotic and Perivascular Glioma Cells. Cell Rep. *20*, 1641–1653.

Johansson, S., Svineng, G., Wennerberg, K., Armulik, A., and Lohikangas, L. (1997). FIBRONECTIN-INTEGRIN INTERACTIONS. Front. Biosci. *2*, 126.

Jokela, T.A., Makkonen, K.M., Oikari, S., Kärnä, R., Koli, E., Hart, G.W., Tammi, R.H., Carlberg, C., and Tammi, M.I. (2011). Cellular Content of UDP-N-acetylhexosamines Controls Hyaluronan Synthase 2 Expression and Correlates with O-Linked N-Acetylglucosamine Modification of Transcription Factors YY1 and SP1. J. Biol. Chem. *286*, 33632–33640.

Kai, F.B., Drain, A.P., and Weaver, V.M. (2019). The Extracellular Matrix Modulates the Metastatic Journey. Dev. Cell *49*, 332–346.

Kakizaki, I., Kojima, K., Takagaki, K., Endo, M., Kannagi, R., Ito, M., Maruo, Y., Sato, H., Yasuda, T., Mita, S., et al. (2004). A Novel Mechanism for the Inhibition of Hyaluronan Biosynthesis by 4-Methylumbelliferone. J. Biol. Chem. *279*, 33281–33289.

Kalluri, R. (2016). The biology and function of fibroblasts in cancer. Nat. Rev. Cancer 2016 169 *16*, 582–598.

Karalis, T.T., Heldin, P., Vynios, D.H., Neill, T., Buraschi, S., Iozzo, R. V., Karamanos, N.K., and Skandalis, S.S. (2019). Tumor-suppressive functions of 4-MU on breast cancer cells of different ER status: Regulation of hyaluronan/HAS2/CD44 and specific matrix effectors. Matrix Biol. *78–79*, 118–138.

Karamanos, N.K., Theocharis, A.D., Neill, T., and Iozzo, R. V. (2019). Matrix modeling and remodeling: A biological interplay regulating tissue homeostasis and diseases. Matrix Biol. *75–76*, 1–11.

Karousou, E., Kamiryo, M., Skandalis, S.S., Ruusala, A., Asteriou, T., Passi, A., Yamashita, H., Hellman, U., Heldin, C.H., and Heldin, P. (2010). The Activity of Hyaluronan Synthase 2 Is Regulated by Dimerization and Ubiquitination. J. Biol. Chem. *285*, 23647–23654.

Kawano, Y., Okamoto, I., Murakami, D., Itoh, H., Yoshida, M., Ueda, S., and Saya, H. (2000). Ras Oncoprotein Induces CD44 Cleavage through Phosphoinositide 3-OH Kinase and the Rho Family of Small G Proteins. J. Biol. Chem. *275*, 29628–29635.

Koltai, T., Reshkin, S.J., Carvalho, T.M.A., and Cardone, R.A. (2021). Targeting the Stromal Pro-Tumoral Hyaluronan-CD44 Pathway in Pancreatic Cancer. Int. J. Mol. Sci. 2021, Vol. 22, Page 3953 *22*, 3953.

Kong, T., Ahn, R., Yang, K., Zhu, X., Fu, Z., Morin, G., Bramley, R., Cliffe, N.C., Xue, Y., Kuasne, H., et al. (2020). CD44 Promotes PD-L1 Expression and Its Tumor-Intrinsic Function in Breast and Lung Cancers. Cancer Res. *80*, 444–457.

Kowanetz, M., Lönn, P., Vanlandewijck, M., Kowanetz, K., Heldin, C.-H., and Moustakas, A. (2008). TGFβ induces SIK to negatively regulate type I receptor kinase signaling. J. Cell Biol. *182*, 655–662.

Koyama, H., Hibi, T., Isogai, Z., Yoneda, M., Fujimori, M., Amano, J., Kawakubo, M., Kannagi, R., Kimata, K., Taniguchi, S., et al. (2007). Hyperproduction of Hyaluronan in Neu-Induced Mammary Tumor Accelerates Angiogenesis through Stromal Cell Recruitment: Possible Involvement of Versican/PG-M. Am. J. Pathol. *170*, 1086–1099.

Kozlova, I., Ruusala, A., Voytyuk, O., Skandalis, S.S., and Heldin, P. (2012). IQGAP1 regulates hyaluronan-mediated fibroblast motility and proliferation. Cell. Signal. *24*, 1856–1862.

Kozono, S., Ohuchida, K., Eguchi, D., Ikenaga, N., Fujiwara, K., Cui, L., Mizumoto, K., and Tanaka, M. (2013). Pirfenidone Inhibits Pancreatic Cancer Desmoplasia by Regulating Stellate Cells. Cancer Res. *73*, 2345–2356.

Kuo, Y.-C., Su, C.-H., Liu, C.-Y., Chen, T.-H., Chen, C.-P., and Wang, H.-S. (2009). Transforming growth factor-β induces CD44 cleavage that promotes migration of MDA-MB-435s cells through the up-regulation of membrane type 1-matrix metalloproteinase. Int. J. Cancer *124*, 2568–2576.

Kusakai, G.I., Suzuki, A., Ogura, T., Miyamoto, S., Ochiai, A., Kaminishi, M., and Esumi, H. (2004). ARK5 Expression in Colorectal Cancer and Its Implications for Tumor Progression. Am. J. Pathol. *164*, 987–995.

Lagendijk, A.K., Goumans, M.J., Burkhard, S.B., and Bakkers, J. (2011). MicroRNA-23 Restricts Cardiac Valve Formation by Inhibiting Has2 and Extracellular Hyaluronic Acid Production. Circ. Res. *109*, 649–657.

Lammich, S., Okochi, M., Takeda, M., Kaether, C., Capell, A., Zimmer, A.K., Edbauer, D., Walter, J., Steiner, H., and Haass, C. (2002). Presenilin-dependent Intramembrane Proteolysis of CD44 Leads to the Liberation of Its Intracellular Domain and the Secretion of an Aβ-like Peptide. J. Biol. Chem. *277*, 44754–44759.

Laurent, T.C., Laurent, U.B., and Fraser, J.R. (1995). Functions of hyaluronan. Ann. Rheum. Dis. *54*, 429–432.

Lefebvre, D.L., and Rosen, C.F. (2005). Regulation of SNARK activity in response to cellular stresses. Biochim. Biophys. Acta - Gen. Subj. *1724*, 71–85.

Lefebvre, D.L., Bai, Y., Shahmolky, N., Sharma, M., Poon, R., Drucker, D.J., and Rosen, C.F. (2001). Identification and characterization of a novel sucrose-non-fermenting protein kinase/AMP-activated protein kinase-related protein kinase, SNARK. Biochem. J. *355*, 297.

Lessard, S.J., Rivas, D.A., So, K., Koh, H.-J., Queiroz, A.L., Hirshman, M.F., Fielding, R.A., and Goodyear, L.J. (2016). The AMPK-related kinase SNARK regulates muscle mass and myocyte survival. J. Clin. Invest. *126*, 560–570.

Li, Y., and Heldin, P. (2001). Hyaluronan production increases the malignant properties of mesothelioma cells. Br. J. Cancer 2001 854 *85*, 600–607.

Li, L., Heldin, C.H., and Heldin, P. (2006). Inhibition of Platelet-derived Growth Factor-BB-induced Receptor Activation and Fibroblast Migration by Hyaluronan Activation of CD44. J. Biol. Chem. *281*, 26512–26519.

Li, L., Asteriou, T., Bernert, B., Heldin, C.-H., and Heldin, P. (2007a). Growth factor regulation of hyaluronan synthesis and degradation in human dermal fibroblasts: importance of hyaluronan for the mitogenic response of PDGF-BB. Biochem. J. *404*, 327–336.

Li, Y., Li, L., Brown, T.J., and Heldin, P. (2007b). Silencing of hyaluronan synthase 2 suppresses the malignant phenotype of invasive breast cancer cells. Int. J. Cancer *120*, 2557–2567.

Ling, H., Vincent, K., Pichler, M., Fodde, R., Berindan-Neagoe, I., Slack, F.J., and Calin, G.A. (2015). Junk DNA and the long non-coding RNA twist in cancer genetics. Oncogene 2015 3439 *34*, 5003–5011.

Liu, S., and Cheng, C. (2017). Akt Signaling Is Sustained by a CD44 Splice Isoform–Mediated Positive Feedback Loop. Cancer Res. *77*, 3791–3801.

Liu, L., Ulbrich, J., Müller, J., Wüstefeld, T., Aeberhard, L., Kress, T.R., Muthalagu, N., Rycak, L., Rudalska, R., Moll, R., et al. (2012). Deregulated MYC expression induces dependence upon AMPK-related kinase 5. Nat. 2012 4837391 *483*, 608–612.

Lokeshwar, V.B., and Bourguignon, L.Y.W. (1991). Post-translational protein modification and expression of ankyrin-binding site(s) in GP85 (Pgp-1/CD44) and its biosynthetic precursors during T-lymphoma membrane biosynthesis. J. Biol. Chem. *266*, 17983–17989.

Lokeshwar, V.B., Fregien, N., and Bourguignon, L.Y. (1994). Ankyrin-binding domain of CD44(GP85) is required for the expression of hyaluronic acid-mediated adhesion function. J. Cell Biol. *126*, 1099–1109.

Lönn, P., Vanlandewijck, M., Raja, E., Kowanetz, M., Watanabe, Y., Kowanetz, K., Vasilaki, E., Heldin, C.H., and Moustakas, A. (2012). Transcriptional Induction of Salt-inducible Kinase 1 by Transforming Growth Factor β Leads to Negative Regulation of Type I Receptor Signaling in Cooperation with the Smurf2 Ubiquitin Ligase. J. Biol. Chem. *287*, 12867–12878.

Lu, P., Weaver, V.M., and Werb, Z. (2012). The extracellular matrix: A dynamic niche in cancer progression. J. Cell Biol. *196*, 395–406.

Lu, Y., Guo, G., Hong, R., Chen, X., Sun, Y., Liu, F., Zhang, Z., Jin, X., Dong, J., Yu, K., et al. (2021). Article 634893 1 (2021) LncRNA HAS2-AS1 Promotes Glioblastoma Proliferation by Sponging miR-137. Front. Oncol *11*, 634893.

Ma, B., Liao, T., Wen, D., Dong, C., Zhou, L., Yang, S., Wang, Y., and Ji, Q. (2016). Long intergenic non-coding RNA 271 is predictive of a poorer prognosis of papillary thyroid cancer. Sci. Reports 2016 61 *6*, 1–13.

Ma, L., Dong, L., and Chang, P. (2019). CD44v6 engages in colorectal cancer progression. Cell Death Dis. 2018 101 *10*, 1–13.

Makkonen, K.M., Pasonen-Seppänen, S., Törrönen, K., Tammi, M.I., and Carlberg, C. (2009). Regulation of the Hyaluronan Synthase 2 Gene by Convergence in Cyclic AMP Response Element-binding Protein and Retinoid Acid Receptor Signaling. J. Biol. Chem. *284*, 18270–18281.

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). The Epithelial-Mesenchymal Transition Generates Cells with Properties of Stem Cells. Cell *133*, 704–715.

Mehić, M., de Sa, V.K., Hebestreit, S., Heldin, C.-H., and Heldin, P. (2017). The deubiquitinating enzymes USP4 and USP17 target hyaluronan synthase 2 and differentially affect its function. Oncog. 2017 66 *6*, e348–e348.

Meyer, K., and Palmer, J.W. (1934). THE POLYSACCHARIDE OF THE VITREOUS HUMOR.

Michael, D.R., Phillips, A.O., Krupa, A., Martin, J., Redman, J.E., Altaher, A., Neville, R.D., Webber, J., Kim, M.Y., and Bowen, T. (2011). The Human Hyaluronan Synthase 2 (HAS2) Gene and Its Natural Antisense RNA Exhibit Coordinated Expression in the Renal Proximal Tubular Epithelial Cell. J. Biol. Chem. *286*, 19523–19532.

Midgley, A.C., Oltean, S., Hascal, V., Woods, E.L., Steadman, R., Phillips, A.O., and Meran, S. (2017). Nuclear hyaluronidase 2 drives alternative splicing of CD44 pre-mRNA to determine profibrotic or antifibrotic cell phenotype. Sci. Signal. *10*.

Monslow, J., Williams, J.D., Norton, N., Guy, C.A., Price, I.K., Coleman, S.L., Williams, N.M., Buckland, P.R., Spicer, A.P., Topley, N., et al. (2003). The human hyaluronan synthase genes: genomic structures, proximal promoters and polymorphic microsatellite markers. Int. J. Biochem. Cell Biol. *35*, 1272–1283.

Monslow, J., Williams, J.D., Fraser, D.J., Michael, D.R., Foka, P., Kift-Morgan, A.P., Dong, D.L., Fielding, C.A., Craig, K.J., Topley, N., et al. (2006). Sp1 and Sp3 Mediate Constitutive Transcription of the Human Hyaluronan Synthase 2 Gene. J. Biol. Chem. *281*, 18043–18050.

Monzen, K., Ito, Y., Naito, A.T., Kasai, H., Hiroi, Y., Hayashi, D., Shiojima, I., Yamazaki, T., Miyazono, K., Asashima, M., et al. (2008). A crucial role of a high mobility group protein HMGA2 in cardiogenesis. Nat. Cell Biol. 2008 105 *10*, 567–574.

Morath, I., Jung, C., Lévêque, R., Linfeng, C., Toillon, R.-A., Warth, A., and Orian-Rousseau, V. (2018). Differential recruitment of CD44 isoforms by ErbB ligands reveals an involvement of CD44 in breast cancer. Oncogene 2018 3711 *37*, 1472–1484.

Morishita, A., Zaidi, M.R., Mitoro, A., Sankarasharma, D., Szabolcs, M., Okada, Y., D'Armiento, J., and Chada, K. (2013). HMGA2 Is a Driver of Tumor Metastasis. Cancer Res. *73*, 4289–4299.

Moustakas, A., and Heldin, C.-H. (2016). Mechanisms of TGFβ-Induced Epithelial–Mesenchymal Transition. J. Clin. Med. 2016, Vol. 5, Page 63 *5*, 63.

Mu, Y., Sundar, R., Thakur, N., Ekman, M., Gudey, S.K., Yakymovych, M., Hermansson, A., Dimitriou, H., Bengoechea-Alonso, M.T., Ericsson, J., et al. (2011). TRAF6 ubiquitinates TGFβ type I receptor to promote its cleavage and nuclear translocation in cancer. Nat. Commun. 2011 21 *2*, 1–11.

Müller, S., Sindikubwabo, F., Cañeque, T., Lafon, A., Versini, A., Lombard, B., Loew, D., Wu, T.-D., Ginestier, C., Charafe-Jauffret, E., et al. (2020). CD44 regulates epigenetic plasticity by mediating iron endocytosis. Nat. Chem. 2020 1210 *12*, 929–938.

Murai, T., Miyauchi, T., Yanagida, T., and Sako, Y. (2006). Epidermal growth factor-regulated activation of Rac GTPase enhances CD44 cleavage by metalloproteinase disintegrin ADAM10. Biochem. J. *395*, 65–71.

Murakami, D., Okamoto, I., Nagano, O., Kawano, Y., Tomita, T., Iwatsubo, T., De Strooper, B., Yumoto, E., and Saya, H. (2003). Presenilin-dependent γ-secretase activity mediates the intramembranous cleavage of CD44. Oncogene 2003 2210 *22*, 1511–1516.

Nagaoka, A., Yoshida, H., Nakamura, S., Morikawa, T., Kawabata, K., Kobayashi, M., Sakai, S., Takahashi, Y., Okada, Y., and Inoue, S. (2015). Regulation of Hyaluronan (HA) Metabolism Mediated by HYBID (Hyaluronan-binding Protein Involved in HA Depolymerization, KIAA1199) and HA Synthases in Growth Factor-stimulated Fibroblasts *. J. Biol. Chem. *290*, 30910–30923.

Nagy, N., Kuipers, H.F., Frymoyer, A.R., Ishak, H.D., Bollyky, J.B., Wight, T.N., and Bollyky, P.L. (2015). 4-Methylumbelliferone Treatment and Hyaluronan Inhibition as a Therapeutic Strategy in Inflammation, Autoimmunity, and Cancer. Front. Immunol. *0*, 123.

Nakamura, H., Suenaga, N., Taniwaki, K., Matsuki, H., Yonezawa, K., Fujii, M., Okada, Y., and Seiki, M. (2004). Constitutive and Induced CD44 Shedding by ADAM-Like Proteases and Membrane-Type 1 Matrix Metalloproteinase. Cancer Res. *64*, 876–882.

Namiki, T., Coelho, S.G., Hearing, V.J., Namiki, T., Coelho, S.G., and Hearing, V.J. (2011). NUAK2: an emerging acral melanoma oncogene. Oncotarget *2*, 695–704.

Namiki, T., Yaguchi, T., Nakamura, K., Valencia, J.C., Coelho, S.G., Yin, L., Kawaguchi, M., Vieira, W.D., Kaneko, Y., Tanemura, A., et al. (2015). NUAK2 Amplification Coupled with PTEN Deficiency Promotes Melanoma Development via CDK Activation. Cancer Res. *75*, 2708–2715.

Naor, D., Sionov, R.V., and Ish-Shalom, D. (1997). CD44: Structure, Function and Association with the Malignant Process. Adv. Cancer Res. *71*, 241–319.

Nieto, M.A., Huang, R.Y.Y.J., Jackson, R.A.A., and Thiery, J.P.P. (2016). EMT: 2016. Cell *166*, 21–45.

Okamoto, I., Kawano, Y., Tsuiki, H., Sasaki, J., Nakao, M., Matsumoto, M., Suga, M., Ando, M., Nakajima, M., and Saya, H. (1999). CD44 cleavage induced by a membrane-associated metalloprotease plays a critical role in tumor cell migration. Oncogene 1999 187 *18*, 1435–1446.

Okamoto, I., Kawano, Y., Murakami, D., Sasayama, T., Araki, N., Miki, T., Wong, A.J., and Saya, H. (2001). Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. J. Cell Biol. *155*, 755–762.

Okamoto, I., Tsuiki, H., Kenyon, L.C., Godwin, A.K., Emlet, D.R., Holgado-Madruga, M., Lanham, I.S., Joynes, C.J., Vo, K.T., Guha, A., et al. (2002). Proteolytic Cleavage of the CD44 Adhesion Molecule in Multiple Human Tumors. Am. J. Pathol. *160*, 441–447.

Okuda, H., Kobayashi, A., Xia, B., Watabe, M., Pai, S.K., Hirota, S., Xing, F., Liu, W., Pandey, P.R., Fukuda, K., et al. (2012). Hyaluronan Synthase HAS2 Promotes Tumor Progression in Bone by Stimulating the Interaction of Breast Cancer Stem–Like Cells with Macrophages and Stromal Cells. Cancer Res. *72*, 537–547.

Olofsson, B., Porsch, H., and Heldin, P. (2014). Knock-Down of CD44 Regulates Endothelial Cell Differentiation via NFκB-Mediated Chemokine Production. PLoS One *9*, e90921.

Olsson, A.-K., and Cedervall, J. (2016). NETosis in Cancer – Platelet–Neutrophil Crosstalk Promotes Tumor-Associated Pathology. Front. Immunol. *0*, 373.

Orian-Rousseau, V., and Sleeman, J. (2014). CD44 is a Multidomain Signaling Platform that Integrates Extracellular Matrix Cues with Growth Factor and Cytokine Signals. Adv. Cancer Res. *123*, 231–254.

Orian-Rousseau, V., Chen, L., Sleeman, J.P., Herrlich, P., and Ponta, H. (2002). CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev. *16*, 3074–3086.

Paget, S. (1889). The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. *8*, 98–101.

Papoutsoglou, P., and Moustakas, A. (2020). Long non-coding RNAs and TGF-β signaling in cancer. Cancer Sci. *111*, 2672–2681.

Papoutsoglou, P., Tsubakihara, Y., Caja, L., Morén, A., Pallis, P., Ameur, A., Heldin, C.H., and Moustakas, A. (2019). The TGFB2-AS1 lncRNA Regulates TGF-β Signaling by Modulating Corepressor Activity. Cell Rep. *28*, 3182-3198.e11.

Papoutsoglou, P., Rodrigues-Junior, D.M., Morén, A., Bergman, A., Pontén, F., Coulouarn, C., Caja, L., Heldin, C.-H., and Moustakas, A. (2021). The noncoding MIR100HG RNA enhances the autocrine function of transforming growth factor β signaling. Oncogene 2021 4021 *40*, 3748–3765.

Pardali, K., and Moustakas, A. (2007). Actions of TGF-β as tumor suppressor and pro-metastatic factor in human cancer. Biochim. Biophys. Acta - Rev. Cancer *1775*, 21–62.

Parra, L.M., Hartmann, M., Schubach, S., Li, Y., Herrlich, P., and Herrlich, A. (2015). Distinct Intracellular Domain Substrate Modifications Selectively Regulate Ectodomain Cleavage of NRG1 or CD44. Mol. Cell. Biol. *35*, 3381–3395.

Pastushenko, I., and Blanpain, C. (2019). EMT Transition States during Tumor Progression and Metastasis. Trends Cell Biol. *29*, 212–226.

Peterson, R.S., Andhare, R.A., Rousche, K.T., Knudson, W., Wang, W., Grossfield, J.B., Thomas, R.O., Hollingsworth, R.E., and Knudson, C.B. (2004). CD44 modulates Smad1 activation in the BMP-7 signaling pathway. J. Cell Biol. *166*, 1081–1091.

Pietras, A., Katz, A.M., Ekström, E.J., Wee, B., Halliday, J.J., Pitter, K.L., Werbeck, J.L., Amankulor, N.M., Huse, J.T., and Holland, E.C. (2014). Osteopontin-CD44 Signaling in the Glioma Perivascular Niche Enhances Cancer Stem Cell Phenotypes and Promotes Aggressive Tumor Growth. Cell Stem Cell *14*, 357–369.

Pinho, S.S., and Reis, C.A. (2015). Glycosylation in cancer: mechanisms and clinical implications. Nat. Rev. Cancer 2015 159 *15*, 540–555.

Platt, V.M., and Francis C. Szoka, J. (2008). Anticancer Therapeutics: Targeting Macromolecules and Nanocarriers to Hyaluronan or CD44, a Hyaluronan Receptor. Mol. Pharm. *5*, 474–486.

Ponta, H., Sherman, L., and Herrlich, P.A. (2003). CD44: From adhesion molecules to signalling regulators. Nat. Rev. Mol. Cell Biol. 2003 41 *4*, 33–45.

Porsch, H., Bernert, B., Mehić, M., Theocharis, A.D., Heldin, C.-H., and Heldin, P. (2012). Efficient TGFβ-induced epithelial–mesenchymal transition depends on hyaluronan synthase HAS2. Oncogene 2013 3237 *32*, 4355–4365.

Porsch, H., Mehić, M., Olofsson, B., Heldin, P., and Heldin, C.H. (2014). Platelet-derived Growth Factor β-Receptor, Transforming Growth Factor β Type I Receptor, and CD44 Protein Modulate Each Other's Signaling and Stability. J. Biol. Chem. *289*, 19747–19757.

Preca, B.-T., Bajdak, K., Mock, K., Lehmann, W., Sundararajan, V., Bronsert, P., Matzge-Ogi, A., Orian-Rousseau, V., Brabletz, S., Brabletz, T., et al. (2017). A novel ZEB1/HAS2 positive feedback loop promotes EMT in breast cancer. Oncotarget *8*, 11530–11543.

Puerta-Guardo, H., Glasner, D.R., and Harris, E. (2016). Dengue Virus NS1 Disrupts the Endothelial Glycocalyx, Leading to Hyperpermeability. PLOS Pathog. *12*, e1005738.

Puerta-Guardo, H., Glasner, D.R., Espinosa, D.A., Biering, S.B., Patana, M., Ratnasiri, K., Wang, C., Beatty, P.R., and Harris, E. (2019). Flavivirus NS1 Triggers Tissue-Specific Vascular Endothelial Dysfunction Reflecting Disease Tropism. Cell Rep. *26*, 1598-1613.e8.

Putavet, D.A., and Keizer, P.L.J. de (2021). Residual Disease in Glioma Recurrence: A Dangerous Liaison with Senescence. Cancers 2021, Vol. 13, Page 1560 *13*, 1560.

Qu, C., Rilla, K., Tammi, R., Tammi, M., Kröger, H., and Lammi, M.J. (2014). Extensive CD44-dependent hyaluronan coats on human bone marrow-derived mesenchymal stem cells produced by hyaluronan synthases HAS1, HAS2 and HAS3. Int. J. Biochem. Cell Biol. *48*, 45–54.

Queisser, K.A., Mellema, R.A., Middleton, E.A., Portier, I., Manne, B.K., Denorme, F., Beswick, E.J., Rondina, M.T., Campbell, R.A., and Petrey, A.C. (2021). COVID-19 generates hyaluronan fragments that directly induce endothelial barrier dysfunction. JCI Insight.

Ramanathan, R.K., McDonough, S.L., Philip, P.A., Hingorani, S.R., Lacy, J., Kortmansky, J.S., Thumar, J., Chiorean, E.G., Shields, A.F., Behl, D., et al. (2019). Phase IB/II Randomized Study of FOLFIRINOX Plus Pegylated Recombinant Human Hyaluronidase Versus FOLFIRINOX Alone in Patients With Metastatic Pancreatic Adenocarcinoma: SWOG S1313. Https://Doi.Org/10.1200/JCO.18.01295 *37*, 1062–1069.

Rodrigues, G., Hoshino, A., Kenific, C.M., Matei, I.R., Steiner, L., Freitas, D., Kim, H.S., Oxley, P.R., Scandariato, I., Casanova-Salas, I., et al. (2019). Tumour exosomal CEMIP protein promotes cancer cell colonization in brain metastasis. Nat. Cell Biol. 2019 2111 *21*, 1403–1412.

Saavalainen, K., Pasonen-Seppänen, S., Dunlop, T.W., Tammi, R., Tammi, M.I., and Carlberg, C. (2005). The Human Hyaluronan Synthase 2 Gene Is a Primary Retinoic Acid and Epidermal Growth Factor Responding Gene. J. Biol. Chem. *280*, 14636–14644.

Saavalainen, K., Tammi, M.I., Bowen, T., Schmitz, M.L., and Carlberg, C. (2007). Integration of the Activation of the Human Hyaluronan Synthase 2 Gene Promoter by Common Cofactors of the Transcription Factors Retinoic Acid Receptor and Nuclear Factor κB. J. Biol. Chem. *282*, 11530–11539.

Schinzel, R.T., Higuchi-Sanabria, R., Shalem, O., Moehle, E.A., Webster, B.M., Joe, L., Bar-Ziv, R., Frankino, P.A., Durieux, J., Pender, C., et al. (2019). The Hyaluronidase, TMEM2, Promotes ER Homeostasis and Longevity Independent of the UPRER. Cell *179*, 1306-1318.e18.

Schmitt, M., Metzger, M., Gradl, D., Davidson, G., and Orian-Rousseau, V. (2015). CD44 functions in Wnt signaling by regulating LRP6 localization and activation. Cell Death Differ. *22*, 677–689.

Siegel, P.M., and Massagué, J. (2003). Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nat. Rev. Cancer 2003 311 *3*, 807–820.

Skandalis, S.S., Kozlova, I., Engström, U., Hellman, U., and Heldin, P. (2010). Proteomic identification of CD44 interacting proteins. IUBMB Life *62*, 833–840.

Skandalis, S.S., Karalis, T.T., Chatzopoulos, A., and Karamanos, N.K. (2019). Hyaluronan-CD44 axis orchestrates cancer stem cell functions. Cell. Signal. *63*, 109377.

Sleeman, J.P., Kondo, K., Moll, J., Ponta, H., and Herrlich, P. (1997). Variant Exons v6 and v7 Together Expand the Repertoire of Glycosaminoglycans Bound by CD44. J. Biol. Chem. *272*, 31837–31844.

Slomiany, M.G., Grass, G.D., Robertson, A.D., Yang, X.Y., Maria, B.L., Beeson, C., and Toole, B.P. (2009). Hyaluronan, CD44, and Emmprin Regulate Lactate Efflux and Membrane Localization of Monocarboxylate Transporters in Human Breast Carcinoma Cells. Cancer Res. *69*, 1293–1301.

Stamenkovic, I., Amiot, M., Pesando, J.M., and Seed, B. (1989). A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell *56*, 1057–1062.

Statello, L., Guo, C.-J., Chen, L.-L., and Huarte, M. (2020). Gene regulation by long non-coding RNAs and its biological functions. Nat. Rev. Mol. Cell Biol. 2020 222 *22*, 96–118.

Stern, R., and Jedrzejas, M.J. (2006). Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action.

Sugahara, K.N., Murai, T., Nishinakamura, H., Kawashima, H., Saya, H., and Miyasaka, M. (2003). Hyaluronan Oligosaccharides Induce CD44 Cleavage and Promote Cell Migration in CD44-expressing Tumor Cells. J. Biol. Chem. *278*, 32259–32265.

Sugahara, K.N., Hirata, T., Hayasaka, H., Stern, R., Murai, T., and Miyasaka, M. (2006). Tumor Cells Enhance Their Own CD44 Cleavage and Motility by Generating Hyaluronan Fragments. J. Biol. Chem. *281*, 5861–5868.

Sundqvist, A., Zieba, A., Vasilaki, E., Herrera Hidalgo, C., Söderberg, O., Koinuma, D., Miyazono, K., Heldin, C.-H., Landegren, U., ten Dijke, P., et al. (2012). Specific interactions between Smad proteins and AP-1 components determine TGFβ-induced breast cancer cell invasion. Oncogene 2013 3231 *32*, 3606–3615.

Sundqvist, A., Vasilaki, E., Voytyuk, O., Bai, Y., Morikawa, M., Moustakas, A., Miyazono, K., Heldin, C.-H., ten Dijke, P., and van Dam, H. (2020). TGFβ and EGF signaling orchestrates the AP-1- and p63 transcriptional regulation of breast cancer invasiveness. Oncogene 2020 3922 *39*, 4436–4449.

Suzuki, A., Kusakai, G.I., Kishimoto, A., Lu, J., Ogura, T., Lavin, M.F., and Esumi, H. (2003a). Identification of a Novel Protein Kinase Mediating Akt Survival Signaling to the ATM Protein. J. Biol. Chem. *278*, 48–53.

Suzuki, A., Kusakai, G., Kishimoto, A., Lu, J., Ogura, T., and Esumi, H. (2003b). ARK5 suppresses the cell death induced by nutrient starvation and death receptors via inhibition of caspase 8 activation, but not by chemotherapeutic agents or UV irradiation. Oncogene 2003 2240 *22*, 6177–6182.

Suzuki, A., Lu, J., Kusakai, G., Kishimoto, A., Ogura, T., and Esumi, H. (2004). ARK5 Is a Tumor Invasion-Associated Factor Downstream of Akt Signaling. Mol. Cell. Biol. *24*, 3526–3535.

Takahashi, N., Knudson, C.B., Thankamony, S., Ariyoshi, W., Mellor, L., Im, H.-J., and Knudson, W. (2010). Induction of CD44 cleavage in articular chondrocytes. Arthritis Rheum. *62*, 1338–1348.

Takai, K., Le, A., Weaver, V.M., Werb, Z., Takai, K., Le, A., Weaver, V.M., and Werb, Z. (2016). Targeting the cancer-associated fibroblasts as a treatment in triple-negative breast cancer. Oncotarget *7*, 82889–82901.

Takasugi, M., Firsanov, D., Tombline, G., Ning, H., Ablaeva, J., Seluanov, A., and Gorbunova, V. (2020). Naked mole-rat very-high-molecular-mass hyaluronan exhibits superior cytoprotective properties. Nat. Commun. 2020 111 *11*, 1–10.

Tan, E.-J., Kahata, K., Thuault, S., Heldin, C.-H., and Moustakas, A. (2015). The high mobility group A2 protein epigenetically silences the Cdh1 gene during epithelial-to-mesenchymal transition. Nucleic Acids Res. *43*, 162–178.

Tan, E.J., Thuault, S., Caja, L., Carletti, T., Heldin, C.H., and Moustakas, A. (2012). Regulation of Transcription Factor Twist Expression by the DNA Architectural Protein High Mobility Group A2 during Epithelial-to-Mesenchymal Transition. J. Biol. Chem. *287*, 7134–7145.

Tang, T.H.-C., Alonso, S., Ng, L.F.-P., Thein, T.-L., Pang, V.J.-X., Leo, Y.-S., Lye, D.C.-B., and Yeo, T.-W. (2017). Increased Serum Hyaluronic Acid and Heparan Sulfate in Dengue Fever: Association with Plasma Leakage and Disease Severity. Sci. Reports 2017 71 *7*, 1–9.

Teriete, P., Banerji, S., Noble, M., Blundell, C.D., Wright, A.J., Pickford, A.R., Lowe, E., Mahoney, D.J., Tammi, M.I., Kahmann, J.D., et al. (2004). Structure of the Regulatory Hyaluronan Binding Domain in the Inflammatory Leukocyte Homing Receptor CD44. Mol. Cell *13*, 483–496.

Thiery, J.P. (2002). Epithelial–mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2002 26 *2*, 442–454.

Thuault, S., Tan, E.J., Peinado, H., Cano, A., Heldin, C.H., and Moustakas, A. (2008). HMGA2 and Smads Co-regulate SNAIL1 Expression during Induction of Epithelial-to-Mesenchymal Transition. J. Biol. Chem. *283*, 33437–33446.

Tian, X., Azpurua, J., Hine, C., Vaidya, A., Myakishev-Rempel, M., Ablaeva, J., Mao, Z., Nevo, E., Gorbunova, V., and Seluanov, A. (2013). High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. Nat. 2013 4997458 *499*, 346–349.

Toole, B.P. (2004). Hyaluronan: from extracellular glue to pericellular cue. Nat. Rev. Cancer 2004 47 *4*, 528–539.

Tremmel, M., Matzke, A., Albrecht, I., Laib, A.M., Olaku, V., Ballmer-Hofer, K., Christofori, G., Héroult, M., Augustin, H.G., Ponta, H., et al. (2009). A CD44v6 peptide reveals a role of CD44 in VEGFR-2 signaling and angiogenesis. Blood *114*, 5236–5244.

Tsubakihara, Y., and Moustakas, A. (2018). Epithelial-Mesenchymal Transition and Metastasis under the Control of Transforming Growth Factor β. Int. J. Mol. Sci. 2018, Vol. 19, Page 3672 *19*, 3672.

Tsukita, S., Oishi, K., Sato, N., Sagara, J., Kawai, A., and Tsukita, S. (1994). ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. J. Cell Biol. *126*, 391–401.

Tzavlaki, K., and Moustakas, A. (2020). TGF-β Signaling. Biomol. 2020, Vol. 10, Page 487 *10*, 487.

Udabage, L., Brownlee, G.R., Waltham, M., Blick, T., Walker, E.C., Heldin, P., Nilsson, S.K., Thompson, E.W., and Brown, T.J. (2005). Antisense-Mediated Suppression of Hyaluronan Synthase 2 Inhibits the Tumorigenesis and Progression of Breast Cancer. Cancer Res. *65*, 6139–6150.

Vallenius, T., Vaahtomeri, K., Kovac, B., Osiceanu, A.-M., Viljanen, M., and Mäkelä, T.P. (2011). An association between NUAK2 and MRIP reveals a novel mechanism for regulation of actin stress fibers. J. Cell Sci. *124*, 384–393.

Vasilaki, E., Morikawa, M., Koinuma, D., Mizutani, A., Hirano, Y., Ehata, S., Sundqvist, A., Kawasaki, N., Cedervall, J., Olsson, A.K., et al. (2016). Ras and TGF-β signaling enhance cancer progression by promoting the ΔNp63 transcriptional program. Sci. Signal. *9*.

Vigetti, D., Genasetti, A., Karousou, E., Viola, M., Moretto, P., Clerici, M., Deleonibus, S., De Luca, G., Hascall, V.C., and Passi, A. (2010). Proinflammatory Cytokines Induce Hyaluronan Synthesis and Monocyte Adhesion in Human Endothelial Cells through Hyaluronan Synthase 2 (HAS2) and the Nuclear Factor-κB (NF-κB) Pathway. J. Biol. Chem. *285*, 24639–24645.

Vigetti, D., Clerici, M., Deleonibus, S., Karousou, E., Viola, M., Moretto, P., Heldin, P., Hascall, V.C., De Luca, G., and Passi, A. (2011). Hyaluronan Synthesis Is Inhibited by Adenosine Monophosphate-activated Protein Kinase through the Regulation of HAS2 Activity in Human Aortic Smooth Muscle Cells. J. Biol. Chem. *286*, 7917–7924.

Vigetti, D., Deleonibus, S., Moretto, P., Bowen, T., Fischer, J.W., Grandoch, M., Oberhuber, A., Love, D.C., Hanover, J.A., Cinquetti, R., et al. (2014). Natural Antisense Transcript for Hyaluronan Synthase 2 (HAS2-AS1) Induces Transcription of HAS2 via Protein O-GlcNAcylation. J. Biol. Chem. *289*, 28816–28826.

Villegas, V.E., and Zaphiropoulos, P.G. (2015). Neighboring Gene Regulation by Antisense Long Non-Coding RNAs. Int. J. Mol. Sci. 2015, Vol. 16, Pages 3251-3266 *16*, 3251–3266.

Vuorio, E., Einola, S., Hakkaramen, S., and Penttinen, R. (1982). Synthesis of underpolymerized hyaluronic acid by fibroblasts cultured from rheumatoid and non-rheumatoid synovitis. Rheumatol. Int. 1982 23 *2*, 97–102.

Wang, J., Zhang, Y., You, A., Li, J., Gu, J., Rao, G., Ge, X., Zhang, K., Fu, H., Liu, X., et al. (2020). HAS2-AS1 Acts as a Molecular Sponge for miR-137 and Promotes the Invasion and Migration of Glioma Cells by Targeting EZH2. Https://Doi.Org/10.1080/15384101.2020.1826237 *19*, 2826–2835.

Wang, W., Zhang, H., Liu, S., Kim, C.K., Xu, Y., Hurley, L.A., Nishikawa, R., Nagane, M., Hu, B., Stegh, A.H., et al. (2017). Internalized CD44s splice isoform attenuates EGFR degradation by targeting Rab7A. Proc. Natl. Acad. Sci. *114*, 8366–8371.

Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K.J., and Werb, Z. (2020). Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat. Commun. 2020 111 *11*, 1–19.

Xu, Y., Gao, X.D., Lee, J.-H., Huang, H., Tan, H., Ahn, J., Reinke, L.M., Peter, M.E., Feng, Y., Gius, D., et al. (2014). Cell type-restricted activity of hnRNPM promotes breast cancer metastasis via regulating alternative splicing. Genes Dev. *28*, 1191–1203.

Yae, T., Tsuchihashi, K., Ishimoto, T., Motohara, T., Yoshikawa, M., Yoshida, G.J., Wada, T., Masuko, T., Mogushi, K., Tanaka, H., et al. (2012). Alternative splicing of CD44 mRNA by ESRP1 enhances lung colonization of metastatic cancer cell. Nat. Commun. 2012 31 *3*, 1–9.

Yamamoto, H., Takashima, S., Shintani, Y., Yamazaki, S., Seguchi, O., Nakano, A., Higo, S., Kato, H., Liao, Y., Asano, Y., et al. (2008). Identification of a novel substrate for TNFα-induced kinase NUAK2. Biochem. Biophys. Res. Commun. *365*, 541–547.

Yamamoto, H., Tobisawa, Y., Inubushi, T., Irie, F., Ohyama, C., and Yamaguchi, Y. (2017). A mammalian homolog of the zebrafish transmembrane protein 2 (TMEM2) is the long-sought-after cell-surface hyaluronidase.

Yang, C., Cao, M., Liu, Y., He, Y., Du, Y., Zhang, G., and Gao, F. (2019). Inducible formation of leader cells driven by CD44 switching gives rise to collective invasion and metastases in luminal breast carcinomas. Oncogene 2019 3846 *38*, 7113–7132.

Yoshida, H., Nagaoka, A., Kusaka-Kikushima, A., Tobiishi, M., Kawabata, K., Sayo, T., Sakai, S., Sugiyama, Y., Enomoto, H., Okada, Y., et al. (2013). KIAA1199, a deafness gene of unknown function, is a new hyaluronan binding protein involved in hyaluronan depolymerization. Proc. Natl. Acad. Sci. *110*, 5612–5617.

Yu, Q., and Stamenkovic, I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev. *14*, 163–176.

Yu, W.-H., Woessner, J.F., McNeish, J.D., and Stamenkovic, I. (2002). CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes Dev. *16*, 307–323.

Yuan, W.-C., Pepe-Mooney, B., Galli, G.G., Dill, M.T., Huang, H.-T., Hao, M., Wang, Y., Liang, H., Calogero, R.A., and Camargo, F.D. (2018). NUAK2 is a critical YAP target in liver cancer. Nat. Commun. 2018 91 *9*, 1–12.

Zagórska, A., Deak, M., Campbell, D.G., Banerjee, S., Hirano, M., Aizawa, S., Prescott, A.R., and Alessi, D.R. (2010). New roles for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion. Sci. Signal. *3*.

Zaytseva, Y.Y., Rychahou, P.G., Gulhati, P., Elliott, V.A., Mustain, W.C., O'Connor, K., Morris, A.J., Sunkara, M., Weiss, H.L., Lee, E.Y., et al. (2012). Inhibition of Fatty Acid Synthase Attenuates CD44-Associated Signaling and Reduces Metastasis in Colorectal Cancer. Cancer Res. *72*, 1504–1517.

Zhang, F.-L., Cao, J.-L., Xie, H.-Y., Sun, R., Yang, L.-F., Shao, Z.-M., and Li, D.-Q. (2018). Cancer-Associated MORC2-Mutant M276I Regulates an hnRNPM-Mediated CD44 Splicing Switch to Promote Invasion and Metastasis in Triple-Negative Breast Cancer. Cancer Res. *78*, 5780–5792.

Zhang, H., Brown, R.L., Wei, Y., Zhao, P., Liu, S., Liu, X., Deng, Y., Hu, X., Zhang, J., Gao, X.D., et al. (2019). CD44 splice isoform switching determines breast cancer stem cell state. Genes Dev. *33*, 166–179.

Zhang, L., Zhou, F., GarcíadeVinuesa, A., deKruijf, E.M., Mesker, W.E., Hui, L., Drabsch, Y., Li, Y., Bauer, A., Rousseau, A., et al. (2013). TRAF4 Promotes TGF-β Receptor Signaling and Drives Breast Cancer Metastasis. Mol. Cell *51*, 559–572.

Zhang, X., Dou, P., Akhtar, M.L., Liu, F., Hu, X., Yang, L., Yang, D., Zhang, X., Li, Y., Qiao, S., et al. (2021). NEU4 inhibits motility of HCC cells by cleaving sialic acids on CD44. Oncogene 2021 4035 *40*, 5427–5440.

Zhang, Y., Jia, S., and Jiang, W.G. (2014). KIAA1199 and its biological role in human cancer and cancer cells (Review). Oncol. Rep. *31*, 1503–1508.

Zhao, H., Tanaka, T., Mitlitski, V., Heeter, J., Balazs, E.A., and Darzynkiewicz, Z. (2008). Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells. Int. J. Oncol. *32*, 1159–1167.

Zhou, X., Liu, S., Cai, G., Kong, L., Zhang, T., Ren, Y., Wu, Y., Mei, M., Zhang, L., and Wang, X. (2015). Long Non Coding RNA MALAT1 Promotes Tumor Growth and Metastasis by inducing Epithelial-Mesenchymal Transition in Oral Squamous Cell Carcinoma. Sci. Reports 2015 51 *5*, 1–10.

Zhu, G., Wang, S., Chen, J., Wang, Z., Liang, X., Wang, X., Jiang, J., Lang, J., and Li, L. (2017). Long noncoding RNA HAS2-AS1 mediates hypoxia-induced invasiveness of oral squamous cell carcinoma. Mol. Carcinog. *56*, 2210–2222.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1768

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-451907

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2021