

The biological role and regulation of versican levels in cancer

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Abstract Increased expression of the proteoglycan, versican is strongly associated with poor outcome for many different cancers. Depending on the cancer type, versican is expressed by either the cancer cells themselves or by stromal cells surrounding the tumor. Versican plays diverse roles in cell adhesion, proliferation, migration and angiogenesis, all features of invasion and metastasis. These wide ranging functions have been attributed to the central glycosaminoglycan-binding region of versican, and to the N-(G1) and C-(G3) terminal globular domains which collectively interact with a large number of extracellular matrix and cell surface structural components. Here we review the recently identified mechanisms responsible for the regulation of versican expression and the biological roles that versican plays in cancer invasion and metastasis. The regulation of versican expression may represent one mechanism whereby cancer cells alter their surrounding microenvironment to facilitate the malignant growth and invasion of several tumor types. A

greater understanding of the regulation of versican expression may contribute to the development of therapeutic methods to inhibit versican function and tumor invasion.

Keywords Versican · Cancer invasion · Extracellular matrix · Tumor stroma

1 Introduction

Although a number of cancer types often show a prolonged natural history and do not require treatment, e.g. indolent prostate cancers [1], a significant number of patients possess more aggressive tumors that metastasize rapidly and lead to death, sometimes within 1 year of initial clinical presentation [2–4]. An increased knowledge of the biology of aggressive versus indolent tumors will be needed to develop appropriate targeting of tumors. In developed countries, cancer continues to contribute to the top three causes of mortality, along with heart disease and stroke [5]. Prevention of the development of metastatic cancer and its treatment remains a significant challenge.

The process of tissue invasion, metastasis and growth of satellite tumors by cancer cells is complicated, involving changes in cellular attachment to extracellular matrix (ECM) components, local proteolysis of the basement membrane and migration through stroma to gain access to the circulation [6, 7]. Within this sequence, an overall decrease in the adhesive capacity of tumor cells at the invasive foci has been noted for a number of human cancers [8, 9]. This loss of cellular adhesion to pericellular matrix molecules, localized at so-called focal adhesion sites, is a complex process mediated by interactions between cellular receptors of both integrin and non-integrin type and their ECM ligands, such as fibronectin, collagen and laminin [10,

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11]. Of the non-integrin cell receptors, there is compelling evidence that proteoglycans play a major role in cell-ECM adhesion interactions during cancer progression [12–14]. Of these proteoglycans, a number of recent studies have demonstrated that versican (also known as *CSPG2* or *VCAN*), a large chondroitin sulfate (CS) proteoglycan, harbors anti-adhesive properties and the ability to modulate proliferation and migration in a number of different cell types, including osteosarcoma cells, astrocytoma cells, smooth muscle cells (SMCs) and various types of tissue fibroblasts. The purpose of this review is to summarize the recently identified regulatory mechanisms and biological roles of versican expression in several cancer types and highlight opportunities for therapeutic intervention.

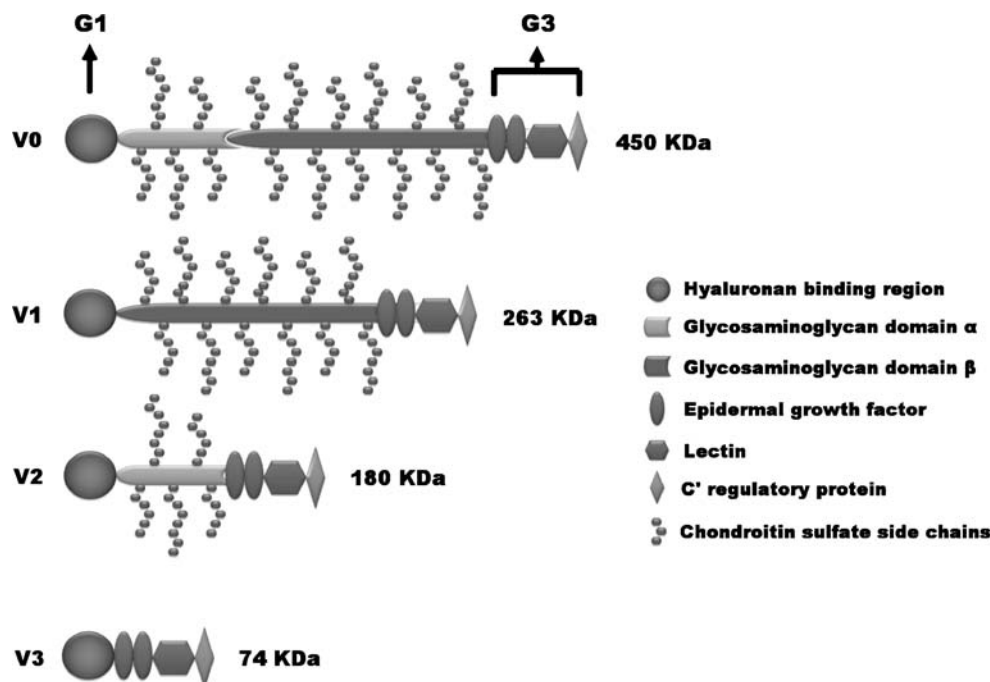
2 The structure and function of versican

Versican is encoded by a single gene and is located on chromosome 5q 12–14 in the human genome. The human *VCAN* gene is divided into 15 exons over 90–100 kb [15]. Versican was initially identified in the culture medium of human IMR-90 lung fibroblasts and has an apparent molecular mass of 900 kDa [16]. Reverse transcription polymerase chain reaction, northern blot analysis and cDNA sequencing demonstrated that the splicing patterns of versican mRNA can generate four versican isoforms, designated V0, V1, V2 and V3, respectively [15, 17] (Fig. 1). Sequence analyses revealed the structural differences between the isoforms are confined to the middle

portion that encodes the GAG attachment region. V0, the largest isoform, contains two alternatively spliced GAG attachment domains designated as GAG- α (12–17 CS side chains) and GAG- β (5–8 CS side chains). In contrast, V1 comprises the GAG- β domain only, whereas V2 contains only GAG- α . The GAG- α and GAG- β domains are both absent in the V3 isoform and consequently this isoform exists as a glycoprotein and not a proteoglycan. All versican isoforms contain globular domains at the amino terminus (G1) and carboxyl terminus (G3) [17, 18]. The G1 domain is composed of an immunoglobulin-like motif, followed by two proteoglycan tandem repeats which bind hyaluronan (HA). The association of versican with HA is mediated by link protein, and both HA and isolated link protein have the ability to bind to the G1 domain of versican [19]. The G3 domain contains two epidermal growth factor-like repeats, a carbohydrate recognition domain (a lectin-like repeat) and complement binding protein-like subdomains with structural similarity to the selectin family [17]. The difference in size of the CS attachment regions between isoforms suggests the actual number and size of attached CS chains varies, indicating the possibility of heterogeneity in the number, length, and molecular structure of CS GAG chains with different structural and functional diversity outcomes.

Versican is able to regulate many cellular processes including adhesion, proliferation, apoptosis, migration and invasion via the highly negatively-charged chondroitin/dermatan sulfate side chains and by interactions of the G1 and G3 domains with other proteins [18, 20]. The wide

Fig. 1 Schematic diagram of versican isoforms



range of molecules that have the ability to interact with versican via both the G1 and G3 and CS side chains have recently been reviewed by Wu *et al* 2005 [21]. In addition to HA, versican binds to ECM components including tenascin-R, type I collagen, fibulin-1 and-2, fibrillin-1, and fibronectin [18, 22–25]. Versican also binds to cell surface molecules, P- and L-selectin, chemokines, CD44, integrin beta1, and epidermal growth factor receptor [21, 26–30]. Studies have also shown that versican can bind to specific chemokines through its CS chains to down regulate chemokine function [29].

3 Distinct functions of versican isoforms

A survey of a large number of adult human tissues showed that <15% of the tissues transcribed significant levels of all four versican isoforms and highlighted a prevalence of V1 mRNA [31]. The V0 isoform is particularly prevalent during early embryonic development [32] but less well represented in adult tissues [31]. There is little information that attributes distinct functions to individual versican isoforms. However, data is now emerging that the V1 isoform may have a different function from the V2 isoform. V1 versican has been shown to enhance cell proliferation and protect NIH-3T3 fibroblasts from apoptosis [33]. By contrast, the V2 isoform exhibits opposing biological activities by inhibiting cell proliferation and lacking any association with apoptotic resistance [33]. Versican V1 and V2 isoforms may also have distinct functions in the brain [34, 35]. V0 and V1 are the predominant isoforms present in cancer tissues [36–39]. There have been no studies to date that have investigated whether V0 and V1 versican have different functions.

The smallest splice variant, V3, consisting only of the amino- and carboxy-terminal G1 and G3 domains and thus a small glycoprotein lacking CS chains, might be expected to have properties considerably different from the other isoforms. However, there have been no studies that have directly compared the biological activity of V3 with the other versican isoforms. The V3 isoform was found to be expressed in primary endothelial cell cultures only following activation by pro-inflammatory cytokines or growth factors [31]. The role of V3 in activated endothelium is not known. The overexpression of V3 in arterial SMCs resulted in their increased adhesion to culture flasks but reduced proliferation and slower migration in scratch wound assays [40]. The overexpression of the V3 isoform in melanoma cancer cells which, although markedly reducing cell growth *in vitro* and *in vivo* [41], actually promoted metastasis to the lung [42]. These findings suggest that the V3 isoform may have a dual role as an inhibitor of tumor growth and a stimulator of metastasis.

4 Versican expression is elevated in cancer cells

Elevated levels of versican have been reported in most malignancies to date, including; brain tumors, melanomas, osteosarcomas, lymphomas, breast, prostate, colon, lung, pancreatic, endometrial, oral, and ovarian cancers [37, 38, 43–56]. Even non-solid cancers, such as human acute monocytic leukemia cells, express and secrete V0 and V1 [57]. Elevated versican levels are associated with cancer relapse and poor patient outcome in breast, prostate, and many other cancer types [37, 46, 51–53, 58–61]. Versican appears most commonly secreted by the peritumoral stromal cells in adenocarcinomas [36, 37, 46, 48, 53, 60–62] although the demonstration that human pancreatic cancer cells can secrete versican challenges this view [63]. Epithelial versican expression has also been described in endometrial cancer and ovarian cancer [48, 61]. In ovarian cancer, high stromal versican levels correlated with serous cancers and were associated with reduced overall survival, whilst high versican levels in the epithelial cancer cells correlated with clear cell histology, early FIGO stage, and increased recurrence-free survival [48].

5 Versican regulates cell adhesion, proliferation, invasion, and migration

The effects of versican on cancer cells are summarized in Table 1. The association between increased levels of versican and progression of cancer to disseminated disease suggests that versican is important in promoting cancer cell motility and invasion. This hypothesis is supported by functional studies demonstrating that versican can increase cancer cell motility [39, 64–68], proliferation [69, 70] and metastasis [42, 67, 71]. In addition, purified versican is able to reduce attachment of prostate cancer cells and melanoma to fibronectin-coated surfaces *in vitro* [47, 72]. Interestingly, the knockdown of versican expression in A549 lung cancer cells by RNA interference significantly inhibited tumor growth *in vivo* but not *in vitro* [69]. V1-induced NIH-3T3 tumor formation in nude mice is associated with apoptotic resistance and down-regulated Fas mRNA and protein levels and unexpectedly, sensitization to a wide range of cytotoxic agents [70].

More recent functional studies have provided evidence that versican-treated cancer cells incorporate versican and HA into a prominent pericellular matrix that promotes their motility [68]. A pericellular sheath of defined polarity is assembled by motile prostate cancer cells following versican treatment. This sheath is particularly visible at the trailing edge of motile prostate cancer cells in wound migration assays, whilst there is no evidence of pericellular sheath at the leading edge of the cells (Fig. 2). The capacity

Table 1 Summary of versican's effects on cancer cells

Function	Effect	References
Cell proliferation	Knockdown of versican by siRNA in lung cancer cells significantly inhibited tumor growth <i>in vivo</i>	[69]
	V1 enhanced proliferation of NIH-3T3 cells whilst V2 inhibited proliferation	[33]
	Overexpression of G1 in leiomyosarcoma cells lead to larger tumor masses	[65]
	Overexpression of G3 versican in breast cancer cells resulted in larger tumors <i>in vivo</i>	[71]
	G1 and G3 induced NIH-3T3 cell proliferation <i>in vitro</i>	[77, 78]
	G3 versican domain promoted proliferation of astrocytoma cells <i>in vivo</i>	[66]
	Overexpression of V3 versican in human melanoma cell lines markedly reduced cell growth <i>in vitro</i> and <i>in vivo</i>	[42]
Apoptosis	V1 induced apoptotic resistance in NIH-3T3 cells	[33, 70]
Adhesion	V0 and V1 versican inhibited prostate and melanoma cell attachment to ECM components	[47, 72]
	G1 but not G3 reduced glioma cell adhesion	[64]
Motility	G1 versican domain promoted motility of glioma cells and lung cancer cells	[64, 67]
	V0+V1 and recombinant V1 increased motility of prostate cancer cells	[68]
Invasion	G1-overproducing sarcoma cells were more invasive than the corresponding G3 mutants and vector transfected cells	[65]
Pericellular matrix formation	V0+V1 and recombinant V1 induced formation of pericellular matrix by prostate cancer cells	[68]
Angiogenesis	G3 enhanced endothelial cell adhesion, proliferation, and migration <i>in vitro</i> and blood vessel formation in nude mouse tumors <i>in vivo</i>	[66]
Metastasis	G1 versican promoted metastasis of lung cancer cells	[67]
	Overexpression of V3 versican in melanoma cells induced lung metastasis in nude mice	[42]
	Overexpression of G3 versican in breast cancer cells promoted metastasis to bones and soft tissues	[71]

to assemble a pericellular sheath correlated with the ability to express membranous HA receptor, CD44. We thus propose that tumor cells form a polarized pericellular sheath through compartmentalized cell surface CD44 expression and subsequent assembly of HA/versican aggregates. The absence of a pericellular sheath at the leading edge of the cell may allow attraction and binding to ECM components such as fibronectin whilst formation of a pericellular sheath at the trailing edge of cells inhibits cellular binding to ECM components. The combination of binding at the leading edge of cells and inhibition of binding at the trailing edge may thus enhance the forward motion of the cells. The descriptions of abundant versican in the trailing tracks of migrating fibroblasts [73] and in the pericellular sheath at the trailing edge of migrating SMCs also supports this model whereby the acquisition of an HA/versican pericellular matrix by cancer cells may aid their dissemination and metastasis *in vivo*. In addition, a study by Yamagata *et al* 1994 found that versican was selectively excluded from podosomes of human osteosarcoma cells and that inhibition of versican biosynthesis by an antisense method suppresses a malignant cell-adhesive phenotype [74]. The formation of a HA pericellular matrix by cancer cells is essential for specific adhesion to bone marrow endothelial cells and may contribute to the preferential bone metastasis by breast and prostate carcinoma cells [75, 76]. The overall consensus is that a pericellular sheath of versican and HA modulates cell attachment to substratum and is a key factor in cancer cell motility which may contribute to the metastatic process.

6 Role of G1 and G3 versican domains in cancer

A large number of studies have focused on the globular domains of versican. Both the G1 and G3 versican domains have been shown to promote cell proliferation of NIH-3T3 fibroblasts and tumor cells [65, 66, 77, 78]. The G1 domain of versican is thought to stimulate proliferation by destabilizing cell adhesion whilst the G3 induced proliferation is mediated, at least in part, by the action of EGF-like motifs in the G3 domain activating EGF receptors. Studies in astrocytoma cancer cell lines have demonstrated that the G1 domain, but not the G3 domain, of versican could enhance migration [64]. Overexpression of G3 versican in astrocytoma cells enhances colony growth in soft agarose gel as well as tumor growth and blood vessel formation in nude mice [66]. Both G1- and G3-overexpressing osteosarcoma cells exhibited enhanced *in vitro* growth when cultured on ECM substrates and in the absence of ECM anchorage [65]. G1-overproducing sarcoma cells were more invasive than the corresponding G3 transfectants and, upon inoculation subcutaneously into nude mice, the G1 transfectants formed larger tumor masses than vector transfected cells. In addition, G1-overexpressing sarcoma cells were resistant to apoptosis. Stable transfection of G1 versican into the H460M lung cancer cell line did not alter tumor growth rate *in vivo*, and interestingly clones expressing low or high levels of G1 versican had opposing effects on cancer cell motility *in vitro* and the incidence of metastasis in nude mice [67]. Only the cells expressing low levels of

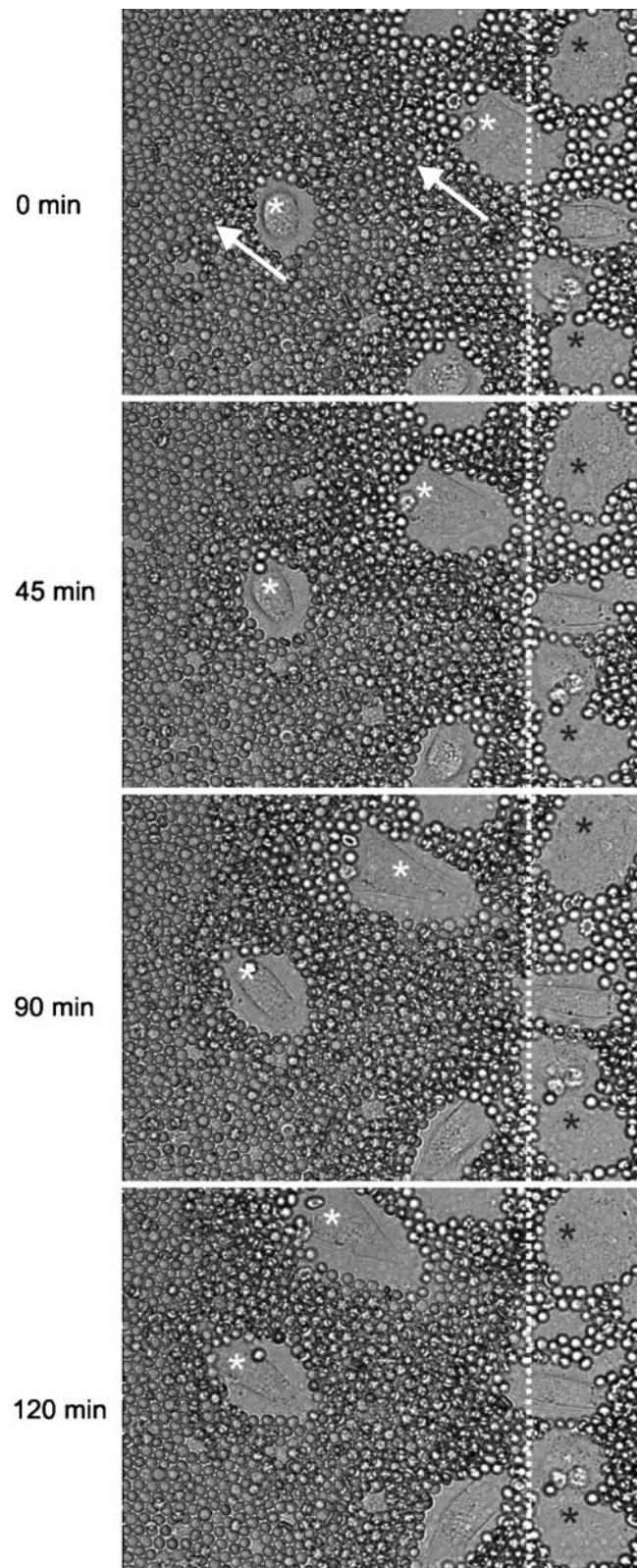
Fig. 2 Time lapse photography of prostate cancer PC3 cells in wound migration assay. The confluent PC3 monolayer was wounded and treated with versican containing media (0.5 U/ml) for 8 h. The white asterisks indicate motile PC3 cells with a polar pericellular sheath observed over a 2 h time period, using a red blood cell exclusion assay. The black asterisks indicate non-motile PC3 cells lacking a pericellular sheath. The white dashed line indicates the edge of the wound and the white arrows indicate the direction of cell movement. Red blood cells diameter=7 μm

G1 versican demonstrated increased motility and metastasis to the lung [67]. Those results suggest that G1 versican can act both as a suppressor or a promoter of metastatic spread. Interestingly, in MT-1 human breast cancer cells, over-expression of G3 versican resulted in larger tumors and the promotion of metastasis to bones and soft tissues [71].

The accumulating data collectively suggest that the G1 and G3 domains of versican may differentially control tumor growth rate and have interactive roles to promote tumor development and metastasis. However the mechanisms that regulate catabolism of versican and the levels of G1 and G3 versican are poorly understood. Several protease families have been shown to cleave versican into smaller fragments. These include ADAMTS (*A Disintegrin And Metalloprotease domain with ThromboSpondin motifs*) and the MMPs (*Matrix MetalloProteinases*). ADAMTS proteases can cleave versican in the GAG β domain of V1 to generate a versican 70 kDa fragment containing the G1 domain [79–81]. An ADAMTS cleavage site in the GAG- β domain generates a G1 domain 64 kDa versican fragment from V0 and V2 versican [82]. Additional ADAMTS cleavage sites in V0, V1 and V2 have also been identified [82]. MMP types -1, -2, -3, -7, and -9, and plasmin, have also been shown to cleave versican *in vitro*, but it has not been confirmed whether they can also do this *in vivo* [83–86]. Unlike the ADAMTS cleavage sites, the MMP cleavage sites in versican have not been characterized. Furthermore, although G3 versican fragments have been found in cancer tissues [66], the specific cleavage site and proteases responsible for generating G3 versican fragments have not yet been described. Versican cleavage by ADAMTS action *in vivo* plays an important role in ovarian function and fertility [80], in vascular SMC function [79] and heart development [81]. ADAMTS cleaved versican may also contribute to the development of cardiovascular disease [87, 88]. The regulation of G1 and G3 versican levels by proteases may be important in regulating cancer cell motility and metastasis.

7 Regulation of versican expression

Studies investigating the 5' flanking region of the human *VCAN* gene [15] have revealed the presence of several



potential regulatory elements. These elements include binding sites for CCAAT binding transcription factor, SP1, cyclic adenosine monophosphate-responsive element-binding protein, a possible negative regulatory element, and

a cluster of binding sites for activating protein 2. The signaling pathways that are involved in the regulation of versican expression are not well understood. A recent review by Rashmani *et al* 2006 [89] has summarized the current knowledge regarding the transcriptional regulation of versican. Versican contains a p53 binding site in its first intron and is a recognized target of p53 [90]. Oligonucleotide-array gene expression analysis revealed that expression of versican correlates with dosage of p53 [90]. Furthermore, use of several *in vitro* and *in vivo* assays has demonstrated that versican can be directly activated by the tumor suppressor p53 [90]. In another study using microarray technology and human embryonic carcinoma cells stimulated with active canonical wingless (Wnt) protein, versican was found to be a target gene of Wnt signaling [91] and regulated via the phosphatidylinositol 3-kinase pathway and the beta-catenin-T-cell factor transcription factor complex in vascular SMCs [92].

Versican expression was found to be up-regulated in normal skin and gingival human fibroblasts treated with TGF β [93, 94]. TGF β is also known to increase versican expression by dividing oligodendrocyte cells [95]. TGF β 1 is a major cancer cell mediator regulating synthesis and secretion of versican by prostatic [36, 96] and mammary fibroblasts (Ricciardelli C, unpublished observations), and glioma cells [39]. TGF β 1 and conditioned medium derived from pancreatic cancer cell lines stimulated the expression of versican in cultured pancreatic stellate cells [97]. TGF β 2 has also been shown to increase V0 and V1 mRNA levels in normal lung fibroblasts, fibrosarcoma and osteosarcoma cells [38, 98]. Versican mRNA levels were also increased in arterial SMCs and gingival human fibroblasts following treatment with platelet-derived growth factor (PDGF) [93, 99–101]. Versican is secreted into the culture medium of both epithelial- and fibroblast-like mesothelioma cell lines and is increased by a number of growth factors including EGF, insulin-like growth factor I and PDGF-BB [102]. Interestingly V0 but not V1 synthesis was increased in bronchial SMCs and arterial SMCs following treatment with leukotriene D4 and EGF [103]. Versican expression is decreased in gingival fibroblasts and vascular SMCs treated with IL-1 α [104, 105], but up-regulated by IL-1 β in human lung fibroblasts [106].

Several studies have revealed that versican expression can also be regulated by steroid hormones and gonadotrophins [58, 107–109]. In a human endometrial cancer cell line, 100 nM medroxy progesterone acetate down-regulated expression of versican [58]. In rodent ovaries, versican mRNA and protein levels are increased in developing follicles and up-regulated during ovulation [107]. Versican mRNA levels are increased up to 10-fold during the periovulatory period after human chorionic gonadotropin treatment in rodent ovaries, but not affected by follicle

stimulating hormone (FSH) treatment alone. In cultured granulosa cells, combined FSH and testosterone treatment increased expression of versican [107]. These observations lead to the conclusion that versican is a matrix component of the granulosa layer throughout folliculogenesis and is enriched in remodeling matrices during ovulation.

In the guinea pig, versican levels in the prostate stromal compartment were found to be negatively regulated by dihydrotestosterone treatment, but were not affected by estradiol treatment [109]. Prostatic versican levels were dramatically increased in castrated guinea pigs but were reduced in intact animals at the onset of puberty, and in castrated animals following treatment with dihydrotestosterone [109]. The view that versican expression in the prostate may be regulated by androgen is supported by the identification of putative androgen response elements on either side of the transcript start site of the human *versican* gene promoter [109]. One putative class II androgen receptor (AR) binding site was identified in the –242 to –228 region of the promoter, and a putative class I AR binding site was identified in the 107–122 region of exon 1. A recent study by Read *et al* 2007 found that the synthetic androgen R1881 could stimulate expression of a versican promoter-luciferase reporter construct in both prostate cancer LNCaP cells and HeLa cells transfected to express AR [108]. Using electrophoretic mobility shift, supershift, and site-directed mutagenesis assays, they have confirmed the presence of a consensus steroid receptor binding site in the versican promoter [108]. Interestingly, R1881 alone could not induce versican promoter activity and mRNA levels in AR-positive prostate stromal fibroblasts, but the overexpression of beta-catenin in the presence of androgen did augment versican promoter activity and enhanced versican mRNA levels [108]. These studies demonstrate that androgens can regulate versican expression and may contribute to prostate cancer progression via tumor-stromal cell interactions.

A study revealed that hypomethylation of the *VCAN* gene occurs in benign and malignant colon cancer compared with normal colon and ulcerative colitis tissues [110]. These changes in methylation may occur prior to malignant transformation but were not associated with any changes in versican mRNA levels in the colon cancer tissues. Interestingly, the demethylating events were found to occur in the host stromal cells rather than the cancer cells. Another study examining methylation status in a colon cancer cell line found that hypermethylation of the *VCAN* promoter was associated with *VCAN* transcriptional suppression [111]. Altered *VCAN* methylation is believed to be an early event and may be involved in the predisposition to tumorigenesis and occur prior to malignant transformation. It is possible that versican expression is silenced in the epithelial cancer cells by hypermethylation, but demethylating events may increase versican levels in the cancer associated stroma.

Collectively, the studies investigating versican regulation suggest that several elements contained within the human VCAN gene promoter might differentially regulate the molecular structure and level of expression of versican during normal and pathological tissue development.

8 Versican as a potential therapeutic target in cancer

8.1 Inhibition of versican synthesis

Inhibiting versican synthesis may be a potential mechanism for reducing versican levels in cancer tissues. Treatment with the tyrosine kinase inhibitor, genistein has been shown to inhibit versican synthesis induced by growth factors in malignant mesothelioma cell lines and vascular SMCs [100, 102]. Genistein can reversibly inhibit PDGF-stimulated versican expression in vascular SMCs in a dose-dependent manner, without affecting the expression of other proteoglycans including decorin and biglycan [100]. Versican levels in vascular SMCs have also been shown to be increased following treatment with angiotensin II [112]. The effect of angiotensin II on versican mRNA levels could be reversed by pretreatment with the tyrosine kinase inhibitor herbimycin A, the mitogen-activated protein kinase inhibitor PD98059 and the EGF receptor inhibitor AG1478. These findings suggest that increased versican synthesis is regulated via the protein tyrosine kinase intracellular and EGF signaling pathways. Versican regulation does not involve the downstream activation of protein kinase C, as treatment of vascular SMCs with phorbol ester 12-O-tetradecanoylphorbol-13-acetate, which can directly activate this kinase, had no effect on versican mRNA expression [100]. No studies to date have investigated whether these inhibitors are effective in inhibiting versican's effects in cancer cell models.

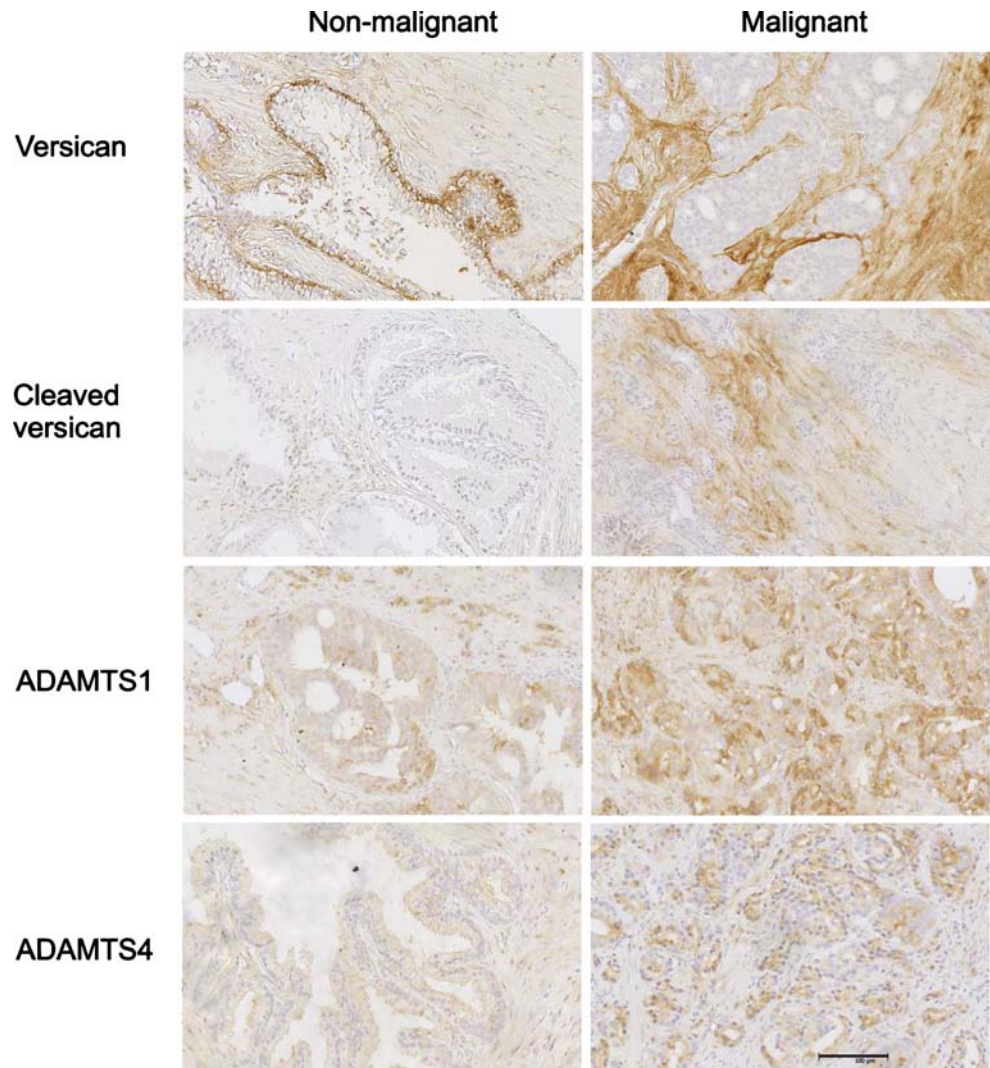
More recent studies have demonstrated that asthma drugs including Budesonide, a glucocorticoid steroid, and Formoterol, a long acting β_2 -adrenergic agonist, could decrease protein levels of a number of proteoglycans including; decorin, biglycan, perlecan, and versican in human lung fibroblasts and airway SMCs [113, 114]. Combination treatment with Budesonide (10^{-8} M) and Formoterol (10^{-10} M) reduced versican levels to a significantly greater extent than either drug alone [114]. The phosphodiesterase 4 inhibitor Roflumilast however was shown to inhibit fibronectin deposition by both the asthmatic and the non-asthmatic airway SMCs, but had no effect on the deposition of versican [113]. Versican synthesis could also be inhibited by the leukotriene receptor antagonist, Montelukast in both bronchial and arterial SMCs [103]. These studies suggest that the changes in versican deposition that occur in cancer tissues, as well as

the asthmatic airway and atherosclerotic lesions, can be inhibited by a number of asthma drugs. The ability of asthma drugs including Budesonide and Formoterol to inhibit versican synthesis need to be evaluated in cancer cell models.

8.2 Inhibition of versican processing

There is increasing evidence that ADAMTS proteases play an important role in catabolism of versican, but the implications and regulation of these cleavage events for cancer cell migration, invasion and metastasis need to be elucidated. The brain-specific lectican, brevican, is dramatically increased in highly invasive gliomas [115] and proteolytic cleavage of brevican by ADAMTS4 has been shown to increase aggressiveness and invasion of glioma cell lines [116, 117]. These studies suggest that both up-regulation and proteolytic cleavage of brevican are critical elements for glioma tumor invasion. More recent studies have shown that brevican secreted by glioma cells is capable of enhancing cell adhesion and motility only after proteolytic cleavage [118]. These findings underscore the important functional implications of brevican processing in glioma progression. The local accumulation of versican fragments generated by ADAMTS digestion may also promote cancer cell motility and invasion. This notion is supported by a recent study demonstrating that an antibody to the specific ADAMTS versican cleavage site inhibited glioma cell migration in a dose-dependent manner [39]. Preliminary studies in our laboratory have demonstrated the presence of intact and cleaved versican using a neoepitope specific versican antibody (V0/V1 neo, DPEAAE) in the peritumoral stroma surrounding prostate cancer cells but not in the interface surrounding non-malignant glands, where intact versican is present (Fig. 3). Consistent with the presence of cleaved versican, we observed higher ADAMTS1 and ADAMTS4 levels in prostate cancer tissue compared to non-malignant glands (Fig. 3). The broad spectrum MMP inhibitor, GM6001 (Galardin) that inhibits the activity of MMPs and ADAMTS proteases has been shown to inhibit cancer cell invasion and metastasis in several model systems [119–121]. One of the more interesting MMP inhibitors is present in the increasingly popular beverage, green tea, which is made from the leaves of *Camellia sinensis* and contains catechin gallate esters. Catechin gallate esters have been shown to selectively inhibit ADAMTS-1, -4, and -5 and inhibit aggrecan catabolism in cartilage tissue [122]. The ability of GM6001 and catechin gallate esters to inhibit versican catabolism and versican-induced motility and metastasis has not been investigated. Manipulation of the versican catabolic pathways may provide novel therapeutic targets for cancer invasion and metastasis.

Fig. 3 Versican, cleaved versican, ADAMTS1 and ADAMTS4 immunostaining in human prostate tissues. Formalin fixed paraffin sections (5 μ m) were immunostained with versican (Vc, 1/500) provided by Assoc Prof Richard Le Baron (Division of Life Science, University of Texas at San Antonio, San Antonio, TX) as previously described [126] or cleaved versican (1/200, raised to DPEAAE neopeptide, Affinity Bioreagents) following digestion with chondroitinase ABC as described previously [46]. ADAMTS1 (1/200, amino acid 806–865, Santa Cruz Biotechnology) and ADAMTS4 (1/200, raised to C V(85) MAHVDPEEP(94) of mouse ADAMTS4, Affinity Bioreagents) immunostaining was achieved using citrate buffer microwave retrieval [127]



8.3 Inhibition of versican-HA pericellular matrix formation with HA oligomers

The formation of a pericellular matrix rich in HA and versican by vascular SMCs and tumor cells can be inhibited following treatment with HA oligomers [123, 124]. Disruption of the HA CD44 interaction with HA oligomers has been shown to markedly inhibit the growth of B16F16 melanoma cells [125]. Treatment with 8mer HA oligosaccharides inhibited the formation of pericellular matrix by osteosarcoma cells and reduced HA accumulation in local tumors, tumor growth, and the formation of distant lung metastases. These findings suggest that the potent antitumor effects of HA oligosaccharides are mediated in part by the blocking of the formation of HA-rich cell-associated matrices. The use of HA oligomers is a potentially attractive reagent to block versican HA interactions as well as local tumor invasion and needs further investigation.

9 Conclusions

Versican is localized to either the peritumoral stromal tissue of many cancers, being secreted into the ECM by host tissue fibroblasts, or expressed by the cancer cells themselves. Studies reveal that multiple mechanisms are responsible for differentially regulating the level, localization and biological function of versican in cancer. Neoplastic modulation of versican, and hence remodeling of the ECM, may be one mechanism by which tumor cells control their microenvironment to facilitate metastasis. Development of therapeutic methods to down-regulate versican expression, decrease its catabolism to more active fragments, or inhibit its function may slow or prevent tumor invasion for many cancer types. A greater understanding of the mechanisms regulating versican expression and activity will assist in the development of specific inhibitors of versican mediated cancer cell metastasis.

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Conflicts of interest There are no conflicts of interest to declare.

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