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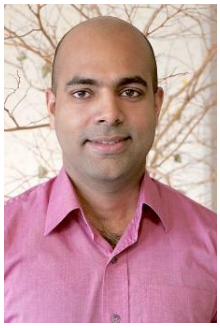
ROLE OF SYNDECAN-1 IN TUMOR CELL PROLIFERATION AND EPITHELIAL-MESENCHYMAL PLASTICITY

Ashish Kumar Singh



Ashish Kumar Singh was born in Raebareli district, India in 1986. He received a Bachelor of Technology from Dr. M.G.R University, Chennai (India) and a Master of Science in Applied Biotechnology at Uppsala University (Sweden). In 2016 he started doctoral studies with Associate professor Katalin Dobra as his main supervisor at the Pathology Division.

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ROLE OF SYNDECAN-1 IN TUMOR CELL PROLIFERATION AND EPITHELIAL-MESENCHYMAL PLASTICITY

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Friday, November 6th, 2020, at 9:00 AM

"There is only one **difference between dream and aim**. **Dream** requires effortless **sleep and aim** requires sleepless efforts. So, **sleep for dreams** and wake up for aims."

-**Swami Vivekananda**

I dedicate this thesis to my family, for their constant support and unconditional love. I love you all dearly.

ABSTRACT

Syndecan-1 (SDC1) is a heparan sulfate proteoglycan (HSPG) intercalated in the cell membrane but also translocated to the cell nucleus in a regulated manner. SDC1 is involved in several malignancy-associated processes such as proliferation and migration. Altered SDC1 expression can induce changes along the epithelial–mesenchymal axis and it may influence the prognosis of cancer.

Transforming growth factor-β (TGF-β) plays a pivotal role in many cellular functions, including epithelial–mesenchymal transition (EMT). In the early stages of tumorigenesis TGF-β inhibits cell growth and induces cell apoptosis, while in the later stages it promotes tumor growth.

The overall aim of this thesis was to study the role of SDC1 in mesenchymal tumors and functions related to the presence or absence of SDC1 in the nucleus. Understanding the role of cell surface and nuclear SDC1 and its interactions could be of importance for the understanding of tumor growth, proliferation, differentiation, and migration in these tumors.

In **paper I** we used fibrosarcoma cell sub-lines to study the functions of SDC1, especially the molecular targets and signaling pathways regulated by its nuclear translocation. The TGF-β pathway was activated by nuclear SDC1, and three genes were altered with the deletion of nuclear localization signal: EGR-1, NEK11 and DOCK8. The study shows the importance of the localization of SDC1 for its effect on tumor cells. The aim of **paper II** was to further study the role of nuclear SDC1 through characterizing its nuclear interactome, using a mesothelioma cell line. SDC1 was immunoprecipitated to identify co-precipitating interacting proteins. The results indicate a previously unknown role for SDC1 in RNA biogenesis. In **Paper III** we investigated if SDC1 plays a role in regulating TGF-β-induced EMT. The knockdown of SDC1 in a carcinoma cell line resulted in decreased expression of E-cadherin, and increased expression of N-cadherin. In fibrosarcoma cells, with its low basic SDC1 levels, overexpression of SDC1 was sufficient to repress N-cadherin and vimentin. The results indicate that SDC1 regulates epithelial-mesenchymal plasticity in tumor cells.

Together, these studies provide new insights into the role of SDC1 in tumors and the functional importance of the transport of SDC1 to the nucleus, as well as the connection between SDC1 and TGF-β.

LIST OF SCIENTIFIC PAPERS

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

I. Tünde Szatmári, Filip Mundt, **Ashish Kumar-Singh**, Lena Möbus, Rita Ötvös, Anders Hjerpe and Katalin Dobra.
Molecular targets and signaling pathways regulated by nuclear translocation of syndecan-1.
BMC Cell Biology 2017, DOI 10.1186/s12860-017-0150-z

II. **Ashish Kumar-Singh**, Jatin Shrinet, Malgorzata Maria Parniewska, Jonas Fuxe, Katalin Dobra, and Anders Hjerpe.
Mapping the Interactome of the Nuclear Heparan Sulfate Proteoglycan Syndecan-1 in Mesothelioma Cells.
Biomolecules 2020, 10, 1034; doi:10.3390/biom10071034

III. **Ashish Kumar-Singh,** Malgorzata Parniewska, Joman Javadi, Wenwen Sun, Katalin Dobra, Anders Hjerpe, and Jonas Fuxe.
Regulation of epithelial-mesenchymal plasticity in tumor cells by syndecan-1.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

CS	Chondroitin Sulfate
DOCK8	Dedicator of Cytokinesis 8
DS	Dermatan Sulfate
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EGR-1	Early Growth Response 1
FACS	Fluorescence-Activated Cell Sorting
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
GAG	Glycosaminoglycan
GO	Gene Ontology
HB-EGF	Heparin-Binding Epidermal Growth Factor
HGF	Hepatocyte Growth Factor
HS	Heparan Sulphate
HSPG	Heparan Sulfate Proteoglycan
MM	Malignant Mesothelioma
N-CAM	Neural-Cell Adhesion Molecule
NEK11	Never-in-mitosis gene a-related Kinase 11
NLS	Nuclear Localization Signal
PDGF	Platelet-Derived Growth Factor
PECAM	Platelet-Endothelial Cell Adhesion Molecule
PG	Proteoglycan
SDC1	Syndecan-1
SDS	Sodium Dodecyl Sulfate
TGF-β	Transforming Growth Factor β
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

1 INTRODUCTION

Cancer is characterized by the uncontrolled growth of cells, which can spread and metastasize to distant parts of the body. According to World Health Organization (WHO), about 9.6 million people die of cancer each year, which is about 16 % of all deaths worldwide. The most common types of cancer in men are lung, prostate, colorectal, stomach and liver cancer, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women. (*https://www.who.int/health-topics/cancer/*).

Cancer is a group of lethal conditions where genetic damages have caused loss of functions that regulate the growth of the original tissue. As normal cells evolve into tumor cells, they acquire capabilities which enable them to evade normal cell proliferation and homeostasis. Those capabilities include supporting proliferative signaling, evading growth suppressors, resisting apoptosis, enabling unlimited replication, inducing angiogenesis, and activating invasion and metastasis. Other possible characteristics are the abilities to deregulate energy metabolism and to evade immune destruction [1].

As the cancer cells invade and spread to form metastasis, they change their phenotype in a process called Epithelial to Mesenchymal Transition (EMT). The result is a more motile and proliferative cell that can enter channels for spreading, such as the lymphatics, veins, and serous cavities. Once settled down or trapped at the metastatic site, a reversed process (MET) will regain its original and more epithelioid tumor phenotype, the appearance of the resulting metastasis being similar to the original tumor.

1.1 SYNDECANS (SDCS)

Proteoglycans (PGs) are proteins that carry glycosaminoglycan (GAG) side chains which are covalently bound to the core protein [2]. They can be classified by their location, their different sizes as well as the nature of their glycosaminoglycan chains. PGs are either found on or in the cell or in the extracellular matrix (ECM) [3].

The first molecule identified as an integral membrane proteoglycan was named syndecan (SDC) from the Greek word *syndein* meaning “to bind together” [4]. The SDCs constitute a family of four type I transmembrane heparan sulfate proteoglycans (HSPGs), which are located in the plasma membrane as a link between intracellular and extracellular surroundings [4, 5]. One central function of SDCs seems to be to regulate the ligand-dependent activation of primary signaling receptors at the cell surface [6]. They interact with extra-cellular ligands and are implicated in cellular signal transduction, which means that they are involved in many cellular processes [7], including cell proliferation, differentiation, adhesion, and migration [8].

SDCs are mainly localized on the cell surface but can also be released through sheddases or accumulate in the cell nucleus, in the tumor stroma, and in body fluids. Through shedding of the ectodomain, the cell-bound SDC is changed into a soluble active ligand [9]. SDC1 is common in epithelial cells, while SDC2 is mainly found on mesenchymal cells, SDC3 in neuronal tissue and cartilage, and SDC4 in all types of cells [4, 5, 10–13]. The SDC proteins has three domains: a N-terminal ectodomain, a transmembrane domain and a C-terminal cytoplasmic domain [14].

1.1.1 Core proteins

Extracellular domain (Ectodomain)

The N-terminal extracellular domain carries glycosaminoglycan (GAG) chains (Figure 1): heparan sulfate (HS) and chondroitin sulfate (CS) [14]. Through the HS chains the ectodomain interacts with many types of ligands in the extracellular matrix, for example growth factors, chemokines, collagens, cytokines, and proteinases [15], while the function of the CS chain is still less clarified. During angiogenesis SDC1 binds to both $\alpha v\beta_3$ and $\alpha v\beta_5$ integrins via the ectodomain, while during reepithelialization of lung tissue it binds to $\alpha v\beta_1$ integrin. This leads to formation of ternary complexes between extracellular molecules, a cell surface receptor and a PG, like the ones associated with growth factors, their receptors and HS chains [16–19]. The DRKE sequence in SDC1 could possibly be essential for oligomerization in the same way as the EKRE motif is needed in the SDC3 ectodomain. However, the function of DRKE is still not clear [20].

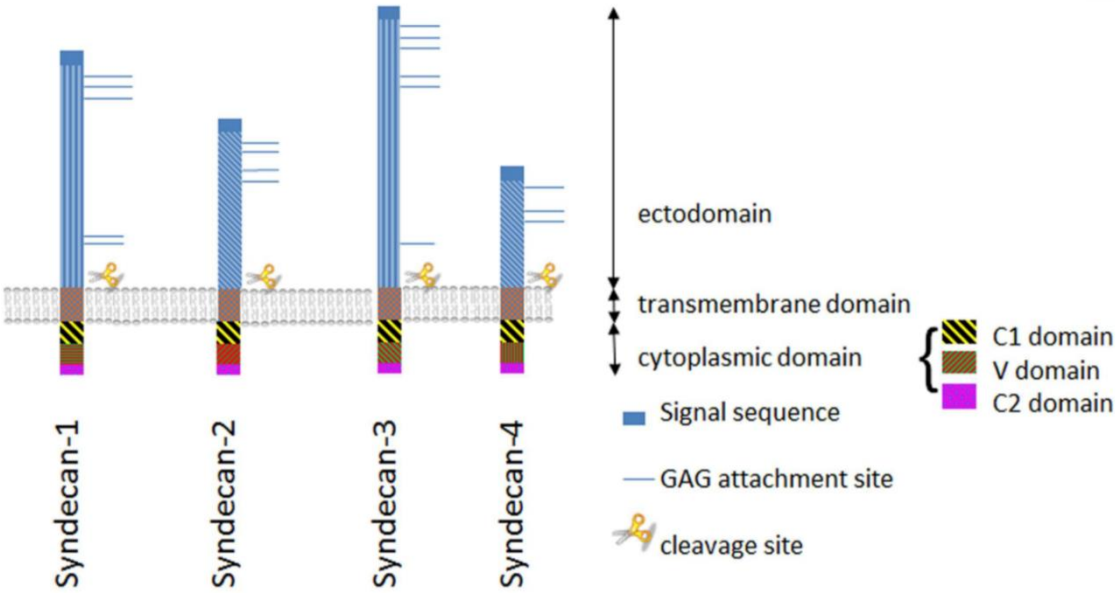
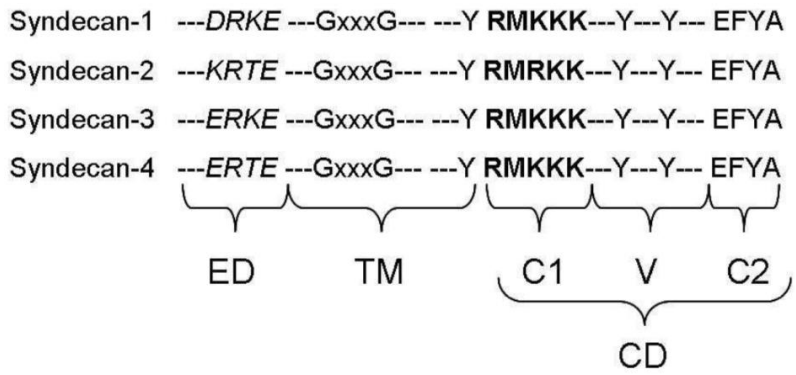


Figure 1. Schematic illustration of structurally related SDC family [21]**.**

Transmembrane and Cytoplasmic domains

The single-pass transmembrane domain is highly conserved in the four SDC family members (Figure 2). Each SDC gene contains the GxxxG motif, which gives rise to dimers formation [22]. The cytoplasmic domain of SDC1 connects with several cytosolic proteins and plays an important role in endocytosis. It has two conserved C1 and C2 sites on each side of the variable region V, which is unique for each SDC family member. The C1 site mediates SDC dimerization and interacts with several intracellular proteins which regulate the organization of the cytoskeleton, for example tubulin, ezrin and cortacin [22, 23]. The RMKKK motif is present at the cytoplasmic domain and plays an important role in nuclear translocation [24].



ED: ectodomain
TM: transmembrane domain
CD: cytoplasmic domain

Figure 2. Conserved amino acid sequences of the SDC family [25].

1.1.2 Glycosaminoglycan (GAG) chains

The extracellular domain of the SDC1 protein core has five serine residues, on which GAG chains can be synthesized. Three of these are located close to the distal N-terminus and the remaining two adjacent to the cell membrane. In the Golgi, the GAGs are synthesized on these serine residues as a repeating disaccharide structure after an initial linkage tetrasaccharide. In this way HS is formed on the three distal sites and CS on the proximal two. The HS and CS chain consists of repeating hexosamine-uronic acid disaccharides with a basic structure of N-acetylglucosamine-glucuronic acid in HS and N-acetylgalactosamine-glucuronic acid in CS. Following synthesis, this basic structure can be modified by deacetylation, uronic acid epimerization and sulfation, which particularly in HS may result in a considerable structural heterogeneity, which seems to be important for different HS interactions [26]. In addition, several post-synthetic changes can occur also at the cell surface: fragmentation of HS by heparanase, selective removal of 6-O sulfate by extracellular endosulfatases and shedding of the entire extracellular domains by sheddases [27, 28]. The fragments which are generated through the selective removal of 6-O sulfate are biologically more active in basic fibroblast growth factor (FGF-2) signaling than the original HS chains [29].

The role of CS in the function of SDC1 is not yet well known. The presence of CS chains on the ectodomain close to the plasma membrane could have an effect on the ability of SDC1 to connect with other transmembrane receptors, change its inclination to cleavage and shedding by proteases, or hamper its clustering [30].

1.1.3 Functional interactions

SDC1 is involved in a large range of biological functions. Changes in SDC1 can influence tumor phenotypes due to alterations in cell proliferation and cell growth, cell survival, cell invasion, metastasis, and angiogenesis [31–33]. The GAG chains bind various protein ligands, depending on their fine structure and position on the core protein. Since they carry various GAG chains that may function together, the ligand binding to proteoglycans is very complex.

SDC1 employs its functions mainly through the HS chain, binding several different morphogens and growth factors with varying affinity to high and low sulfated regions [6, 34]. (Table 1), For example, it binds fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), Wnt, hepatocyte growth factor (HGF), and their receptors. It thereby helps to stabilize the growth factor/growth factor receptor complexes [35–38].

Table 1. Selected HS-binding ligands

Growth factors, Growth factor receptors	FGF family, FGFR-1, HGF, HB-EGF, VEGF, PDGF
Extracellular-matrix proteins	Fibronectin, Laminin, Collagens, Thrombosponin, Tenascin
Cell-cell adhesion molecules	L-selectin, N-CAM. PECAM, Mac-1

The selection is partly based on Carey *et al.*[6].

Because of their fine structure, HS chains have been shown to affect the signals imposed on the cell by the growth factor. The HS chains on SDC1, rather than the ectodomain of the core protein, mediate the paracrine fibroblast growth factor 2 (FGF-2)-dependent signaling to stimulate growth in stromal fibroblasts of breast carcinomas [38]. On the other hand, CS chains also bind to matrix proteins and soluble molecules [39]. Separate domains of the core proteins can also bind or relate to ECM components, cell surface and cytoplasmic ligands, thereby modulating various cellular processes (Figure 3).

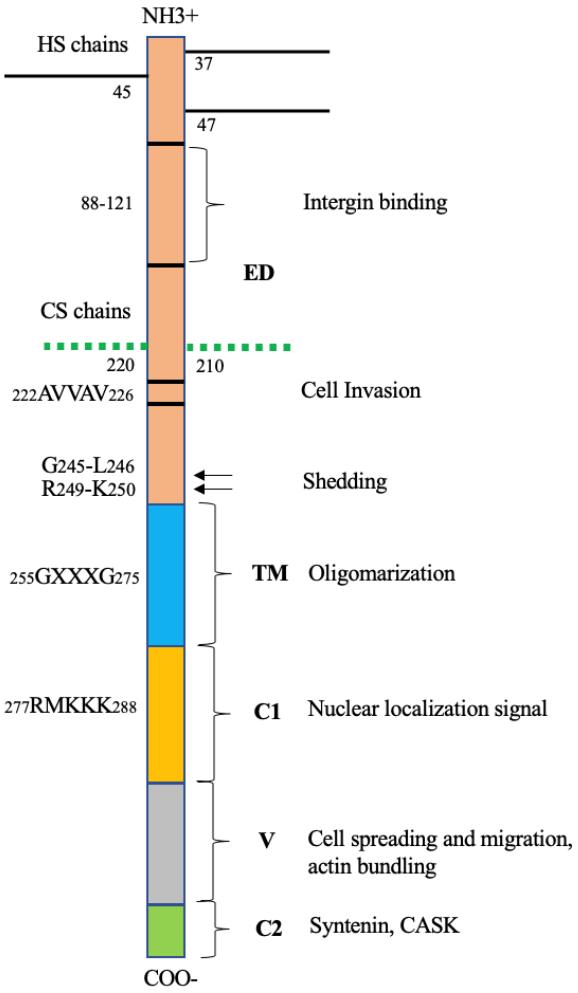
Through an indirect interaction with β1 integrin, the ectodomains of SDC2 and -4 promote mesenchymal cell adhesion [40]. A similar property of a stretch of 5 hydrophobic amino acids -$_{222}$AVAAV$_{226}$- in the ectodomains close to the plasma membrane is critical for SDC1-mediated inhibition of cell invasion [41].

On their own, the transmembrane and cytoplasmic domains of SDCs do not have intrinsic kinase or catalytic activity, but through multimerization or interaction with different intracellular components like GTPases or kinases, they are essential in generating the signal transduction [42].

The SDC core proteins could be brought closer together through oligomerization, which enlarges their interaction surfaces and thereby increases the probability of binding to other membrane proteins [43]. This oligomerization is crucial for triggering the cytoplasmic domain for downstream signaling [44, 45].

The cytoplasmic region influences the actin cytoskeleton and membrane trafficking through binding cytoskeletal and PDZ-domain proteins. This controls SDC recycling through endosomal compartments, promotes internalization of accompanying proteins and regulates cell adhesion as well as different signaling systems [46].

The C-terminal EFYA domain of C2 binds to PDZ-binding proteins, for example synbindin, synectin, CASK [47], CASK/LIN-2 and syntenin. Syntenin is essential for vesicular transportation, neuronal migration, synaptic signaling, adhesion, and metastasis formation [48, 49]. The cytoplasmic domain of SDC1 also interacts with $\alpha_6\beta_4$ integrin and through this regulates activation of ErbB2 [50].



ED: ectodomain
TM: transmembrane domain

Figure 3. The structure of SDC1, showing the localization of certain peptide sequences or domains with corresponding ligand interactions or functions [25].

1.1.4 Cellular distribution and nuclear localization

In adult tissues, SDC1 is the major SDC on the basolateral surface of epithelial cells. During embryogenesis development, it is transiently expressed by mesenchymal cells [4]. SDC1 is expressed by most epithelial cells, where it supports adhesion and regulates the biological activity of HS-binding growth factors [51].

Traditionally, SDCs are considered to function on the cell surface, but a substantial amount of SDC1 has been detected also in the nucleus [52]. More recent findings indicate that the functions of SDCs relate not only to its presence on the cell surface but also in the cytoplasm and nucleus. By using confocal laser microscopy to study the subcellular distribution, the nuclear translocation of SDC1 was shown to be time- and tubulin dependent. Following seeding of the mesothelioma cells, SDC1 is first present in the cytoplasm and then in the nucleus. Only later it can be seen in the cell membrane, often at contact sites between cells. The nuclear translocation of SDC1 takes place in a time- and tubulin-dependent manner. This has been shown in different types of carcinomas, in neuroblastoma and in benign mesothelial and endothelial cells [52]. Interference with tubulin integrity will inhibit the transport of SDC1 to the nucleus [52] and affect cell proliferation. Our recent study also shows that SDC1 plays a part in the formation and export of RNA **(Paper II).** SDC1 can be found inside the cell nuclei of several types of cancers. It has been found in the nucleus of mesothelioma, adenocarcinoma, breast cancer, hepatocellular carcinoma, neuroblastoma and myeloma cells [52–54].

Various studies of the presence of PGs and GAGs in the nucleus show that they play a role in the control of cell division, the transportation of FGF-2 into the nucleus [55] and suppression of DNA topoisomerase I activity [56].

The minimal structural requirement for the nuclear translocation of SDC1 is the conserved juxta-membrance RMKKK motif, which works as a nuclear localization signal (NLS), essential for tubulin-dependent nuclear translocation of SDC1 [53]. Substitution of the arginine in the RMKKK sequence decreases in the proportion of cells with nuclear SDC1 considerably, indicating the central role of arginine in this nuclear translocation. Removal of this highly conserved sequence prevents the nuclear translocation of SDC1. The MKKK sequence is also involved in raft-dependent endocytosis [24].

Questions that remain to be answered are for example, which molecules accompany them along the way and the role they play in the nucleus. Since SDC1 has been found in the nucleus [52], it could possibly function as a transcription factor and impact gene regulation affecting cancer pathogeneses.

It has been shown that loss of SDC1 from the nucleus of myeloma cells results in increased histone acetyltransferase (HAT) activity as well as increased expression of genes driving tumor progression [57]. However, we are only at the beginning of understanding the route and functional significance of this nuclear transport.

It has recently been found that also shed SDC1 may translocate to the nucleus of both tumor cells and bone-marrow-derived stromal cells [57]. An important factor regulating the level of both HS and SDC1 in the nucleus is heparanase, an endoglycosidase that cleaves HS and causes a considerable decrease in nuclear SDC1 levels in a concentration-dependent manner. This implies that HS chains are of importance for the nuclear translocation or degradation of SDC1. In many types of cancer, overexpression of heparanase is related to poor prognosis [58]. Depending on the concentration, overexpression, or addition of heparanase, this reduces the amount of SDC1 in the nucleus of myeloma cells. This indicates that also HS chains are

important for the nuclear translocation of SDC1 [54]. In malignant mesothelioma cells TGF-β inhibits the nuclear translocation of SDC1, with an antiproliferative effect as result [59].

1.1.5 Ectodomain shedding

Shedding is a process in which the SDC ectodomains are released from the surface of the cell by endogenous proteolytic cleavage [60]. One of the cleavage sites is G245-L246, which is placed about 7 amino acids from the cell membrane in human SDC1 [61]. During shedding SDC is converted from receptors on the membrane to soluble HSPG effectors, which compete with their equivalents on the cell surface in binding ligands [62]. The enzymes that regulate SDC1 shedding are matrix metalloproteinases (MMPs), including MMP-7, MMP-9, membrane-bound metalloproteinases (MT-MMP1) as well as disintegrin and metalloproteases (ADAM10, ADAM17) [61, 63–66]. Also, heparanase has been found to accelerate MMP-9 mediated shedding of SDC1 [35]. SDC1 expression is actually known to suppress the level of MMP-9 and inhibit cell invasion into type I collagen [67, 68]. However, the process of shedding is not yet fully known [69].

Patophysiological disorders, like tissue injury and inflammation [63, 70] or cancer forms like multiple myeloma [71], lung cancer [72] and Hodgkin's lymphoma [73], can cause acceleration in SDC shedding. Accelerated shedding can also be generated by physiological stimuli, such as chemokines, bacteria, growth factors, and cellular stress [29]. Heparanase increase SDC1 shedding by stimulating protease expression, which could explain the stimulation of tumor growth and metastasis [74, 75]. Chemotherapy can actually activate shedding of SDC1, especially through ADAMs, which leads to a more aggressive and therapy resistant phenotype [76].

SDC shedding has functional effects on cancer cells (Figure 4). For example, in breast cancer cells, SDC1 has different roles when in soluble form compared to when bound to the cell membrane. In adenocarcinoma cells, overexpression of SDC1 on the cell surface stimulates proliferation and inhibits invasion; on the other hand, overexpression of shed SDC1 has the opposite effect [77].

Soluble SDC1 also promotes growth of myeloma tumor cells, since the cells lacking its transmembrane and cytoplasmic domains proliferate much faster than cells with full-length SDC1 [78].

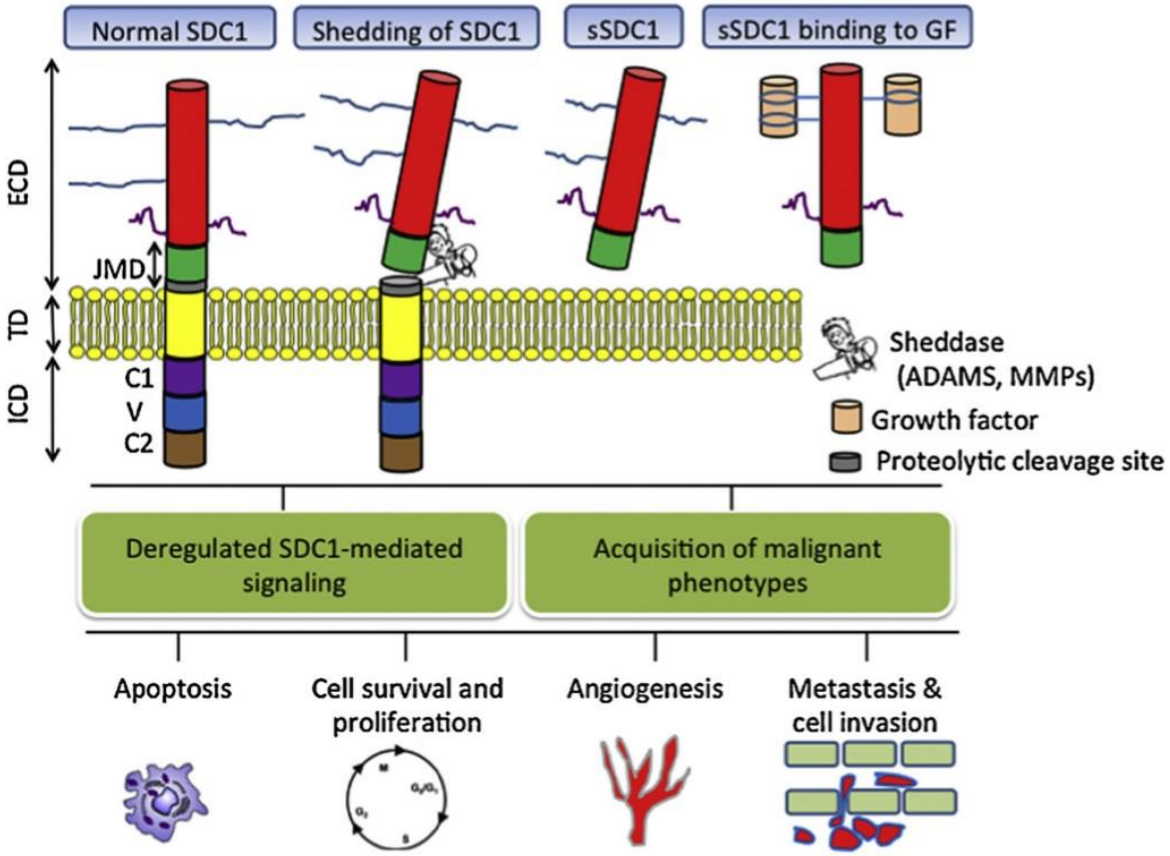


Figure 4. Schematic representation of the influence of SDC1 on tumor cells. SDC1 is cleaved by sheddases (e.g. ADAMS and MMPs), resulting in soluble SDC1 (sSDC1), which binds to growth factors and stimulate their signaling [33]. *(Reprinted with permission of Elsevier)*

1.1.6 SDC1 in cancers

The expression of SDC1 is related to the inhibition of tumor cell growth and invasiveness, as well as the maintenance of epithelial morphology, and changes in this expression can influence tumor cell behavior. SDC1, like other proteoglycans, affects tumorigenesis by altering the process of cell proliferation, apoptosis, angiogenesis etc. [79]. Suppression of SDC1 expression has been shown to change the tumor cell into a mesenchymal phenotype with loss of E-cadherin and altered cell morphology [80]. SCD1 expression also regulates cell differentiation in malignant mesothelioma [8, 81].

In several cancers, for example ovarian [82], breast [83], colorectal carcinomas [84] and mesothelioma [8, 85] the SDC1 expression is dysregulated and gradually lost [86]. In other types of human cancer SDC1 is highly expressed and in multiple myeloma it can even be used as a diagnostic biomarker [87]. Low SDC1 expression is correlated with a worse prognosis in head and neck tumors [88], as well as colorectal [89] and lung [90] cancer. In both lung cancer and mesothelioma, the presence of cell membrane-bound SDC1 is related to better prognosis [8, 90], whereas the shed soluble SDC1 correlates to worse prognosis. Down-regulation of SDC1 has been shown in mesenchymal tumors like human fibrosarcoma [91]. Low cell surface SDC1 level is associated with poor prognosis, for example in lung cancer. This means that SDC1 may have two different roles in different types of cancer, either as an inhibitor or promoter of tumor progression [85].

The process through which SDC1 regulates the survival and proliferation of tumor cells is dependent on the cell type. Different signaling pathways are related to SDC1 as a co-receptor. SDC1 null mice have been found resistant against Wnt-1 induced mammary tumorigenesis, with SDC1 acting as a co-receptor for Wnt signaling in an HS-dependent manner [36]. Another pathway is the signaling of Hepatocyte growth factor (HGF) through its receptor Met, which is significant for cancer development [92, 93]. For example, HGF can bind HS chains of SDC1 in myeloma [37]. Heparanase induces SDC1 shedding in myeloma cells via MMP-9 upregulation [75] and increases HGF expression [94].

The role of shed SDC1 in the tumor stroma can conflict with the role of cell bound SDC1, depending on the type of cell. Stromal SDC1 enhances the proliferation of breast epithelial cells [95]. It is also related to a poor prognosis in oral [96], ovarian [82] and gastric carcinoma [97]. Ectodomain shedding of SDC1 from stromal fibroblasts enhances tumor cell growth by activating FGF2 signaling [38]. In mammary tumor cells SDC1 overexpression in fibroblasts also leads to increased cancer cell proliferation [98]. This means that stromal SDC1 can save and dispense heparin-binding growth factors such as EGFs, FGFs and HGF to stimulate cancer cell proliferation [86].

SDC1 regulates tumor cell apoptosis in different ways depending on cell type. In myeloma cells SDC1 silencing engenders apoptosis [99], which could be caused by decreased levels of cell surface SDC1 as co-receptors for growth factor signaling. On the other hand, in MCF-7 breast cancer cells the reverse effect has been shown, when apoptosis was enhanced by adding human recombinant SDC1 ectodomain [100]. The varying effect that SDC1 can have on growth and apoptosis in different tumor cells, could partly be explained by their different requirements for growth factors. Also, each tumor has a unique microenvironment and SDC1 HS chains could be modified, with the effect of either stimulating or inhibiting tumor cell growth and apoptosis [101]. The opposing functions of SDC1 in tumor cell apoptosis may also be caused by the bell-shaped activity curve of HS in regulating HS-binding ligands [86]. HS can bring the ligand and its binding partner closer together and facilitates the interaction by increasing the local concentration of the ligand. Depending on whether HS-binding is inhibiting or stimulating the ligand, the bell-shaped curve may be concave or convex [86].

Integrins are essential receptors in cell adhesion, proliferation, and migration [102]. SDC1 can interact with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and trigger their signaling through the involvement of α_v ligands such as vitronectin, laminins, collagens, and fibronectin [16]. Also, integrins and SDC1 can form a link between the ECM and the cytoskeleton, stabilizing the focal adhesions, which inhibits cell migration and increases adhesion [86, 103].

Myeloma cell invasion into type I collagen gel is inhibited by SDC1 expression [68]. Within the SDC1 ectodomain, an invasion regulatory domain has been shown to inhibit the invasion of myeloma cells [41]. Overexpression of full-length SDC1, or a truncated variant without the ectodomain, stimulates metastasis of fibrosarcoma in a mouse model [104].

It has been shown that membrane-bound and soluble SDC1 play different roles in breast cancer progression [77]. While cell proliferation is increased by overexpression of full-length SDC1, it is decreased by shed SDC1. On the other hand, membrane-bound SDC1 inhibits invasiveness, while shed SDC1 promotes cell invasion. This means that the SDC1 shedding involves a change from a proliferative to an invasive phenotype.

1.2 MESENCHYMAL TUMORS

The focus in this PhD study is on the role of SDC1 in malignancy and changes in phenotype and biological behavior, in particular the process of EMT. For this purpose, we have chosen two mesenchymal tumors: malignant mesothelioma (MM) and fibrosarcoma- both with low endogenous expression of SDC1 and for comparison a cell line with high expression level **(Paper III)**. Both these tumors often have biphasic growth, i.e., they may have simultaneous presence of both epitheliod and sarcomatoid characteristics [105]. They can undergo a reversible morphological transition between these two phenotypes, which facilitates the study of how cell differentiation and tumor progression are regulated.

1.2.1 Malignant Mesothelioma (MM)

MM begins as a localized tumor, but it spreads rapidly along mesothelial surfaces [106]. Pleural MM primarily emerges from the surface serosal cells or the fibroblast like mesothelial cells just beneath the surface of the pleura [107]. As the MM cells infiltrate the pleura, they cause the formation of an effusion. This leads to difficulties to breathe due to reduced space for lung expansion [108]. Because of lack of early symptoms, MM is usually diagnosed at an advanced stage and the patient's life expectancy is quite short. Although MM is most common in the pleura, it may also appear in the other serous cavities of the body, such as in the peritoneum, pericardium and in the tunica vaginalis testis [109, 110].

Most MMs are caused by exposure to asbestos or asbestos-like minerals such as erionite [111, 112]. Asbestos is a carcinogenic mineral fiber, which may penetrate the pleura after having been inhaled. It has been suggested that the asbestos fibers interfere with the mitotic spindle, leading to aneuploidy [113]. A perhaps more important hypothesis is that the asbestos crystals, that attract iron-containing elements (hemosiderin, ferritin) to form the ferruginous bodies, through these iron deposits cause reactive oxygen species, that cause DNA damage and subsequent oncogenesis [107].

Asbestos has now been prohibited in Western countries, but due to the long latency for MM development– 20 to 50 years – of MM and the fact that asbestos is still used in other parts of the world, MM remains an increasing problem [109, 114, 115]. Ten percent of MM cases may relate to other asbestos-like materials such as erinonite or to genetic predispositions [116, 117]. The prognosis for this tumor is poor, the survival time being around 12 months after diagnosis. Mesotheliomas are often resistant to treatment, average improvement from chemotherapy being only a few months prolonged survival [137, 138].

SDC1 expression in MM is lower than in epithelial malignancies, which may suggest SDC1 to be useful as a biomarker in MM, related to better prognosis. Epithelioid MM expresses

higher levels of SDC1, while the less differentiated sarcomatoid MM shows low or negative expression of SDC1 [8, 81].

In culture SDC1 overexpression in MM cells inhibits tumor growth [120] and migration, and enhances cell adhesion [24]. Downregulation of SDC1 leads to a more sarcomatoid phenotype, and a decreased level of SDC1 on these cells indicates EMT transition, which would correspond to worse prognosis [81].

1.2.2 Fibrosarcoma

Fibrosarcoma is a somewhat less common malignant tumor that may relate to exposure to radiation [121]. This tumor expresses different GAGs and PGs [122]. The level of SDC1 is usually low but some cell lines can still express this PG [91, 120]. The location seems to be crucial for the effect of SDC1 on fibrosarcoma cells. While cell membrane bound SDC1 inhibits migration on collagen, migration is enhanced by membrane type I metalloprotease (MT1-MMP) mediated shedding [61].

Fibrosarcoma cells with an overexpression of SDC1 have higher growth rates and develop more lung metastases [104]. However, SDC1 overexpression has different effects depending on type of fibrosarcoma cells. The proliferation and migration of sarcomatoid fibrosarcoma cells is inhibited [24, 120], while epithelioid fibrosarcoma cells are enhanced with SDC1 overexpression together with SDC2 [104, 123].

1.3 TGF-β IN CANCER

Transforming growth factor-β (TGF-β) is a cytokine which maintains tissue homeostasis and is important during embryogenesis [124]. TGF-β can be produced by the tumor cell itself or cells in its microenvironment, such as stromal cells, macrophages and platelets [125–127]. Also, secreted TGF-β stored in the ECM can be released through the increase in matrix degradation factors associated with tumors [128–130].

TGF-β plays a role in many cellular functions, such as cell proliferation, differentiation, and control of apoptosis. It hampers the proliferation of various human cell lines and tissues, such as hepatocytes, thyroid, mammary and colon epithelial cells [131]. In cancer cells, TGF-β cannot induce changes in myc, ID-1, and ID-2 because of somatic mutations in parts of the TGF-β signaling pathway [131, 132]. It has been shown that TGF-β produced by tumor cells acts on itself in an autocrine manner to stimulate the suppression of tumors. Some studies indicate that TGF-βRI and TGF-βRII are genes that inhibit tumor growth. Ovarian, cervical, gastric, head and neck carcinomas can be related to mutations in TGF-βRI and TGF-βRII [133]. TGF-βRIII is expressed in epithelial cells and can suppress cancer progression. On the other hand, loss of TGF-βRIII is associated with disease progression and poor prognosis [131, 132, 134].

TGF-β also participates in EMT [135–138]. It regulates cellular microenvironment and prevents tissues from becoming malignant [139]. Paradoxically, TGF-β also promotes cell migration, invasion, and metastasis, mainly through induction of EMT [140]. It can also promote the colonization of the malignant cells to form metastases through the reversed mesenchymal-to-epithelial transition (MET) and up-regulation of metastatic niche genes.

When breast cancer cells metastasize to the lung, TGF-β promotes MET via an ID1-Twist1 signaling axis [141]. In this way, TGF-β plays dual roles in cancer (Figure 5). In the early stages of tumorigenesis TGF-β inhibits cell growth and induces cell apoptosis, while in the later stages it promotes tumor growth. As the tumor cells undergo EMT their growth is no longer inhibited by TGF-β, which instead supports invasion and metastasis [142]. Studies have shown a resemblance between MM and EMT, indicating that the three mesothelioma phenotypes (epithelioid, sarcomatoid and biphasic) could be an illustration of the EMT axis [143, 144]. In epithelioid to sarcomatoid mesothelioma tissues, a change from epithelial to mesenchymal markers can be seen moving towards EMT (e.g. loss of E-cadherin and increase in N-cadherin and vimentin) [144]. Although mesothelioma can express both E-cadherin and N-cadherin, the mesothelial marker NCAM is central in both phenotypes [145] and Vimentin is often expressed in epithelioid mesotheliomas [146].

Although TGF-β can suppress cancer initiation, in cancer that do develop there has been noted an increased expression of TGF-β ligands in breast [147, 148], lung [149], colorectal [150], and pancreatic cancers [151]. In these cancers, higher TGF-β ligand levels have been shown locally and systemically, with increased levels in the circulation. In lymph node metastasis, a higher level of TGF-β has been demonstrated, compared with primary tumors or in tumors that eventually metastasize [147, 149–154].

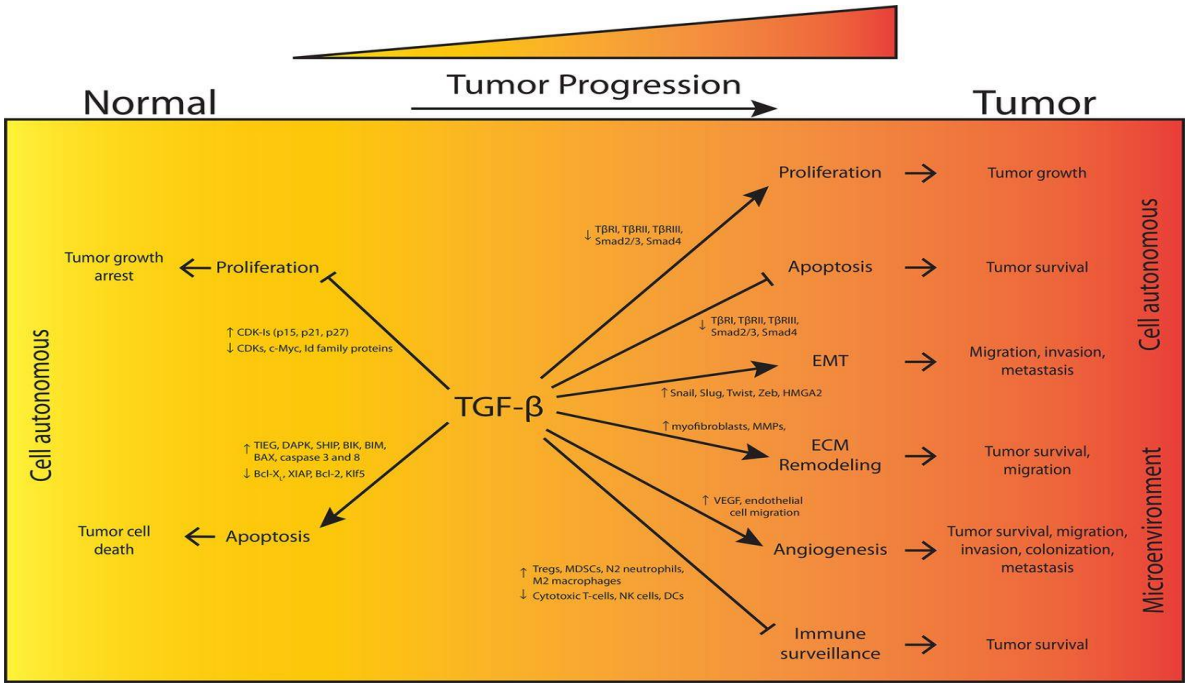


Figure 5. TGF-β has a dual role in cancer as a tumor suppressor and promoter, controlling proliferation, apoptosis and EMT [155]. *(Reprinted with permission of Portland press)*

1.3.1 TGF-β-induced EMT

During malignant transformation, cancer progression and metastasis, normal epithelial cells go through molecular and morphological changes, which give them mesenchymal characteristics and a migratory phenotype (Figure 6). The transcriptional repression of

epithelial markers is one of the first essential stages in this epithelial-mesenchymal transition (EMT) [156, 157], which results in loss of E-cadherin and SDC1 [80, 158]. SDC1 is necessary to maintain the epithelial phenotype. EMT can be induced in various cell types through TGF-β, which plays an essential role in the activation of various EMT inducing transcription factors [157]. For a complete EMT, cooperation between TGF-β and other pathways, such as Ras, is necessary [159–162]. SMAD signaling is also necessary for TGF-β-induced EMT [161]. The binding of TGF-β to its receptors leads to phosphorylation of SMAD2/3, partnering with SMAD4 and translocating to the nucleus where the expression of target genes is controlled by SMAD transcriptional complexes. Cells read TGF-β signals differently and the range of SMAD cofactors active at the time affects the outcome of the response [162]. SMAD complexes could either stimulate activation or repression of target genes, depending on the cofactors present. It has been shown that several transcription factors promoting EMT, such as Snail, Zeb1 and Zeb2, can act as SMAD cofactors. The interaction of SMAD with these transcription factors generates EMT-promoting SMAD complexes [163].

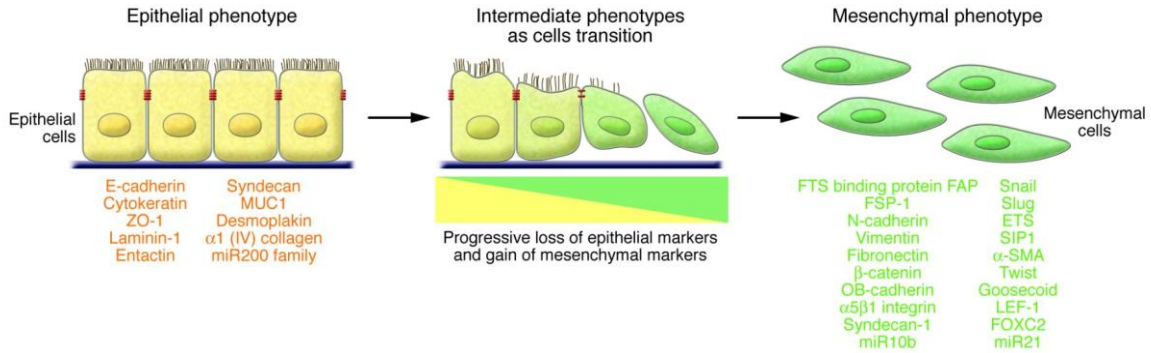


Figure 6. During EMT epithelial cells transform step by step from an epithelial to a mesenchymal phenotype [157]. *(Reprinted with permission of American Society for Clinical Investigation)*

TGF-β induces EMT either through canonical or non-canonical pathways. TGF-β promotes cytoskeleton reorganization by inducing focal adhesion kinase signaling, smooth muscle actin expression and stress fiber formation [164]. These EMT-associated changes make cells lose their connection to other epithelial cells, obtain a fibroblast phenotype, and migrate from their initial location, promoting cancer metastasis.

The gene expression analysis of tumor-propagating breast cancer cell populations expressing the cell surface markers $CD44^{+}/CD24^{low}$ indicates a role of TGF-β-induced EMT in human cancer. Cancer cells from different patients showed a common gene expression pattern, which suggests the presence of an active TGF-β pathway [165].

The TGF-β signaling pathway is very important in metazoan biology, and its misregulation can result in tumor development. It also modulates processes such as cell invasion, immune regulation, and microenvironment modification that cancer cells can use to their advantage [139]. There is a regulatory mechanism in which TGF-β, and the SDC1 cytoplasmic domain coordinate to induce cell surface expression of SDC1 in epithelial cells [166].

The differentiation of mesothelioma is dependent on the expression of SDC1 on the cell. The SDC1 level is usually low in mesenchymal cells, but during embryonal morphogenesis it increases [167–171]. At the same time, there is a loss of SDC1 in the adjacent epithelium. This low level of SDC1 in mesenchymal tumors is the reason why the expression and function SDC1 is less studied than in carcinomas.

SDC1 is the principal SDC of epithelial malignancies, while in mesenchymal tumors its cell surface expression level is normally low, but its expression is related to epithelioid differentiation. On the other hand, loss of cell surface SDC1 expression correlates with shorter survival [8, 172]. For a detailed review see [31, 33].

2 AIM OF THE STUDY

The overall aim of this thesis was to study the role of SDC1 in mesenchymal tumors and the functional importance of the transport of SDC1 to the nucleus. Particular attention is paid to what effects can be associated with tumor cell proliferation. Deeper knowledge of the role of cell surface and nuclear SDC1 and its interactions might make it possible to control tumor growth, proliferation, differentiation, and migration in these tumors. Such knowledge may be of fundamental importance in case SDC1 functions can be targeted in a therapeutic context.

2.1 SPECIFIC AIMS

- **Paper I:** To study the downstream effects of cell surface and nuclear SDC1
- **Paper II:** To study the role of SDC1 by characterizing its nuclear interactome in a mesothelioma cell line
- **Paper III:** To investigate whether SDC1 plays a role in regulating TGF-β1-induced EMT

3 METHODOLOGY

3.1 SDC1 CONSTRUCTS

To separately study the role of the cell surface and nuclear SDC1 two constructs had been designed: a full-length SDC1 and a truncated variant lacking the RMKKK nuclear localization signal (NLS) [24]. The deletion of the RMKKK sequence makes it possible to dissect functions related to the cell surface and nuclear SDC1, respectively. Mesenchymal cells were transfected with the constructs above. To obtain stable transfectants, cells carrying the integrated plasmid had been selected by the antibiotic geneticin. Verification of SDC1 expression on RNA and protein level was performed by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Both membrane and intracellular SDC1 were also detected by fluorescence-activated cell sorting (FACS) analysis or fluorescence microscopy.

3.2 CELL LINES

To study mesenchymal-epithelial transdifferentiation, we compare well-established mesothelioma and fibrosarcoma cell lines, which grow with epithelioid or sarcomatoid phenotypes, were compared. Both cell lines have mesenchymal origin and are selected based on their low endogenous SDC1 expression, providing the opportunity to study effects of added SDC1.

For the study of **Paper I**, human fibrosarcoma cell line B6FS [173] was used. This cell line can differentiate into either an epithelioid or a fibroblast-like phenotype. **Paper II** is based on the mesothelioma cell line STAV-AB, originally established from a pleural effusion [174]. When grown in human AB-serum, this cell line has a predominantly epithelioid morphology [81]. Both these cell lines have a comparatively low basic expression of SDC1. In **Paper III** the B6FS cell line was compared to a human lung adenocarcinoma cell line, A549, obtained from American Type Culture Collection (ATCC).

These mesenchymal tumor cell lines were used for transfection of the different constructs. Stable transfections were achieved in both STAV-AB and B6FS cell lines by geneticin selection; the B6FS transfectants were further selected by FACS.

3.3 LABORATORY ASSAYS

3.3.1 Fluorescence Activated Cell Sorting (FACS)

This method was used to validate SDC1 overexpression in **Papers I, II, and III**. The technique allows the identification of various parameters of single cells, physical properties like size and granularity, as well as specific fluorescent antibody labels.

3.3.2 Gene silencing

Transient silencing of SDC1 **(Papers II, and III)** was performed using siRNA constructs for the corresponding gene [175]. Scrambled siRNA sequences were used as negative control. Silencing was validated on mRNA and/or protein level using qPCR and/or western blot. The benefit of using siRNA silencing is that the effect is achieved after only a couple of days.

3.3.3 RT-qPCR and Western Blot

Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) was used for the gene expression **(Paper I and Paper II)**. RT-qPCR was performed with the Platinum SybrGreen qPCR SuperMix-UDG kit (Invitrogen) and DNA-polymerase, with a set of sense/antisense primers (CyberGene AB, Sweden).

The primers were designed based on gene sequences from GeneBank (NCBI), except for GAPDH [176] and SDC1 [87]. All PCR reactions were performed with an iCycler machine (CFX96TM Real Time PCR Detection System, BioRAD Hercules, CA, USA). The analyses were performed with Bio-Rad CFX Manager Software 2.0 (BioRad Laboratories 2008). Data were analyzed with the $2^{-\Delta\Delta Ct}$ method. Each target was normalized to GAPDH, as the reference gene, and the fold-change in expression was measured for each target with respect to the corresponding controls.

Western blotting was used to demonstrate expression of different proteins (**Papers I, II and III),** using HRP-conjugated secondary antibodies together with the more sensitive Odyssey Imaging System (LI-COR). To compare the expression of the protein in question, a stable loading control GAPDH was used.

3.3.4 Immunoprecipitation

Immunoprecipitation (IP) was used to isolate specific proteins with their interacting partners. This technique is used in **Paper II**. After confirming the presence of SDC1 in the sample by Western blotting, the immunoprecipitates were subjected to proteomic screening using mass spectrometry for the identification of interacting partners of SDC1.

3.3.5 Proteomic analysis

In **Paper I** isoelectric focusing was used to fractionate our Tandem Mass Tag (TMT) 10-plexes, to reduce the complexity of the proteome. The recently developed, high resolution, isoelectric focusing method (HiRIEF [177]), with an immobilized pH gradient of 3.7 to 4.9 (provided by GE healthcare, Uppsala, Sweden), was applied. The fractions were then injected separately on a Q Exactive mass spectrometer. This procedure has been described earlier in more detail [177].

The proteomic screening was performed at the Karolinska Bioinformatic Center **(Paper II).** On-bead reduction, alkylation and digestion (trypsin, sequencing grade modified, Pierce) were performed followed by SP3 peptide clean-up of the resulting supernatant [178]. Each sample was separated and analyzed using a Thermo Scientific Dionex nano LC-system in a 3 hr 5-40 % ACN gradient coupled to Thermo Scientific High Field QExactive. The software Proteome Discoverer (vs.1.4) including Sequest-Percolator for improved identification was used to search the *Homo sapiens* Uniprot database for protein identification, keeping false discovery rate of 1%. Total 750 proteins were identified in the sample using the SDC1-specific antibody.

3.3.6 Bioinformatic analysis

In **Paper I** a differential gene expression analysis was performed, based on Affymetrix data, with OCplus package provided in R software (*http://www.R-project.org/*). For each pair of data, the functional analysis on the previously established, differentially expressed genes was applied. In addition to a differential analysis of the fold-change, a global network analysis of functional coupling was performed to reveal the involvement of genes with specific biological functions, which were apparent when SDC1 was overexpressed with or without NLS. This allowed investigation of functional relationships between differentially expressed genes and highlighted differentially expressed genes that might be direct binding partners of SDC1.

We used two approaches for identifying the sub-cellular compartments of the SDC1 interacting proteins **(Paper II).** First, already known sub-cellular locations of the human proteins were extracted from the Human Protein Atlas database and after matching the protein IDs/names, the sub-cellular localization was assigned to these proteins. Secondly, DeepLoc-1.0 tool was used to find the sub-cellular localization of the remaining proteins.

Further, SDC1-interacting proteins were classified into different pathways using Webgastalt web server and KOBAS (KEGG Orthology Based Annotation System). The pathway analysis was performed in two steps; (1) pathways analysis of all identified interacting proteins; (2) pathways analysis of the nuclear proteins. Three pathways, namely Spliceosome, Ribosome and RNA transport, were identified as significant in these two analyses. As our interest was to understand the role of SDC1 in the nucleus, the nuclear proteins which interact with SDC1 were identified for further analysis. We selected top two proteins EWSR1 and FUS based on their higher average peak area values. The nuclear localization of both the proteins and their interaction with SDC1 were then validated by western blotting and immunofluorescence.

4 SUMMARY OF PAPERS

4.1 PAPER 1

Molecular targets and signaling pathways regulated by nuclear translocation of syndecan-1

The cell-surface heparan sulfate proteoglycan SDC1 is important for tumor cell proliferation, migration, and cell cycle regulation in various types of malignancies. However, recent studies have shown that SDC1 also translocates to the cell nucleus, where it might regulate various molecular functions. To dissect the functions of SDC1 related to the nucleus and separate them from functions related to the cell-surface, we used two fibrosarcoma cell sub-lines.

Multiple, unsupervised global transcriptome and proteome profiling approaches were implemented and combined functional assays to disclose the molecular mechanisms that govern nuclear translocation and its related functions. Nuclear translocation of the full-length SDC1 hampered the proliferation of fibrosarcoma cells compared to the NLS-deleted SDC1. The growth inhibitory effect of nuclear SDC1 corresponded to a significant accumulation of cells in the G0/G1 phase, which indicated a possible G1/S phase arrest.

Genes and pathways related to the nuclear compartment were also identified with network enrichment analysis of the transcriptome and proteome. The TGF-β pathway was activated by nuclear SDC1, and three genes were considerably altered with the deletion of nuclear localization signal: EGR-1 (early growth response 1), NEK11 (never-in-mitosis gene a-related kinase 11), and DOCK8 (dedicator of cytokinesis 8). These genes were related to growth and cell-cycle regulation. Nuclear translocation of SDC1 influenced the activity of several other transcription factors, such as E2F, NFκβ, and OCT-1. The transcripts and proteins affected by SDC1 were very similar in their corresponding biological processes. These processes were dominated by protein phosphorylation and post-translation modifications, which suggests changes in intracellular signaling. We also identified molecules involved in the known functions of SDC1, including extracellular matrix organization and transmembrane transport. Removal of nuclear translocation of SDC1 resulted in a set of changes clustering in distinct patterns, which shows the functional importance of nuclear SDC1 in hampering cell proliferation and the cell cycle. This study accentuates the importance of the localization of SDC1 when considering its effects on tumor cell fate.

4.2 PAPER 2

Mapping the Interactome of the Nuclear Heparan Sulfate Proteoglycan Syndecan-1 in Mesothelioma Cells

SDC1 regulates many different signaling pathways which control the proliferation and migration of malignant mesothelioma and other types of cancer. It has previously been shown that SDC1 can translocate to the nucleus in mesothelioma cells through a tubulin-dependent transport mechanism. The aim of this study was to gain further knowledge about the role of nuclear SDC1 through characterizing its nuclear interactome in a mesothelioma cell line.

SDC1 was co-immunoprecipitated in the mesothelioma cell line to identify interacting proteins. The precipitates contained many different proteins, which showed the recovery of protein networks. Through proteomic analysis with a focus on nuclear proteins, an association with pathways related to cell proliferation and RNA synthesis, splicing and transport was revealed. The top RNA splicing candidates were verified to interact with SDC1 by Co-IP and subsequent Western blot analysis. Further experiments examining the loss and gain of function revealed that SDC1 effects RNA levels in mesothelioma cells. The results show a proteomic map of SDC1 nuclear interactors in a mesothelioma cell line and indicate a previously unknown role for SDC1 in RNA biogenesis. These results could be a basis for further studies on the role of nuclear SDC1 in different types of normal and cancer cells.

4.3 PAPER 3

Regulation of epithelial-mesenchymal plasticity in tumor cells by syndecan-1

SDC1 is a cell surface proteoglycan that also may translocate into the nucleus. In tumor cells this PG plays multiple roles that may relate to cancer progression. Loss of SDC1 can induce epithelial-mesenchymal transition (EMT), a latent developmental process, which, when activated in tumors, promotes tumor cell invasion, migration, and metastasis. The cytokine transforming growth factor beta 1 (TGF-β1) is a forceful inducer of EMT and is frequently overexpressed in cancer tissues. Our previous study indicates a link between SDC1 and the TGF-β1 signaling pathway.

The aim of the present study was to investigate whether SDC1 plays a role in regulating TGF-β1-induced EMT. To study this, we knocked down SDC1 in a cellular model of TGF-β1-induced EMT in human A549 lung adenocarcinoma cells. This resulted in decreased expression of E-cadherin, and increased expression of N-cadherin and nuclear staining of the core EMT transcription factor Zeb1 after TGF-β1 exposure. Consequently, loss of SDC1 sensitized A549 cells to TGF-β1-induced EMT. Further experiments showed that overexpression of SDC1 was sufficient to repress N-cadherin and vimentin in B6FS fibrosarcoma cells. The results of this study indicate that SDC1 plays a role as a regulator of epithelial-mesenchymal plasticity in tumor cells.

5 RESULTS AND DISCUSSION

This thesis provides some new insights into the role of SDC1 in tumors and the study of the functional importance of the internalisation and transport of SDC1 to the nucleus, as well as the connection between SDC1 and TGF-β.

SDC1 plays an important role in tumor cell proliferation and migration in many types of cancer. Its effects are very different depending on the origin of the tumors and their tissues [24, 120, 179–182]. In the first study we tested the functions and molecular pathways related to nuclear translocation of SDC1, with focus on cell growth. To investigate the functions of SDC1 in the nucleus and on the cell surface we used a fibrosarcoma model, with the nuclear localization preserved and hampered respectively [24] We mapped the molecular mechanisms governing these functions, through combining transcriptomic and proteomics approaches.

Our research group has previously demonstrated that SDC1 translocates to the nucleus in a regulated manner [52]. Here it was shown for the first time that the nuclear translocation of SDC1 has anti-proliferative effects. We found that cell proliferation is inhibited after transfection with full length SDC1 carrying the nuclear localization signal RMKKK, as well as when SDC1 expression is suppressed. Also, cells with nuclear SDC1 accumulated more in the G0/G1 phase of the cell cycle then those with hampered nuclear localization.

SDC1 has been found to both enhance and inhibit cell apoptosis depending on the cell type. For example, SDC1 inhibited apoptosis in myeloma [183], while knock-down SDC1 had the opposite effect in myeloma [99] and carcinoma cells [184]. In our experiments, the anti-proliferative effect of nuclear SDC1 was combined with inhibition of apoptosis, which suggests that these two mechanisms might be connected.

The subcellular localization of SDC1 generated various molecular changes, which were classified and analyzed through bioinformatics. Network analyses mainly showed changes in genes and pathways connected to the nuclear compartment. SDC1 overexpression was found to change cell cycle regulation and TGF-β signaling pathways, which corresponds with our earlier data [175, 185, 186].

We also identified three genes that were significantly enhanced by the nuclear translocation of SDC1: EGR-1, NEK11 and DOCK8. EGR-1 and NEK11 are both proteins present in the nucleus, which play many roles in the cell cycle. EGR-1 is a transcription factor activated by different extracellular stimuli and apoptotic signals. It has a dual role, sometimes promoting tumor growth and inhibiting apoptosis, while having the opposite effect in other tumor types [187]. In non-small lung cancer, the TGF-β-induced EMT was hampered by EGR-1 [188]. In our study, nuclear SDC1 generated EGR-1 expression, which was related to activation of the TGF-β pathway and hampering of apoptosis. The NEK11 protein is DNA damage-responsive, and its kinase activity is needed for DNA damage-induced G2/M arrest [189]. DOCK8 is a guanine nucleotide exchange factor, which plays a role in the regulation of cell morphology, intracellular signaling, tumor cell invasion [190] and metastasis [191].

This study shows that the upregulation of SDC1 inhibits cell proliferation and that the localization of SDC1 might affect how tumor cells develop. The results indicate that transcription factors such as EGR-1 and NEK11 are targets for SDC1 in the cell nucleus.

In the second study we aimed to discover more about the role of nuclear SDC1 through identifying its nuclear interactome in a mesothelioma cell line. Nuclear proteins associated with cell proliferation were found in the SDC1 precipitate, but also that SDC1 was related to proteins involved in RNA synthesis.

The results suggest that the regulatory effect on cell proliferation, which we found in study one, is related to nuclear SDC1. Of the eight pathways, where SDC1 interacts with nuclear proteins, five were related to different aspects of RNA transcription and export. Two of these, mRNA surveillance and Spliceosome, are linked together through common members of the Exon junction complex (EJC). The EJC regulates translation, as well as mRNA surveillance and localization [192–194]. These results indicate that SDC1 might regulate the binding of EJC and mRNA, and play a role in the degradation of the mRNA through different mechanisms [192]. SDC1 was also found to interact with three EJC inner core factors, Y14, MAGOH and eIF4AIII. Two other interactors of SDC1were identified, EDC3 and EDC4 (enhancer of mRNA-decapping protein 3 and 4), which also affect mRNA degradation [195, 196]. Similar to the previously found effects on cell proliferation, both the up- and downregulation of SDC1 reduced the total cellular amount of RNA. In short, the protein networks which interact with nuclear SDC1 regulate two main functions: cell proliferation and RNA transport and transcription modification. Decreasing or increasing SDC1 inhibit these functions in a regulatory manner. Among these proteins, EWSR1 and FUS were identified as co-precipitating with SDC1. A previous study has shown that MM is related to recurrent EWSR1/FUS fusions [197].

In the third study we have shown that knockdown of SDC1 sensitizes A549 cells to TGF-β1-induced EMT. After knockdown of SDC1 E-cadherin was more repressed and N-cadherin more induced. Also, nuclear localization of Zeb1 was increased, suggesting a role for SDC1 in regulating the activity of core EMT transcription factors. This was obvious in the carcinoma cell line with its high basic production of SDC1, while no effect could be seen when modulating the SDC1 expression in the sarcomatous cells with their lower levels of SDC1. As recent research has identified Zeb1 as a suppressor of SDC1 in prostate cancer cell lines with EMT [198], there is possibly a feedback loop by which they inhibit the activity of each other. In B6FS fibrosarcoma cells, overexpression of SDC1 suppressed the levels of N-cadherin and vimentin, which are proteins related to a mesenchymal phenotype.

Taken together, these studies show the different roles of SDC1 in the process of EMT and in malignancy in general.

6 CONCLUSIONS

Paper I: Molecular targets and signaling pathways regulated by nuclear translocation of syndecan-1

- Proliferation and cell cycle are hampered by nuclear syndecan-1 (nSDC1)
- These effects are regulated by multiple actors in related signaling pathways, where TGF-β1 seems to play a central role
- nSDC1 enhances expression of EGR1, NEK11, DOCK8
- Activity of several other transcription factors such as NFκβ and E2F-1 is augmented by nSDC1
- Network analysis shows overlap of biological processes in transcripts and proteins affected

Paper II: Mapping the Interactome of the Nuclear Heparan Sulfate Proteoglycan Syndecan-1 in Mesothelioma Cells

- The results suggest a previously unknown role for SDC1 in RNA biogenesis
- SDC1 plays a role in various pathways, from cell proliferation to RNA synthesis and transport
- This study is a first step towards discovering the role of nuclear SDC1 in normal and cancer cells of different origin

Paper III: Regulation of epithelial-mesenchymal plasticity in tumor cells by syndecan-1

- SDC1 knockdown sensitizes A549 cells to TGF-β1-induced EMT
- SDC1 plays a role in regulating the activity of core EMT transcription factors
- Overexpression of SDC1 in B6FS cells leads to suppression of N-cadherin and vimentin, which are associated with a mesenchymal phenotype

7 FUTURE PERSPECTIVES

The overall aim of this thesis was to study the role of SDC1 in malignancy, in particular its effect on EMT, and the functions of SDC1 nuclear transportation.

Our results from the first study indicate that EGR-1, NEK11 and other transcription factors are SDC1 targets in the nucleus. However, it is not yet clear if they are direct targets or mediators of the effect of SDC1 in the nucleus. It might also be interesting to further study whether SDC1 could be regulating the binding of EJC and mRNA and helping in the degradation of the mRNA. We know that EDC3 and EDC4 assist in mRNA decapping and degradation, but the exact process and the role of SDC1 is yet to be proved in vitro and/or in vivo.

Our research indicates that SDC1 plays a role in various pathways, including cell proliferation and RNA synthesis and transport. However, the results are based on SDC1 interacting proteins in a single mesothelioma cell line, which makes it difficult to draw generalized conclusions about the SDC1 nuclear interactions in other cell types. Further research is needed to define the exact molecular mechanisms of nuclear SDC1 and its involvement in these pathways, as well as to study its possible importance in different types of cancer like MM.

Previous studies have indicated SDC1 as a powerful suppressor of the TGF-β pathway in mesothelioma. We have found that SDC1 is involved in regulating TGF-β1-induced EMT, but more research is needed to fully investigate the molecular links in this process. It would also be interesting to further study the role of SDC1 in epithelial-mesenchymal plasticity during metastasis.

Overall, the findings of this thesis may serve as a basis for further study of the function of SCD1 and even make improved future cancer therapy possible.

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"All of us do not have equal talent. But, all of us have an equal opportunity to develop our talents."

- **Dr APJ Abdul Kalam**

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