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Review

Roles of versican in cancer biology - tumorigenesis, progression and metastasis

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Summary. Versican, a large extracellular matrix proteoglycan accumulates in tumor stroma and plays a key role in both malignant transformation and tumor progression. Increased versican expression has been observed in a wide range of malignant tumors, and has been associated with both cancer relapse and poor patient outcomes in breast, prostate, and many other cancer types. Through negatively-charged chondroitin and dermatan sulfate side chains or interactions of the G1 and G3 domains, versican is able to regulate many cellular processes including cell adhesion, proliferation, apoptosis, migration, angiogenesis, invasion and metastasis. In this review, the biological roles that versican plays in cancer development are presented. Therapeutic targeting of versican in malignant tumors is also discussed.

Key words: Tumorigenesis, Metastasis, Invasion, Apoptosis, Versican

Introduction

Despite advances in prevention, diagnosis, and treatment, malignant tumor formation remains a leading cause of mortality. Although some cancer types need only active surveillance in the early stages and others can be eradicated by surgical, chemo-, radio-, or medical- therapeutic treatments, many cancer types progress rapidly, metastasizing to distant organs. Knowledge of tumorigenesis, tumor progression and metastasis is thus critical for developing cancer therapeutics. An increased understanding of the molecular determinants of cancer is one of the key strategies that can be exploited for therapeutic approaches.

Tissue neoplastic transformation, cancer progression and metastasis are complicated, systemic multi-step processes, which are affected by both tumor cell characteristics and the microenvironment surrounding the tumor. The extracellular matrix (ECM) is involved in both of these aspects, exerting important functions that actively contribute to various processes. Versican (also known as VCAN or CSPG2), a chondroitin sulfate proteoglycan, is one of the main components of the ECM, ubiquitously expressed in almost all tissues. While versican plays a role in normal tissue development, elevated levels of versican have been reported in most malignancies to date, including non-solid tumors, such as human acute monocytic leukemia (Ricciardelli et al., 2002; Makatsori et al., 2003; Nikitovic et al., 2006). Depending on the cancer type, increased versican expression occurs in either the tumor cells themselves or the stromal cells surrounding the tumor (Ricciardelli et al., 2002; Voutilainen et al., 2003; Mauri et al., 2005; Kodama et al., 2007b). Emerging evidence indicates that increased versican expression is strongly associated with poor outcomes for many different cancer types (Ricciardelli et al., 1998, 2002; Hanekamp et al., 2003; Pukkila et al., 2004, 2007; Pirinen et al., 2005; Kodama et al., 2007a,).

In this review, we focus on recently identified mechanisms responsible for regulating versican expression. We then describe the biological roles that

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versican plays in tumor development, progression and metastasis. Finally, we discuss potential strategies for therapeutic targeting of versican in malignant tumors.

Structure and function of versican

Within the human genome, versican is encoded by the VCAN gene located on chromosome 5q 12-14. Human versican is encoded by 15 exons spanning over 90-100 kb (Naso et al., 1994). Typically, the N-terminal globular domain (G1 domain) of versican contains an immunoglobulin-like motif and two proteoglycan tandem repeats which bind hyaluronan (HA). The C-terminal globular domain (G3 domain) contain two EGF-like repeats, a complement regulatory protein-like repeat and a C-type lectin domain (Wight and Merrilees, 2004). Analysis by reverse transcription polymerase chain reaction, cDNA sequencing and northern blot has demonstrated that the structural diversity of versican originates from alternative splicing processes. Designated V0, V1, V2 and V3, the respective sizes of the four versican isoforms are 370 kDa, 263 kDa, 180 kDa, and 74 kDa (Dours-Zimmermann and Zimmermann, 1994; Naso et al., 1994). Each isoform contains different lengths of glycosaminoglycan (GAG) binding regions with an accompanying variation in the number of attached GAG chains. Specifically, these isoforms differ by the presence or absence of two GAG attachment regions, GAG-α and GAG-ß (Ito et al., 1995). The V0 isoform contains both GAG-α and GAG-ß, while V1 contains GAG-ß, V2 contains GAG-α, and V3 contains no GAG attachment domains (Kenagy et al., 2006).

The GAG attachment regions carry 5 and 23 chondroitin-/dermatan-sulfate (CS/DS) chains depending on the versican isoform. The isoforms also have variable chondroitin 6-sulfate to chondroitin 4-sulfate ratios (Schonherr et al., 1991; Wight, 2002). It has been recognized that the number, length, and molecular structure of GAG chains may also be affected by the G1 and G3 domains. In particular, the attachment of GAG chains has been reported to be inhibited by the versican G1 domain and promoted by the G3 domain (Yang et al., 2000; Wu et al., 2005b).

Versican regulates a variety of cell activities including cell adhesion, proliferation, apoptosis, migration and invasion via the chondroitin and dermatan sulfate side chains and the G1 and G3 domains (Zhang et al., 1998a; Ang et al., 1999; Xiang et al., 2006; Sheng et al., 2007; Wu et al., 2009). In addition, the versican G1 and G3 domains can interact with various intracellular or extracellular molecules (Lebaron, 1996). To date, a wide range of molecules have been reported to interact with versican through either the G1 or G3 domains, or the GAG attachment region (Wu et al., 2005b). It is known that the association of the versican G1 domain with HA is mediated by link protein (LP). Both HA and LP protein have the ability to bind to the G1 domain of versican (Matsumoto et al., 2003). In addition to HA (Lebaron, 1996), versican has been shown to associate with tenascin-R (Aspberg et al., 1995), fibulin-1 and -2 (Aspberg et al., 1999), fibrillin-1 (Isogai et al., 2002), fibronectin (Yamagata et al., 1986), P- and L-selectin (Kawashima et al., 2000, Zheng et al., 2004a), and various chemokines (Wu et al., 2005b). Versican also binds to cell surface proteins including epidermal growth factor receptor (EGFR) (Wu et al., 2005b), CD44 (Kawashima et al., 2000), and integrin ß1 (Wu et al., 2002). Recently, versican has been shown to act on macrophages through toll-like receptors, TLR2 and TLR6, leading to the production of inflammatory cytokines, and the promotion of tumor cell metastasis (Kim et al., 2009).

Regulation of versican expression

Versican is encoded by 15 exons encompassing over 90-100 kb of continuous DNA, with considerable conservation of both exon and intron sequences (Naso et al., 1994). Versican expression is regulated by a promoter region harboring a TATA box located approximately 16 base pairs upstream of the transcription start site. Among its various regulatory mechanisms, there are potential binding sites for transcription factors, including TCF-4, AP1 and CCAAT enhancer protein (Domenzain-Reyna et al., 2009). The versican gene can also be bound by the tumor suppressor p53 at its first intron causing direct activation in a dose-dependent manner (Naso et al., 1994; Yoon et al., 2002). The versican gene is a reported target of several signalling pathways, including the Wnt pathway (Willert et al., 2002), GSK-3 beta pathway (Rahmani et al., 2005), and PI3K pathway (Hamamura et al., 2008).

Versican expression is known to be regulated by a number of cytokines including TGF-ß, PDGF, IL-1α and IL-1ß. TGF-ß has been found to up-regulate synthesis of versican in glioma, osteosarcoma and fibrosarcoma cells (Serra et al., 2005; Nikitovic et al., 2006; Arslan et al., 2007). Additionally, TGFß1 is one of the major regulators of versican expression within the tumor stroma (Cross et al., 2005; Arslan et al., 2007). The induction of versican expression by TGF-ß in benign prostatic hyperplasial (BPH) stromal cells is complemented with the negative effects of TGF-ß on a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) -1, -5, -9, and -15. When coupled with increases in the TGF-ß inhibitor TIMP-3, versican accumulates in the stromal cells of the prostate during BPH and prostate cancer (Cross et al., 2005). TGFß2 has also been shown to increase versican expression in normal lung fibroblasts, fibrosarcoma and osteosarcoma cells (Berdiaki et al., 2008). With regard to other cytokines, platelet-derived growth factor (PDGF) treatment has been shown to increase versican expression in arterial smooth muscle cells (SMC) and human gingival fibroblasts (Haase et al., 1998). Furthermore, versican expression is up-regulated in lung fibroblasts treated with IL-1ß (Tufvesson and

Westergren-Thorsson, 2000) and down-regulated in vascular SMCs treated with IL-1α (Lemire et al., 2007). The expression of versican can be also modified by Epidermal Growth Factor (EGF), insulin-like growth factor I and PDGF-BB in malignant mesothelioma cells (Syrokou et al., 1999). In addition, steroid hormones and gonadotrophins also modulate versican expression (Russell et al., 2003). Androgen receptors regulate the versican gene through an androgen response element located in the promoter region (Read et al., 2007).

There is increased evidence that the accumulation of proteolytic fragments of versican play an important role in cancer progression. The regulation of G1 and G3 versican levels by proteases is known to be important in regulating cancer cell motility and metastasis. A number of proteinase families are capable of generating the proteolytic fragments of versican. For example, matrix metalloproteinase (MMP)-1 (Perides et al., 1995), -2 (Passi et al., 1999), -3 (Perides et al., 1995), -7 (Halpert et al., 1996), and -9 (Passi et al., 1999) have been shown

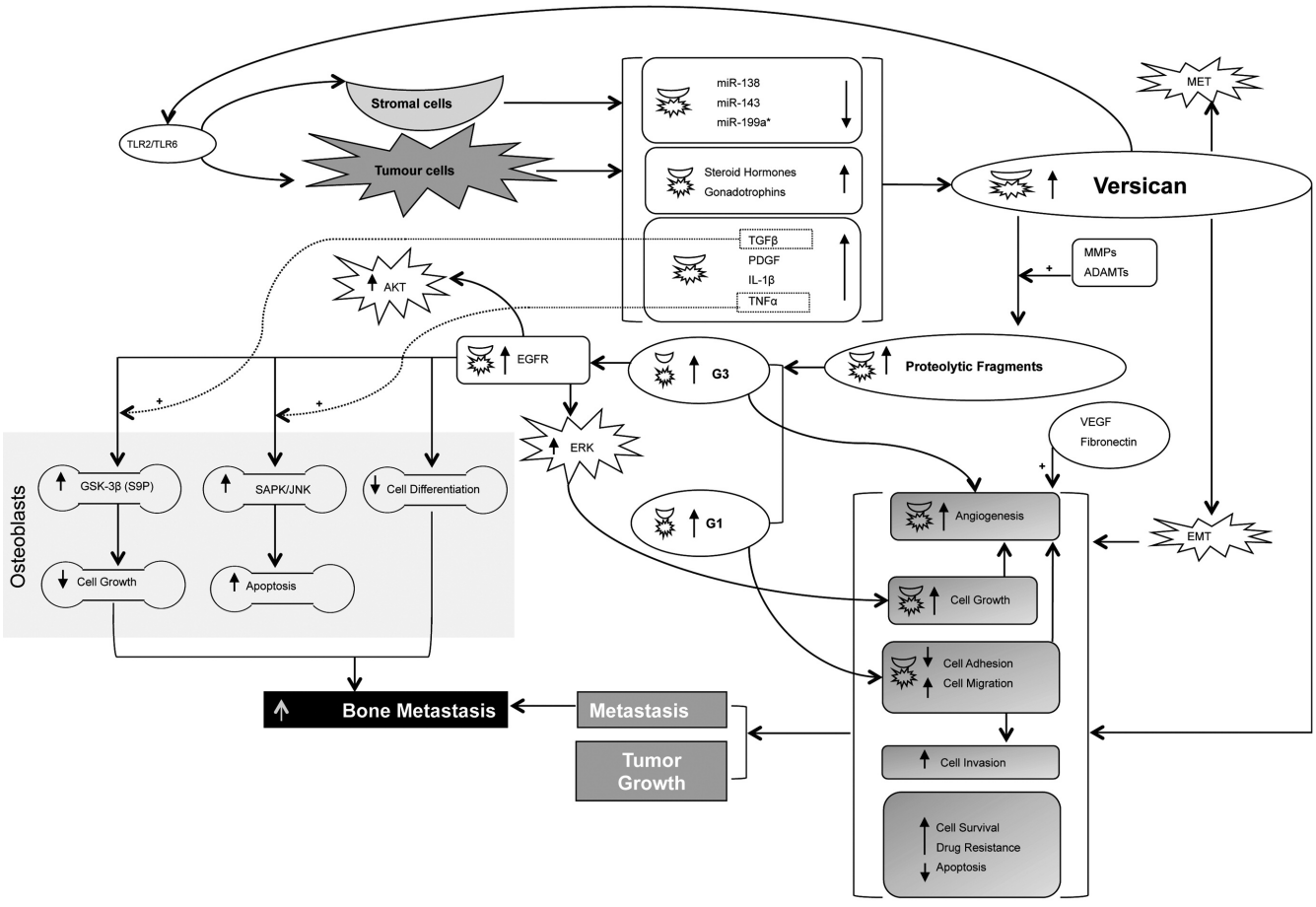

Fig. 1. Biological functions of versican in cancer progression. The interaction between cancer cells and stromal cells up-regulates expression of a number of cytokines including PDGF, TGF-ß, IL-1ß and TNFα, steroid hormones and gonadotrophins, and down-regulates certain onco-suppressing microRNAs which target versican 3'UTR molecules, thus promotes expression of versican in the tumor and stroma. Expression of versican represses cancer cell adhesion and apoptosis whereas enhances cancer cell survival, cell growth, migration, invasion, angiogenesis, drug resistance, and metastasis. Upregulated expression of versican and it G3 fragment promote pro-angiogenic arterial smooth muscle cells proliferation and migration of these cells which contributes angiogenesis. The G3 fragment also can directly binds to fibronectin and forms a complex together with VEGF, which enhances angiogenesis. The proteoglycan versican appears to regulate the transformation of EMT phenotypes at various stages of development, which supports cancer cell growth, migration, invasion, angiogenesis, and metastasis. Versican can strongly enhance cancer metastatic growth by activating TLR2/TLR6 complexes and a number of growth factors. Similarly, the ligation of TLR2/TLR6 on endothelial cells and fibroblasts by versican may also activate these cells and trigger the secretion of inflammatory cytokines, providing a link between inflammation and cancer metastasis. Enhanced activity of proteinase family members MMPs and ADAMTs are capable of generating the proteolytic fragments of versican which play an important role in cancer progression. The G1 fragment is known enhance motility of cancer cells and reduce cell adhesion. The G3 fragment enhances cancer cell proliferation, migration and invasion by up-regulating EGFR/ERK signaling, and enhances cell survival and drug resistance via the EGFR/AKT pathway. Expression of the versican G3 fragment also enhances osteoblast apoptosis, and inhibits osteoblast growth and differentiation when cultured in TGF-ß or TNFα, contributing to cancer cell bone metastasis.

to degrade native, purified versican in vitro. ADAMTS-1 (Sandy et al., 2001), -4 (Sandy et al., 2001), -5 (Cross et al., 2005), and -9 (Somerville et al., 2003) have been reported to cleave either native versican or versican peptide substrates.

Recently, expression of versican has been found to be regulated by a number of microRNAs. For example, miR-138 is expressed in specific domains of the zebrafish heart and functions partially by repressing versican during heart development (Morton et al., 2008). Likewise, miR-143 expression induced by the transcription factor myocardin lowers versican levels within smooth muscle cells (Wang et al., 2010). Recently, it has been found that the versican 3'UTR can antagonize endogenous microRNAs which target versican, thereby enhancing versican expression. In a transgenic mice model, expression of the versican 3'UTR induced organ adhesion by modulating miR-199a* activities (Lee et al., 2009, 2010). MiR-199a* is considered to be an onco-suppressor which is downregulated during various malignancies. By enhancing versican expression, mir-199a* contributes, at least in part to tumor growth.

Versican regulates cell proliferation

Versican is highly expressed in proliferating tumor and tumor-associated stromal tissues. Immunohistochemical studies of breast tumors have shown that versican localizes within HA-rich portions of proliferating interstitial tissue. This indicates that versican expression is in association with tumor cell growth and invasion (Nara et al., 1997). Within vascular SMCs, endogenous tyrosine kinase activity of PDGF, TGF-,1 and EGF receptors stimulate versican synthesis at both the transcript and protein levels (Evanko et al., 2001). Molecules that associate with versican such as HA and CD44 are also upregulated by these growth factors (Evanko et al., 2001), causing an increase in the pericellular matrix and expansion of the ECM. By producing a highly malleable extracellular environment, the cell-shape change necessary for cell proliferation and migration is facilitated by these growth factors (Lee et al., 1993). Versican can also stimulate cell proliferation via other mechanisms: through two EGF-like motifs in the G3 domain which play a role in stimulating cell growth, and through the G1 domain, which destabilizes cell adhesion and facilitates cell growth (Zhang et al., 1998a, 1999; Yang et al., 1999; Du et al., 2010). Consistent with these results are the function of the G3 domain in chondrogenesis (Zhang et al., 1998b, 2001). Through various mechanisms, versican expression is associated with a high rate of proliferation (Gulyas and Hjerpe, 2003).

Versican regulates cell survival, apoptosis and drug resistance

For a malignant transformed clone to survive, genetic or epigenetic modifications in its apoptotic signaling machinery must facilitate cell survival and growth (Igney and Krammer, 2002). Along with its effects on proliferative capacity, versican has been shown to enhance cell survival and apoptotic resistance (LaPierre et al., 2007). Expression of the G1 and G3 domains of versican protects cells from apoptosis induced by death receptor ligands or cytotoxic drugs (Cattaruzza et al., 2004). In this study, versican G1-overproducing sarcoma cells appeared resistant to both cytotoxic drug-induced and Fas-dependent programmed cell death. This resistance implicated mitochondrial apoptotic genes (Cattaruzza et al., 2004).

The G3 domain binds to integrin-ß1, increasing focal adhesion kinase activation, and protecting cells against apoptosis (Wu et al., 2002). Following treatment with hydrogen peroxide, G3-expressing cells have shown resistance to apoptosis (Wu et al., 2005a). Resistance to Doxorubicin and Epirubicin has shown to be enhanced by up-regulating pERK and GSK-3ß (S9P). Increased expression of pSAPK/JNK and decreased expression of GSK-3ß (S9P) also promoted cell apoptosis induced by C2-ceramide or Docetaxel (Du et al., 2011). Indeed, GSK-3ß (S9P) appears to function as a key check-point in the balance of apoptosis and anti-apoptosis (Du et al., 2011). Inhibited endogenous versican expression, achieved by siRNA or linking G3 with the versican 3'UTR, both prevented G3 modulated cell apoptosis (Du et al., 2011). The dual role of G3 in modulating breast cancer cell resistance to chemotherapeutic agents may in part explain a potential mechanism for breast cancer cell resistance to chemotherapy and EGFR therapy.

The V1 isoform can enhance cell survival in serum-free conditions, down-regulate the expression of the proapoptotic proteins, Bad and Fas, modulate cell cycle progression and protect cells from apoptosis (Sheng et al., 2005). The V2 isoform did not appear to contribute to apoptotic resistance (Sheng et al., 2005). However, the combination of selective apoptotic resistance and sensitivity is often seen in cancer cells. The deregulated proliferation of tumor cells is well documented as a potent apoptotic inducer. V1 expressing cells showed increased sensitivity to a wide range of cytotoxic agents and UV radiation. High resting levels of p53 and murine double minute-2 (MDM2) in these cells were also correlated with apoptotic sensitivity (LaPierre et al., 2007). Loss of the p21 response to apoptosis induction coupled with high resting levels of proapoptotic p53 may be partially involved in premature cell death following cytotoxic treatment (LaPierre et al., 2007). The dual roles of versican in modulating cancer cell survival and apoptosis, reveals the complexity of apoptosis regulation in tumor development and progression.

Versican regulates cell adhesion, migration and invasion

Studies have shown that versican localized in proliferating interstitial tissues, particularly in HA-rich

portions were associated with carcinoma cell growth, and also accumulated in perivascular elastic tissues involved in cancer invasion (Nara et al., 1997). These functional studies have provided evidence supporting the proposed role of versican as a proliferative, anti-adhesive and pro-migratory molecule that promotes cancer cell motility (Ricciardelli et al., 2007). Versican is able to reduce the attachment, and promote both cancer cell migration and invasion (Touab et al., 2002; Sakko et al., 2003; Skandalis et al., 2006; Ghosh et al., 2012; Kusumoto et al., 2012). Induction of stromal versican expression is correlated with higher tumor grade and invasiveness in carcinomas, and with associated with tumor progression (Mukaratirwa et al., 2004; Labropoulou et al., 2006; Skandalis et al., 2006; Kusumoto et al., 2012). In addition, elevated expression is always correlated with altered levels of HA in tumor cell or stromal tissues (Mukaratirwa et al., 2004). Cancer cells recruit stromal components to remodel their pericellular environment and promote their motility (Ricciardelli et al., 2007). High levels of HA and versican in the peritumoral stroma are associated with metastatic spread of clinical prostate cancer (Ricciardelli et al., 2007). In advanced endometrial cancer, it was shown that increased expression CD44 and Versican was associated with loss of expression of both Progesterone Receptor (PR) and E-cadherin (Hanekamp et al., 2003). Integration of HA and versican within the pericellular sheath is a prerequisite for proliferation and migration of vascular smooth muscle cells (Ricciardelli et al., 2007). Cancer cells can form a polarized pericellular sheath through compartmentalized cell-surface CD44, HA and versican aggregates that promotes their motility (Ricciardelli et al., 2007). Elevated versican expression in the tumor associated stroma results in reduced numbers of intraepithelial CD8-positive T cells and enhanced cancer cell local invasion (Gorter et al., 2012).

Versican enhances motility of cancer cells and reduces cell adhesion through its G1 domain. G1-overproducing sarcoma cells were more invasive than the corresponding G3 expressing cells, and their locomotion was perturbed by exogenous HA (Cattaruzza et al., 2004). Studies in astrocytoma cancer cell lines have demonstrated that the G1 domain, but not the G3 domain, of versican could enhance migration (Ang et al., 1999). The versican G3 domain appears to be important in local and systemic tumor invasiveness of human breast cancer (Yee et al., 2007). Within breast cancer cells, G3 expression enhanced cell proliferation and migration by up-regulating EGFR signaling, and enhanced chemotactic cell motility to bone stromal cells. This observed motility was prevented by inhibitor AG 1478 (Du et al., 2010). The expression of both versican G3 and G1 domains is positively related to the Ki67 index of carcinoma cells and tumor size, respectively (Takahashi et al., 2012).

Increased versican V0 and V1 expression in tumor vessels and decreased expression of these two isoforms in glioma ECM may be related to the marked local invasivity and rarity of extracranial metastasis of gliomas (Paulus et al., 1996). Cell motility and migration are significantly enhanced by V1 isoform transfection (Wasa et al., 2012). Versican has the capacity to form extensive cell-associated matrices, increasing the aggressive behaviour of cancer cells (Ricciardelli et al., 2007; Wasa et al., 2012). *In vitro* studies also revealed that versican V0/V1 silencing caused increased adhesion to type I collagen, laminin and fibronectin. This was coupled with reduced cell migration in both wound healing assays and Transwell chambers (Hernandez et al., 2012).

Versican in angiogenesis

Angiogenesis is a normal and vital process in growth, development, and wound healing. It is also one

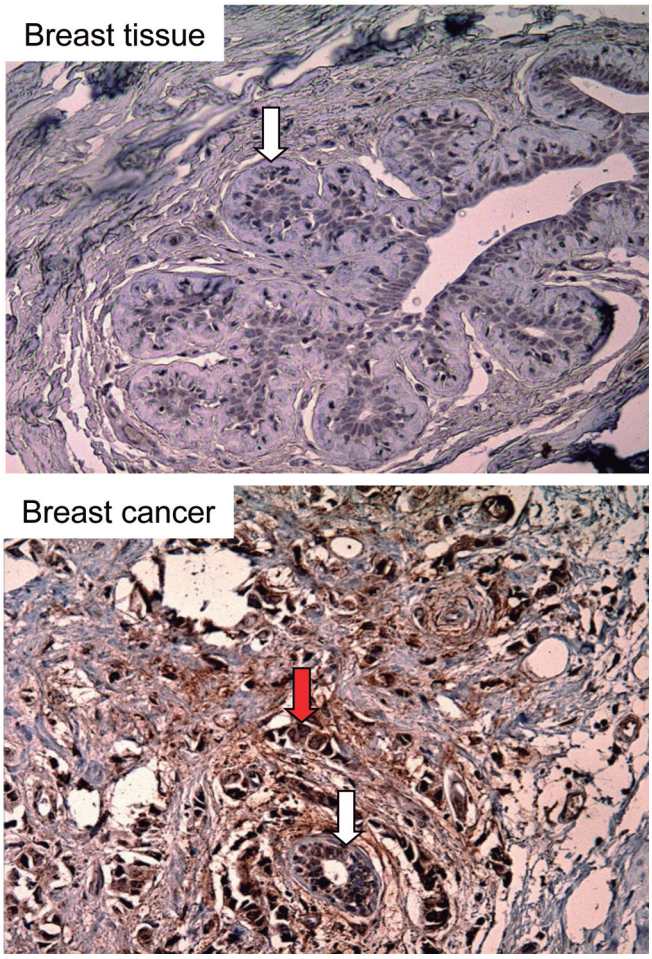

Fig. 2. Expression of versican in breast cancer cells. Normal breast tissue and breast cancer tissue were subjected to immunostaining for versican expression. Breast cancer cells (red arrow) were found to express increased level of versican as compared with the normal ductal structure of the breast tissue (white arrow).

of the fundamental steps in the transition of tumors from a dormant state to a malignant one. Versican is one the important ECM components at the center of the angiogenesis-associated network (Rivera et al., 2012). Upregulated expression of versican was found in pro-angiogenic arterial SMCs which contributed to the proliferation and migration of these cells (Evanko et al., 1999, 2001; Kreutziger et al., 2012). The deposition of versican has been found to be linearly correlated with the number of microvessels in tumour stroma (Labropoulou et al., 2006; Ghosh et al., 2012). Indeed, versican accumulation in germ cell tumours is related to both metastatic potential and neovascularization. Versican expression may be a useful marker for testicular malignancy (Labropoulou et al., 2006). Versican is actively processed during the early stage of Vascular endothelial growth factor A (VEGF-A)-induced pathological angiogenesis in tumors (Fu et al., 2012). VEGF-A initiates enlarged "mother vessel" (MV) formation from preexisting venules, in part, by inducing the expression of endothelial cell proteases such as ADAMTS-1 and MMP-15. These proteases act in concert to degrade venular basement membrane versican (Fu et al., 2012). In addition, increased expression of versican is often associated with elevated levels of HA in the vascular and perivascular elastic structures in malignant tumors (Koyama et al., 2007; Nara et al., 1997). It is believed that versican acts as a key player in HA-mediated angiogenesis by enhancing recruitment of host stromal cells (Koyama et al., 2007). A recent study has documented that versican can activate resident fibroblasts and endothelial cells in the tumor stroma through both TLR2 and its co-receptors TLR6 and CD14. This elicits the production of proinflammatory cytokines including interleukin-8, a proinflammatory CXC chemokine that potentiates neutrophil infiltration, angiogenesis and metastatic growth.

The versican G3 domain has been found to enhance angiogenesis both *in vitro* and *in vivo* (Zheng et al., 2004b). G3 domain expresion enhanced endothelial cell adhesion, proliferation, and migration *in vitro* and blood vessel formation in nude mouse tumors (Zheng et al., 2004b). G3-expressing cells and tumors formed by these cells express elevated levels of fibronectin and vascular endothelial growth factor (VEGF) (Zheng et al., 2004b). In the presence of versican, fibronectin and VEGF, endothelial cell adhesion, proliferation, and migration were found to be significantly enhanced. Removal of the complex containing these molecules reversed these processes (Zheng et al., 2004b). The above study indicated that the G3 domain directly binds to fibronectin and forms a complex together with VEGF (Zheng et al., 2004b). Potential effects on angiogenesis include enhancing vascular endothelial proliferation, migration, and vessel formation. The interactions between tumor cells, surrounding stromal components and neo-vascularization in breast cancer may include interactions among versican, VEGF and fibronectin (Yee et al., 2007).

Versican in tumor metastasis

High expression of versican in a number of human tumors, including breast, prostate and epithelial ovarian cancers, is prognostic, being predictive of relapse, and negatively impacts overall survival rates (Ricciardelli et al., 2002, 2007; Casey et al., 2003; Suwiwat et al., 2004; Lancaster et al., 2006; Du et al., 2010; Kusumoto et al., 2012). Versican participates in cell adhesion, migration, and angiogenesis, all features facilitating invasion and metastasis (Wang et al., 2009). Functional studies have demonstrated that versican can increase cancer cell proliferation, cell aggregation, motility and metastasis (Cattaruzza et al., 2004; Zheng et al., 2004b, 2006; Arslan et al., 2007; Ricciardelli et al., 2007). Versican, HA and CD44 form a macromolecular complex which promotes the motility of prostate cancer cells and leads to tumor invasion and metastasis (Ricciardelli et al., 1998, 2007). In addition, the formation of the HA enriched pericellular matrix of the cancer cells is essential for the specific adhesion of cancer cells to bone marrow endothelial cells and may contribute to preferential bone metastasis by breast and prostate carcinomas (Simpson et al., 2001; Draffin et al., 2004).

The structure of CS chains also plays an important role in the interactions of selectins with their ligands and may affect the signal transduction into cells (Kawashima et al., 2000). Versican has been known to interact with E-selectin, L-selectin, P-selectin through the CS chains (Luo et al., 2001). Selectin-mediated binding of cancer cells to leukocytes, platelets and vascular endothelium can regulate their hematogenous and lymphogenous spread. CS chains are also major P-selectin ligands on metastatic breast cancer cell lines such as 4T1 cells (Monzavi-Karbassi et al., 2007). Advanced cancer cells can usurp components of the resident innate immune system to generate an inflammatory microenvironment hospitable for metastatic growth (Kim et al., 2009). The ECM components act as a depot of cytokines and growth factors, mobilized by enzymes originating from inflammatory white blood cells and promoting blood-vessel formation during cancer development and progression (Hanahan and Weinberg, 2000; Mantovani, 2009).

Collectively, the accumulated data suggest that both G1 and G3 domains of versican may differentially control tumor growth rates and have interactive roles to promote tumor development and metastasis (Ricciardelli et al., 2009). A recent study revealed that versican G3 not only inhibited osteoblast differentiation, but also enhanced osteoblast cell apoptosis in the condition medium (containing TGF-ß1 and TNF-α), facilitating breast cancer bone metastasis.

Versican in tumorigenesis

Increased expression of versican has been recorded in wide ranges of malignant tumors, including: melanomas, osteosarcomas, lymphomas, brain tumors,

leukemia, breast, prostate, colon, lung, pancreatic, endometrial, oral, and ovarian cancers (Paulus et al., 1996; Rottiers et al., 1998; Ricciardelli et al., 2002, 2007; Touab et al., 2002; Casey et al., 2003; Makatsori et al., 2003; Voutilainen et al., 2003; Mukaratirwa et al., 2004; Skandalis et al., 2004a,b, 2006; Pirinen et al., 2005; Lancaster et al., 2006; Nikitovic et al., 2006; Pukkila et al., 2007). Elevated versican levels are associated with cancer relapse and poor patient outcome in breast, prostate, and many other cancer types (Ricciardelli et al., 1998, 2002; Hanekamp et al., 2003; Pukkila et al., 2004, 2007; Pirinen et al., 2005; Kodama et al., 2007a,b). Cell growth, adhesion, migration, invasion, angiogenesis and metastasis are all hallmarks of malignant tumor growth, indicating that expression of versican may be involved in tumorigenesis. Recent studies indicate that alterations in the profile of versican may be positively linked to the formation of tumors, but further research and clinical investigations are required to conclusively establish such a link.

The "nevi - dysplastic nevi - melanoma" transition reveals that versican expression can gradually increase during a multistep carcinogenesis sequence. Versican was absent in benign melanocytic nevi, weakly expressed in dysplastic nevi, but abundantly expressed in advanced phases of the tumor and in metastatic lesions (Touab et al., 2002; Domenzain et al., 2003). Excessive exposure to UV radiation is a major risk factor for developing skin cancer. A study has reported that inflammatory responses, particularly neutrophil infiltration and versican up-regulation, are closely involved in UVB/ROS-induced skin tumorigenesis (Kunisada et al., 2012). In addition, versican may be involved in the morphogenesis of neoplastic epithelium and mesenchymal tissues in odontogenic tumors (Ito et al., 2002).

In functional assays, versican promoted tumorigenesis by inhibiting cell death. Overexpression of versican is sufficient to promote tumorigenesis by limiting cell death (Dondeti et al., 2012). Astrocytoma U87 cells expressing the versican G3 mutant lost the hallmark of cell transformation *in vitro* and *in vivo*, indicating the role of G3 in tumorgenesis (Wu et al., 2004). Recent studies have highlighted the interaction between versican and TLR2 linked inflammation and induced malignancy transformation. Ligation of TLR2 present on endothelial cells and fibroblasts by versican activates these cells and triggers the secretion of multiple inflammatory cytokines and growth factors (Kim et al., 2009; Wang et al., 2009). The resulting inflammation is often associated with cancer initiation and transformation. Inflammatory cells are regarded as critical mediators in the development of malignancies (Kim et al., 2009; Wang et al., 2009). Thus, collectively, current studies support the idea that versican expression is not only a secondary event of malignancy transformation, but also a primary and major one, acting as a direct promoter of malignant conversion.

Versican in cancer cell epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET)

Initiation of tumor cell metastasis involves enhancement of cell invasion, which has many phenotypic similarities to epithelial-mesenchymal transition (EMT), including a loss of cell-cell adhesion and an increase in cell mobility, which are mainly mediated by E-cadherin repression. The role of versican as a proliferative, anti-adhesive and pro-migratory molecule that promotes cancer cell motility has been well established (Ricciardelli et al., 2007). Elevated levels of versican synthesized by embryonic stem cells (ESCs) within embryoid body (EB) microenvironments are associated with EMT processes and play a key role in ESC differentiation (Shukla et al., 2012). Versican plays an essential role in the EMT of the endocardial mesenchymal cushion (Kern et al., 2006), mesenchymal condensation and hair induction (Kishimoto et al., 1999). Expression of versican has been known to be responsible for the EMT in mammary tumors, which are revealed as ectopic cartilage formation within the tumors (Erdelyi et al., 2005).

However, versican is also identified as a putative indicator of mesenchymal-epithelial transition (MET), which by comparison, is the opposite mechanism to EMT (Soltermann et al., 2008). Expression of versican is sufficient to induce MET in NIH3T3 fibroblasts and reduced versican expression decreases MET in metanephric mesenchyme (Sheng et al., 2006). Molecular analysis showed that V1 promoted a "switch" in cadherin expression from N- to E-cadherin, resulting in repressed vimentin levels and enhanced occludin levels, an epithelial-specific marker (Sheng et al., 2006). Thus versican may be an important protein with both EMT and MET potentials, implicated in both the initiation and progression of malignancy. MET is known to participate in the establishment and stabilization of distant metastases by allowing cancerous cells to regain epithelial properties and integrate into distant organs (Yang and Weinberg, 2008). These studies have highlighted that MET may be one of potential therapeutic targets in the prevention of metastases. The multifaceted role of versican in modulating cancer cell EMT and MET throughout various processes warrant ongoing study.

Strategies for therapeutic targeting of versican

Targeting versican in synthesis

Targeting versican synthesis is believed to be a potential measure in reducing the biological functions of this tumor-promoting agent. The effects of growth factor and cytokine signaling such as PDGF, TGF-ß, EGFß VEGF and IL-1ß which promote versican expression, can be reversed by following treatment with various

tyrosine kinase inhibitors (Shimizu-Hirota et al., 2001). Blocking these growth factors and cytokines with specific tyrosine kinase inhibitors has been effective in certain cases. The tyrosine kinase inhibitor genistein has been shown to block versican expression induced by growth factors in malignant mesothelioma cell lines (Syrokou et al., 1999). In vascular SMC, genistein inhibits PDGF-stimulated versican expression in a dose-dependent manner (Schonherr et al., 1997). In SMC, the tyrosine kinase inhibitor herbimycin A, the mitogen-activated protein kinase inhibitor PD98059, and the EGF receptor inhibitor AG1478 have been reported to reduce angiotensin II enhanced versican expression (Shimizu-Hirota et al., 2001). In addition to selective tyrosine kinase inhibitors, these growth factors can also be inhibited at the translational level by antisense oligonucleotides or blocked via monoclonal antibodies which inhibit the ligand-receptor interaction (Theocharis, 2008). However, there is no studies report whether these therapeutic approaches are effective in modulating *in vivo* versican expression.

Chronic inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease are characterized by airway remodeling with altered ECM deposition, especially versican (Bensadoun et al., 1997; Huang et al., 1999; Passi et al., 1999). Recent studies have revealed that alteration of versican deposition in asthmatic airways can be inhibited by a number or asthma drugs. Montelukast, a leukotriene receptor antagonist, inhibits versican expression in both bronchial and arterial SMCs (Potter-Perigo et al., 2004). Other asthma drugs such as Budesonide and Formoterol can repress versican deposition in human lung fibroblasts and airway SMCs (Burgess et al., 2006). Further studies are needed to evaluate the effects of these drugs on expression of versican in malignancy.

Expression of versican can be modulated post-transcriptionally by a number of microRNAs such as miR-143, miR-138 and miR-199a-3p (Morton et al., 2008; Lee et al., 2009). Theses microRNAs are considered to be tumor-suppressors, often downregulated in malignant tumors (Migliore et al., 2008; Lee et al., 2009). MiRNA-based therapeutic strategy for targeting versican can be introduced by short double-stranded synthetic RNA loaded into an RNA-induced silencing complex. Another strategy utilizes the expression of hairpin pre-miRNA in a viral vector system, which leads to increased expression of these tumor-suppressor microRNAs.

Targeting versican in processing

With increased knowledge of the roles of versican proteolytic fragments in cancer progression, current studies highlight targeting versican processing as a novel strategy to prevent and control cancer cell invasion and metastasis. Antibodies against to the ADAMTS specific versican cleavage site inhibit glioma cell migration in a dose-dependent manner (Arslan et al., 2007). GM6001 (Galardin), a MMPs and ADAMTS proteases inhibitor, has been shown to inhibit cancer cell invasion and metastasis in several kinds of carcinoma (Casey et al., 2003; Almholt et al., 2008; Nakamura et al., 2007). Other protease inhibitors such as catechin gallate esters, present in natural sources (green tea) have been shown to selectively inhibit ADAMTS-1, -4 and -5 catabolism (Vankemmelbeke et al., 2003). Versican G3 fragments have been known to enhance cancer cell growth, invasion, metastasis, and chemical resistance via EGFR signaling (Du et al., 2010, 2011). The selective EGF receptor inhibitor, AG1478 prevents G3 fragment enhanced cell growth, migration, invasion and chemical resistance *in vitro* (Du et al., 2010, 2012). Application of versican specific protease inhibitors or proteolytic fragments involved in cell signaling pathways can be further explored. Preventing versican catabolism and proteolytic fragment accumulation may provide novel therapeutic targets for cancer invasion and metastasis.

Targeting versican and its binding molecules

The unique and versatile structure of versican lends itself to multiple types of interactions through either protein-protein or protein-carbohydrate interactions. As mentioned previously, the versican G1 domain can interact with HA and CD44, forming a polarized pericellular sheath mediated around tumor cells, thus promoting their motility, invasion and metastasis (Matsumoto et al., 2003; Ricciardelli et al., 2007). It has been found that tumor cell formation of the pericellular matrix with HA and versican can be inhibited by treatment with HA oligomers (Evanko et al., 1999). HA oligomers can block the interaction between HA and versican, and are promising inhibitors of cancer dissemination (Ween et al. 2012). Disruption of the HA CD44 interaction with HA oligomers has been reported to significantly inhibit the growth of B16F16 melanoma cells (Zeng et al., 1998). Therefore the application of HA oligomers is an attractive agent for inhibiting the formation of vesicant-HA-CD44 complexes, providing valuable targets against cancer metastasis.

Targeting versican chondroitin sulfate (CS) chain

Increased evidence has revealed that versican CS plays an important role in cancer biology as it is involved in interactions with tumor cells and related molecules, such as growth factors and cytokines (Asimakopoulou et al., 2008). The roles of CS in promoting tumor growth, invasion and metastasis, can be abolished by chemically modified CS, or the use of CS with altered sulfation patterns (Asimakopoulou et al., 2008). A recent study showed that modified CS injected directly into nude mice breast tumors reduced or abolished cancer cell growth without apparent toxic effects to adjacent tissue (Pumphrey et al., 2002). CS targeted anticancer drugs delivered by cationic liposomes may represent a potentially useful strategy to

prevent local tumor growth and metastasis (Lee et al., 2002).

Conclusion

The multifaceted roles of versican in regulating cell behaviour are critical in tumour development. Key processes such as tumorigenesis, angiogenesis and metastasis have all been shown to be mediated through versican. In addition to understanding the tissue and isoform specific functions of versican, it will be important to identify strategies for the therapeutic targeting of this proteoglycan. Understanding versican's wide array of regulatory mechanisms will provide a rational basis for further clinical development.

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