Versican in the Tumor Microenvironment

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Abstract

Versican is an extracellular matrix proteoglycan with nonredundant roles in diverse biological and cellular processes, ranging from embryonic development to adult inflammation and cancer. Versican is essential for cardiovascular morphogenesis, neural crest migration, and skeletal development during embryogenesis. In the adult, versican acts as an inflammation "amplifier" and regulator of immune cell activation and cytokine production. Increased versican expression has been observed in a wide range of malignant tumors and has been associated with poor patient outcomes. The main sources of versican production in the tumor microenvironment include accessory cells (myeloid cells and stromal components) and, in some contexts, the tumor cells themselves. Versican has been implicated in several classical hallmarks of cancer such as proliferative signaling, evasion of growth suppressor signaling, resistance to cell death, angiogenesis, and tissue invasion and metastasis. More recently, versican has been implicated in escape from tumor immune surveillance, e.g., through dendritic cell dysfunction. Versican's multiple contributions to benign and malignant biological processes are further diversified through the generation of versican-derived bioactive proteolytic fragments (matrikines), with versikine being the most studied to date. Versican and versican-derived matrikines hold promise as targets in the management of inflammatory and malignant conditions as well as in the development of novel predictive and prognostic biomarkers.

Keywords

Proteoglycans · Tumor matrix · Versican · Tumor microenvironment · Myeloid regulatory cells · Versican proteolysis · Versikine · ADAMTS · Dendritic cells · Macrophages · Biomarkers · Immunotherapy · Inflammation · Metastasis · Tumor progression

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4.1 Introduction: Structure, Isoforms, and Key Intermolecular Interactions

Versican is a chondroitin sulfate (CS) matrix proteoglycan with crucial, nonredundant roles in organ development and disease [117]. In humans, it is encoded from a single locus on chromosome 15q14.3 [50]. Its amino acid sequence is 89% identical between mouse and human [76] highlighting the highly conserved nature of this proteoglycan. The locus-encoding versican (*VCAN*, *CSPG2*) comprises 15 exons, which are arrayed over 90 kb of contiguous genomic DNA. Versican core protein consists of an N-terminal G1 domain, a C-terminal G3 domain, and CS chain-binding regions (Fig. 4.1). The G1 domain is composed of an immunoglobulin (Ig)-like module, followed by two hyaluronan (HA)-binding domains (link modules). The G3 domain of versican consists of two epidermal growth factor (EGF)-like repeats, a carbohydrate recognition (lectin-like, CRD) domain, and a complement binding protein (CBP)-like motif [124]. The expression of versican gene is regulated by a promoter that harbors a typical TATA box. Successful cloning of the gene in man, mouse, cow, and chicken has revealed the existence of at least four splice variants of versican, which differ in the size of the core protein and the number of glycosaminoglycan (GAG) chains. The central, glycosaminoglycan (GAG)-bearing domain of the versican core protein is coded by two large exons, GAG-α and GAG-β, which can be alternately spliced at exon 7 (which codes for the GAG-α region) and exon 8 (which codes for the GAG-β region). When both exons 7 and 8 are present and no splicing occurs, versican V0 isoform is formed. When exon 7 is spliced out, versican V1 is generated. When exon 8 is spliced out, versican V2 is formed. When both exons 7 and 8 are spliced out, versican V3 is formed. Since V3 contains no GAG (CS) chains and is solely composed of the G1 and G3 domains, it cannot be considered a proteoglycan, but it is frequently grouped with proteoglycans and studied as such [117, 118].

Versican is a crucial partner in extracellular matrix (ECM) assembly through key protein-protein or protein-carbohydrate interactions. One of the most studied interactions is between the amino-terminal domains of versican (G1 domain) to HA, mediated through link modules [118]. Versican interacts with diverse ECM components that are important in inflammation, such as TNF-stimulated gene-6 (TSG-6), fibulins and fibrillin, inter-alpha-trypsin inhibitor (IαI), fibronectin, tenascin-R and tenascin-C. Tenascin-R binds to versican at its C-terminal lectin-like domain (CRD) through protein-protein interactions [8]. Versican binds to fibulin-2 and fibrillin-1 through its C-terminal lectin-like domain in a calcium-dependent manner [51, 80]. Fibulin also may serve as a bridge between versican and fibrillin, forming highly ordered multimolecular structures important in the assembly of elastic fibers [117]. Versican also interacts with fibronectin, as well as collagen type I [109, 126]. Moreover, versican G3 domain can form complexes with fibronectin and vascular endothelial growth factor (VEGF). This complex was found to stimulate endothelial cell adhesion, proliferation, and migration. Disrupting the complex through anti-fibronectin antibody reversed G3's enhancing effects on endothelial cell activities [121]. Finally, versican binds to adhesion molecules on the surface of inflammatory leukocytes such as L- and P-selectins through oversulfated sequences [52, 53].

4.2 Versican and Versican Proteolysis in Embryonic Development

Versican has been implicated in cardiovascular morphogenesis, neural crest cell migration, and skeletal development. The ADAMTS protease family includes several versican-degrading members (versicanases) that are active during remodeling of the embryonic provisional matrix, especially during sculpting of versican-rich tissues [75]. Versican is cleaved at specific peptide bonds by ADAMTS proteases, and the proteo-

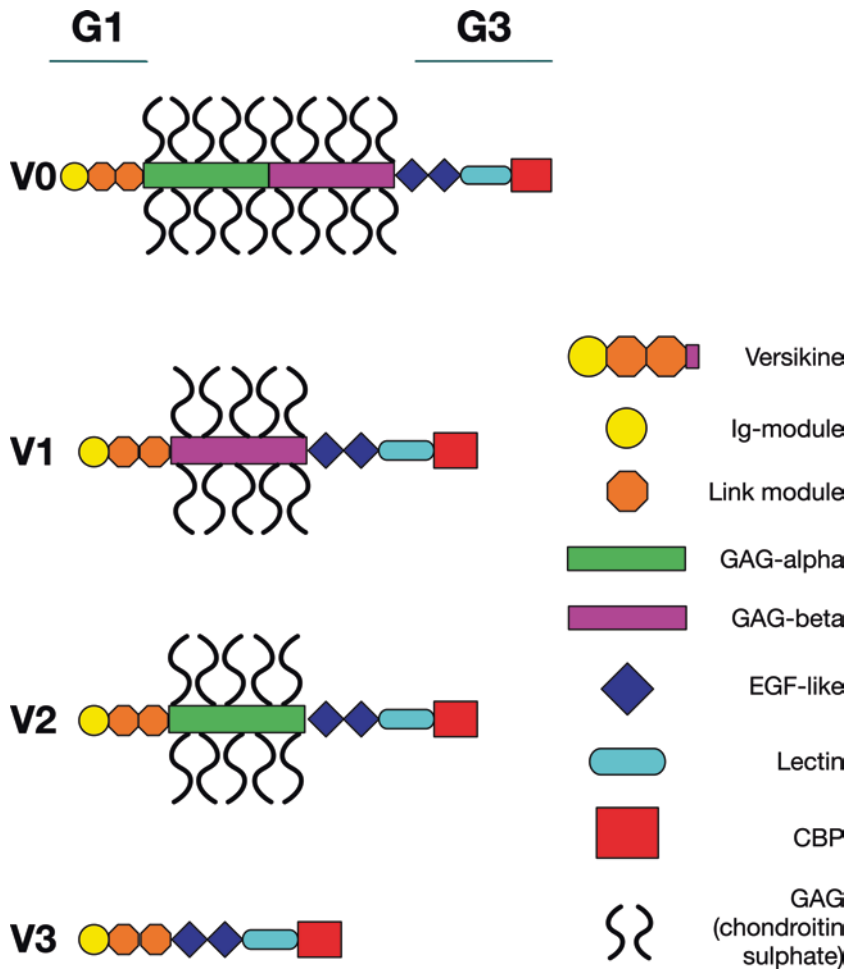

Fig. 4.1 Structure of versican, its isoforms, and its proteolytic product, versikine. Ig immunoglobulin, GAG glycosaminoglycan, EGF epidermal growth factor, CBP complement-binding protein

lytic products are detectable by neo-epitope antibodies. The developmental significance of versican's proteolytic processing has been elucidated at the sites of the most dramatic shaping of the provisional matrix such as interdigital webs, sculpting, redirection and migration of the secondary palate shelves prior to their midline fusion, resorption of cardiac jelly during myocardial compaction, and remodeling of endocardial cushions to form mature heart valve leaflets. Collectively, several studies have illustrated how proteolysis of versican deposited early in the embryo could be a regulator of morphogenetic processes during subsequent development [23, 55, 67, 88].

In cardiac development, versican is essential to the formation of endocardial cushion mesenchyme by epithelial-mesenchymal transformation (EMT). Versican proteolytic fragments generated through the actions of ADAMTS proteases can be detected in the cardiac cushions [56]. Later in development, endocardial cushions are rapidly remodeled to achieve their mature structure, and cleaved versican is broadly distributed around cushion mesenchyme cells. Congenital valve anomalies associated with accumulation of versican were seen in both *Adamts9*$^{+/-}$ mice and *Adamts5*$^{-/-}$ mice and were attributed mostly to subtle developmental alterations in extracellular matrix remodeling or defects in adult homeostasis [55, 57].

Versican proteolysis by ADAMTS9 in vascular endothelium and by ADAMTS20 in palate mesenchyme drives palatal shelf sculpting and extension. Cooperation of ADAMTS9 and ADAMTS20 contributes to secondary palate closure [23]. Reduced sculpting of the shelves and decreased growth were accompanied by accumulation of ECM and reduced cell density, with decreased cell proliferation in palate mesenchyme of the *Adamts9*$^{+/-}$ and *Adamts20*$^{bt/bt}$ mutant mice. Moreover, the palates of these embryos showed a clear reduction of processed versican as evident from reduced anti-DPEAAE staining (a neo-epitope generated by cleavage of V1 versican) [23]. *Vcan* haploinsufficiency in the *Adamts20*$^{bt/bt}$ background also led to cleft palate, demonstrating that versican was a necessary partner of ADAMTS proteases during palate closure, possibly by providing a bioactive fragment, versikine [23].

Versikine, a bioactive N-terminal fragment generated by V1 versican cleavage, is implicated in induction of apoptosis in the context of web regression. Specifically, when Affi-Gel beads were soaked in conditioned medium from HEK293 cells stably overexpressing versikine, they could induce apoptosis in ADAMTS-deficient interdigital tissues [67]. Thus, versican itself and its proteolytic derivative are essential for web regression.

4.3 Versican in Tissue Inflammation and Immunity

Versican is a major component of the inflammatory response cascade. Its production is highly regulated by inflammatory cytokine networks and, in turn, regulates downstream inflammatory mediators to amplify the response [132]. Upon extravasation in the subendothelium, leukocytes encounter ECM structures enriched in versican and HA that act as scaffold for leukocytes having an impact on their cell adhesion and subsequent retention and activation [119]. Versican interacts with receptors on the surface of leukocytes such as P and L selectins and then provides intrinsic signals that influence immune and inflammatory phenotypes [52, 53, 124, 133]. Once bound to the versican-containing ECM, leukocytes degrade the ECM to generate pro-inflammatory fragments, mostly derived from laminin, elastin, and IV collagen that further drive the inflammatory response by increasing monocyte-/macrophage-dependent secretion of proteases and pro-inflammatory cytokines [1, 6, 98, 111]. Versican, which binds to HA, can also bind to CD44 via chondroitin sulfate (CS) GAGs [52], suggesting that both versican and HA may strengthen CD44-dependent interactions and subsequent CD44-dependent signaling in inflammatory cells. On the other hand, versican binding to HA may interfere with the binding of HA to CD44 on immune cells, such as T lymphocytes [26], and attenuate the immune response. Versican proteolysis can also drive new blood vessel formation as part of inflammatory events associated with tissue repair. For instance, injection of an adenoviral vector expressing $VEGF_{164}$ into the skin induces a robust angiogenic response by increasing ADAMTS-1 and versican's proteolytic fragment, versikine [28].

Versican appears to have a role in monocyte adhesion. ECMs that did not support monocyte adhesion were deficient in versican but enriched in HA. In support of this notion, treating a monocyte-attractant ECM with an antibody against the N-terminal region of versican before adding monocytes blocked monocyte adhesion to that ECM [85]. Versican also controls inflammatory cytokine release by myeloid cells. Versican acts as a danger-associated molecular pattern (DAMP) molecule that interacts with Toll-like receptors (TLRs), such as TLR2 on alveolar macrophages, to promote production of inflammatory cytokines, including tumor necrosis factor-α (TNFα), IL-6, and other pro-inflammatory cytokines [31, 38, 114, 120].

A major source of versican production in the inflammatory milieu is macrophages. Versican gene is differentially expressed in M1 macrophages, as opposed to M2 macrophages. Matrix metalloproteinases (MMP) degrade ECM proteins [39, 43, 44]; however, ECM degradation is neither the sole nor predominant function of these enzymes. Versican produced by macro-

phages can form complexes with MMPs [65], such as MMP-9, implying possible roles for versican in controlling the activity of matrix-degrading enzymes. Such activity suggests that versican could assist myeloid cells in shaping their own microenvironment [119]. Versican can also alter the inflammatory milieu through chemokine regulation. Versican expression is elevated in CD14+ monocytes isolated from patients with systemic sclerosis, and this elevated expression is accompanied by increased expression of CCL2 [66]. Earlier studies had also shown that CCL2 binds to versican and impacts inflammation in a model of neuronal inflammation hyperalgesia [12]. In the setting of lung infection, versican and HA are increased in the lung during acute inflammation associated with *E. coli* pneumonia. Bacterial activation of TLR4 led to synthesis of versican which can itself interact with TLR4 to further modulate the inflammatory response [16].

Versican is also a crucial mediator of chronic inflammation. Versican accumulates in chronic lung diseases that involve persistent inflammation such as pulmonary fibrosis, acute respiratory distress syndrome, asthma, and chronic obstructive pulmonary disease [5, 10, 46, 72]. Versican, which is mainly secreted by fibroblasts throughout the airway tree, contributes to airway remodeling in asthma, leading to persistent airway obstruction and subsequent decline in lung function [2]. Altered deposition of proteoglycan in the asthmatic lung seems to vary between asthma phenotypes and severities [77, 84]. Interestingly, fibroblasts isolated from bronchial biopsies from asthmatic patients with the greatest degree of hyperresponsiveness produced larger amounts of versican [116]. Patients with fatal asthma had increased versican content in the internal area of large and small airways compared with controls [18]. Versican is also implicated in chronic obstructive pulmonary disease (COPD), a chronic lung condition characterized by loss of elastic fibers from small airways and alveolar walls. Fibroblasts in distal airways from COPD patients bear modifications in proteoglycan production that may contribute to disease development: there is a higher rate of versican production/accumulation compared to degradation [34]. Versican in the alveolar wall is also negatively correlated to elastin and elastin-binding protein (EBP), a molecular chaperone important in processing of elastin [69]. In versican-rich microenvironment, new formation of elastic fibers is hampered. The association between elastic fiber loss and accumulation of versican suggests that modulation of versican influences elastic fiber deposition [47, 70].

In a seminal study by the Stambas group, versican was implicated in regulation of antigen-specific, adaptive immunity. Accumulation of versican in *Adamts5*-knockout mice, which lack ADAMTS5 versicanase, causes impaired influenza virus clearance and prevents CD8+ T cell egress, leading to compromised antiviral immunity. However, when $Adamts5^{-/-}Vcan^{+/hdf}$ (versican-haploinsufficient) mice were infected with influenza virus, T cell function was restored. The authors showed that V0/V1 versican accumulation impedes migration of CD8+ T cells from draining lymph nodes to the periphery, which is critically important for the establishment of full effector function and eventual clearance of the viral pathogen [68].

4.4 Versican in Cancer

Versican is of central relevance to several hallmarks of cancer [35] and plays important roles in both malignant transformation and tumor progression (Fig. 4.2). 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.

4.4.1 Source of Versican Production in the Tumor Bed

There are at least four major sources of versican production in the tumor bed: the tumor cells, the stromal cells, the tumor-associated myeloid cells, and the tumor-infiltrating lymphoid cells. Versican sources are often context-specific and not necessarily mutually exclusive.

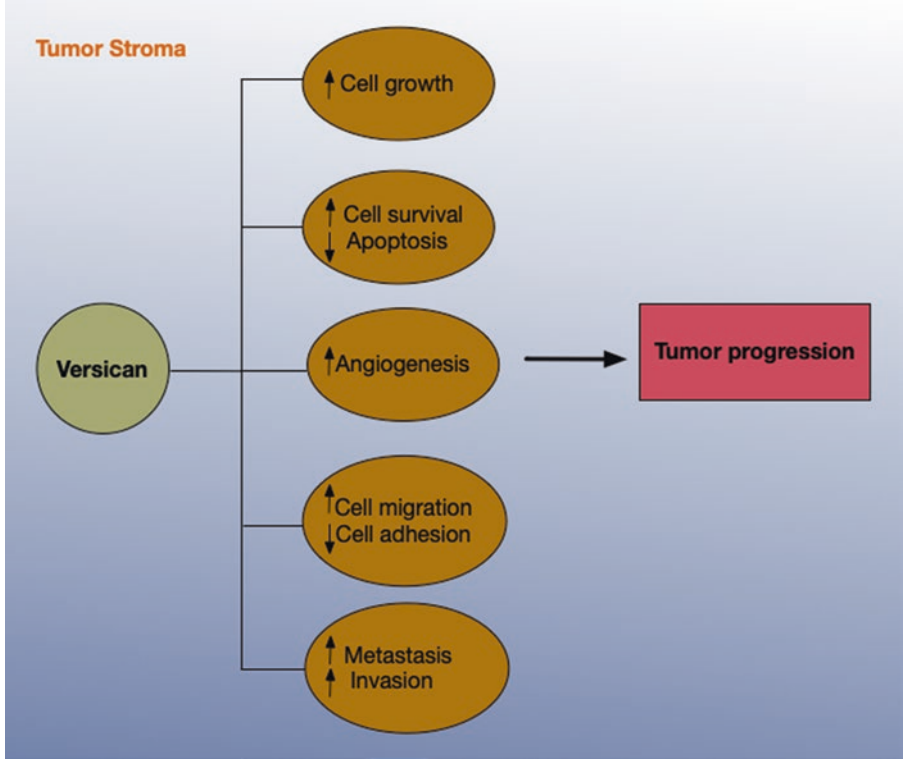

Fig. 4.2 Synopsis of the actions of versican on tumor progression

In lung cancer, versican's main source of secretion is the tumor cell. Versican secretion by the experimental lung cancer model Lewis Lung carcinoma (LLC) is necessary for metastatic spread to the lung, liver, and adrenal gland, a process that depends on TLR2-mediated myeloid cell activation and TNF-α production [58]. Tumor cells show also an elevated expression of versican in ovarian cancer [63], leiomyosarcoma [54], hepatocellular carcinoma [125], colon carcinoma [14], glioma [45], and bladder cancer [95]. Several of these studies find a direct correlation between tumor versican expression and tumor grade.

In other contexts, stromal cells constitute the main source of versican production, such as in breast cancer [17, 59, 89, 105], colon cancer [49], pharyngeal cancer [87], ovarian cancer [129], and prostate cancer [90, 96, 97]. Stromal versican is often accompanied by increased HA in the tumor bed. The increased amounts of versican and associated polysaccharide (HA) expand pericellular matrix volume and as a result distend the ECM [117]. Peritumoral versican expression is induced in stromal cells by factors secreted by carcinoma cells [13, 89, 97]. Versican, which is not expressed in normal breast tissue, gets upregulated with progressive premalignancy and frank malignancy [17]. Strong versican expression was also observed in primary pharyngeal tumors, whereas in metastatic tumors, stromal versican staining in the metastatic site was found to be significantly more intense compared to the primary tumor [87]. TGF-β has been found to induce strong stromal versican expression in breast cancer [112] as well as other types of cancer [81]. Intriguingly, TGF-β can also induce the production of versican by the tumor cells themselves, e.g., in prostate cancer [79]. In some cancers, such as endometrial and cervical cancers, tumor and stromal cells can both be the source of versican production. The combination of tumor and stromal expression of versican correlates with

shortened disease-free survival and overall survival [60].

Myeloid cells are a major source of versican production in the tumor microenvironment in certain cancer types. Studying spontaneous breast cancer murine models, Gao and colleagues showed that $CD11b^{+}Ly6C^{high}$ monocytic cells (but not the tumor cells or other stromal cells) produces versican that subsequently promotes mesenchymal to epithelial transition and metastasis [29]. Likewise, in breast cancer, versican derived from myeloid cells is crucial for tumor metastatic potential [30]. Interestingly, co-culture of myeloid cells with bladder carcinoma cells in vitro results in upregulation of versican in the myeloid cells, suggesting that the source of versican in bladder tumors includes myeloid cells [95]. Finally, in patients with acute myeloid leukemia (AML) post-cord blood stem transplantation, macrophages were the major versican-producing cells in the bone marrow (BM) [100]. Consistent with the latter observation in the hematopoietic context, our group has demonstrated that macrophages are the major source of versican in the bone marrow of patients with multiple myeloma [42].

4.4.2 Role of Versican in Cancer

4.4.2.1 Tumor Cell Proliferation and Self-Renewal

Versican is a crucial mediator of tumor cell proliferation and, in some cases, proliferation of essential tumor-accessory components. Versican enhanced proliferation rate of melanoma cells [109]. The G1 domain of versican is thought to stimulate proliferation by destabilizing cell adhesion [127], while the G3 domain mediates proliferation through two EGF-like motifs, which play a role in stimulating cell growth [22, 130, 131]. The EGF motifs were also shown to mediate breast cancer cell self-renewal [21]. Overexpression of the versican G3 domain enhanced breast cancer self-renewal through EGFR/Akt/GSK-3β signaling and conferred enhanced resistance to chemotherapeutic drugs. Of interest, versican G3-overexpressing tumors not only showed high levels of 4B6, pEGFR, pAKT, and GSK-3β (S9P), all of which were related with tumor invasiveness, but also expressed high levels of tumor stem cell markers Sox2, Sca-1, and ALDH1 [21]. Finally, siRNA against versican isoform V1 decreased tumor cell proliferation in human glioma cells [81].

Versican also regulates the proliferation of crucial tumor-accessory components. For example, platelet-derived growth factor (PDGF) upregulates versican expression in arterial smooth muscle cells and promotes the expansion of the pericellular ECM, which is required for the proliferation and migration of these cells [24, 25, 99].

4.4.2.2 Tumor Cell Survival and Apoptosis

Genetic or epigenetic modifications in tumor apoptotic signaling machinery facilitate tumor cell survival [48]. V1 versican overexpression has been reported to cause either selective apoptotic resistance or selective apoptotic sensitization. This combination of selective apoptotic resistance and sensitivity is often seen in cancer cells. Intriguingly, murine NIH 3T3 fibroblasts overexpressing V1 versican (V1 cells) were shown to have concurrent high resting levels of p53, which confers apoptotic sensitivity and Mdm2, which is a crucial negative regulator of p53 [62]. Expression of the G1 and G3 domains of versican protects cells from apoptosis induced by death receptor ligands or cytotoxic drugs [15]. The G3 domain of versican interacts also with beta-1 (β1) integrin and protects glioma cells against free radical-induced apoptosis [122]. Furthermore, versican protects cells from oxidative stress-induced apoptosis through an enhancement of cell-matrix interactions and increased cell attachment and expression of beta-1 integrin and fibronectin [123]. However, versican has also been implicated in proapoptotic signaling. siRNA-mediated versican knockdown prevented G3-modulated cell apoptosis in human breast cancer cell lines. The somewhat contradictory roles of versican in modulating cancer cell survival and apoptosis underscore the complexity of

apoptosis regulation in tumor development and progression.

4.4.2.3 Tumor Angiogenesis

Angiogenesis is the creation of new blood vessels from the branching of preexisting ones. Tumor neo-angiogenesis provides nascent tumors with adequate oxygen and nutrients. A recent study illustrated the impact of stroma-derived versican in tumor growth and vascularization [7]. The investigators showed that the major source of versican production was the tumor stroma in B16F10 (melanoma) and LLC tumors and compared vasculature density of B16F10 tumors in *Vcan* $^{hdf/+}$ mice (haploinsufficient for versican) and wild-type littermates. A significant reduction of tumor volume as well as capillary formation in the *Vcan* $^{hdf/+}$ mice at 10 days and 13 days post-tumor inoculation compared to wild-type mice was observed [7]. Thus, genetically manipulated reduction of versican attenuates tumor angiogenesis by impairing vascular invasion into the tumor core, at the same time as exerting cell-autonomous growth regulatory effects on tumor cells [7].

In the context of the well-vascularized tumor glioblastoma, versican appears to exert a pro-angiogenic effect. The versican G3 domain enhanced angiogenesis both in vitro and in vivo. G3-expressing cells and tumors formed by these cells expressed very high levels of fibronectin and VEGF. Furthermore, the G3 domain directly interacted with fibronectin and formed a complex together with VEGF. This complex promoted angiogenesis-associated activities in endothelial cells, and its disruption inhibited these processes [134]. Consistent with the observation that G3 domain binds fibronectin, the V2 versican isoform promoted extensive vasculature formation by upregulating and binding to fibronectin [128]. Silencing fibronectin expression by siRNA abolished V2 versican's effect in enhancing vascular tube-like structure formation [128].

Pericytes also participate in normal and tumoral angiogenesis. Type 2 pericytes in particular have been shown to possess angiogenic potential and play an important role in stabilizing blood vessels in the microvasculature [11]. RT-PCR has demonstrated abundant versican message in cultured pericytes in vitro [20]. Thus, type 2 pericyte-derived versican might participate in new blood vessel formation during tumor angiogenesis.

4.4.2.4 Tumor Cell Motility and Local Invasion

Versican is associated with local tumor invasion [94]. Elevated levels of versican in the pericellular stroma is an indicator for disease relapse following surgery for clinically localized prostate cancer [90–92] and breast cancer [89, 104]. Versican has been shown to impede cell adhesion to ECM substratum, and this activity is attributed to the G1 domain: for example, versican enhances locomotion and reduces cell adhesion of astrocytoma cells through the binding of its G1 domain to hyaluronan and link protein [3, 127]. More recent studies have demonstrated that purified versican from cultured human prostatic fibroblasts inhibited adhesion of prostate cancer cells to a fibronectin substratum in vitro, highlighting the key anti-adhesive regulatory role of versican in prostate cancer [96]. Moreover, the formation of an HA/versican pericellular matrix promoted prostate cancer motility in Boyden chamber motility assays using fibronectin as a chemoattractant. Thus, prostate cancer cells in vitro have the ability to recruit versican produced by prostatic stromal cells to promote their motility [93]. These findings suggest that the formation of a pericellular sheath in vivo by prostate cancer cells utilizing versican laid down by prostate stromal cells may contribute to the development of locally invasive disease.

Silencing versican by a specific siRNA against isoform V1, but not V3, significantly decreased migration in human glioma cell lines and primary cultures in vitro [81]. Induction of stromal versican expression correlated with higher tumor grade and invasiveness in carcinomas and was associated with tumor progression [61, 101]. Elevated versican expression in tumor-associated stroma resulted in reduced numbers of intraepithelial CD8-positive T cells and enhanced cancer cell local invasion in cervical cancer [32],

whereas increased expression of CD44 and versican was associated with loss of expression of both progesterone receptor (PR) and E-cadherin [36]. Moreover, in vitro silencing of V0/V1 versican caused increased adhesion to type I collagen, laminin, and fibronectin. This was coupled with reduced cell migration in both wound-healing assays and transwell chamber assays [37].

Ovarian cancer cells have the ability to recruit stromal ECM components such as versican and HA to form a pericellular matrix which in turn promotes ovarian cancer cell motility and invasion. By using modified chemotaxis assays, treatment with versican-rich conditioned media in vitro promoted ovarian cancer cell motility and invasion and enhanced their migratory potential. However, HA oligomers (six to ten disaccharides) were able to significantly block formation of pericellular matrix by ovarian cells, as well as the increased motility and invasion induced by recombinant versican. Thus, HA oligomers could be a promising adjuvant treatment tool, administered intraperitoneally together with chemotherapy drugs to ovarian cancer patients following debulking surgery, to inhibit residual ovarian cancer cells from repopulating and invading peritoneal sites [115].

4.4.2.5 Tumor Systemic Metastasis

Versican accumulation has been associated with tumor metastasis to distant organs. Versican expression was upregulated in patients with clear cell renal carcinoma (ccRCC), and this upregulation was associated with poor prognosis and high rate of metastasis [71]. In a study of 84 matched sporadic ccRCC and normal renal tissues, patients with high versican expression had a significantly worse 5-year OS (overall survival) (p-value = 0.007) and a higher rate of systemic metastasis than those with low versican expression (p-value = 0.0139). Mechanistically, versican promoted ccRCC cell migration and invasion via MMP7 and CXCR4 [71]. In breast cancer, versican derived from CD11b+ Ly6C^{high} myeloid cells is critical in promoting metastasis to the lung in a TGF-β-dependent manner [29].

Karin and colleagues showed that versican binds TLR2 and its co-receptors TLR6 and CD14 on myeloid cells in a highly metastatic lung cancer model (Lewis Lung carcinoma, LLC). Upon activating TLR2-TLR6 complexes and inducing TNF-α secretion by myeloid cells, versican strongly enhanced LLC metastatic growth. TLR2 was absolutely necessary for metastatic growth, since no metastatic enhancement was seen in *Tlr2*$^{-/-}$ mice [58]. On the other hand, TNF-α is one of the major pro-metastatic factors produced by host myeloid cells. TNF-α can suppress the apoptosis of cancer cells and stimulate their proliferation through NF-κB activation [64]. In addition, by increasing vascular permeability [110], TNF-α can enhance recruitment of leukocytes as well as intravasation and extravasation of cancer cells. Since TLR2 is absolutely necessary for versican to exert its metastasis-enhancing abilities and TNF-α is a product of activated myeloid cells after interacting with versican, either or both of these targets could provide a useful point for anti-metastatic intervention.

4.4.2.6 Interplay Between Versican and Immune Cells in the Tumor Microenvironment (TME)

Dendritic cells (DCs) play a crucial role in the regulation of the balance between CD8+ T cell immunity vs. tolerance to tumor antigens. Cross-priming, a process which DCs activate CD8+ T cells by cross-presenting exogenous antigens, plays a critical role in generating antitumor CD8+ T cell immunity [102]. However, DC-mediated cross-presentation of tumor antigens in tumor-bearing hosts often induces T cell tolerance instead of immunity. There is accumulated evidence that the TME modulates tumor-infiltrating DCs and other antigen-presenting cells such as macrophages, leading to impairment of their function in initiating potent antitumor immunity and even promotion of tumor progression [27, 78].

Importantly, tumor-derived versican leads to DC dysfunction through TLR2 activation. TLR2 ligation not only stimulated secretion of autocrine IL-10 and IL-6 but also led to sustained

elevation of the cell-surface receptors for these cytokines, which decreased the threshold concentration required to activate STAT3. This amplification loop reprogrammed DCs to produce high amounts of IL-10 rather than IL-12 and IL-1β when stimulated with LPS, a classic pro-inflammatory stimulus. Thus, versican impeded immunogenic DC activation and conceivably downstream Th1 and cytotoxic lymphocyte (CTL) differentiation [107, 108]. In multiple myeloma, versican is abundantly expressed and processed in the bone marrow [42]. We have previously proposed a model in which versican activates myeloma-associated monocytes/macrophages through TLR2/TLR6 signaling, thus generating trophic IL-1β and IL-6 induction [42]. The significance of versican pathway for human myeloma is further underscored by two recent reports: first, the high-resolution analysis of the human immune microenvironment in MM showing that myeloid-derived versican transcription was very strongly associated with MM progression and loss of protective T cell stemlike (Tcf1+) memory in favor of dysfunctional/exhausted T effectors [9] and, second, the demonstration that immunosuppressive macrophages (expressing versican, ENTPD1, and STAB1) were associated with persistence of minimal residual disease post-autologous stem cell transplant for myeloma, thus promoting relapse [4].

In the setting of mesothelioma, tumor-derived versican promotes tumor progression by shaping a tumor-conducive inflammatory milieu, mainly by blunting macrophage antitumor activities [83]. Mice harboring versican-deficient tumors presented fewer tumor/pleural macrophages and neutrophils and fewer pleural T-regulatory cells, compared to the control animals. Moreover, macrophages co-cultured with versican-deficient mesothelioma cells were polarized toward M1 antitumor phenotype and demonstrated increased tumor cell phagocytic capacity, compared to macrophages co-cultured with control tumor cells [83]. Overall, the critical cross-talk created by versican among different types of immune cells leads to an immunosuppressive TME that promotes cancer progression and metastasis.

4.5 Versican Proteolysis and Versican-Derived Matrikines in Inflammation and Cancer

Regulated proteolysis of versican by ADAMTS proteases at the Glu^{441}-Ala^{442} bond of the V1 isoform is associated with robust CD8+ infiltration in MM BM [19, 41] as well as solid tumors [40]. This proteolytic event is predicted to release a 441-aa-long N-terminal fragment, *versikine* (Figs. 4.1 and 4.3). We previously showed that versikine induces IRF8-dependent interferon-stimulated genes [41]. Versikine promotes IRF8-dependent Batf3-DC [33, 73] generation from Flt3L-mobilized BM in vitro [40] and Batf3-DC density in vivo (our unpublished data, see next paragraph). Enhanced Batf3-DC at the tumor site could provide a conceptual link between versikine and CD8+ infiltration because Batf3-DC, in addition to their role in cross-presenting tumor antigen for priming CD8+ effectors, orchestrate chemokine networks that enhance intratumoral CD8+ infiltration [102].

In order to investigate the effects of versikine in DC intratumoral composition in vivo, we utilized a transplantable Ras-driven multiple myeloma model (VQ) as well as transplantable solid tumor models (LLC and 4T1 mammary carcinoma). Tumor cells were stably engineered to secrete versikine vs. empty vector control, and they were then implanted into syngeneic recipients. Versikine influenced the DC milieu in the tumor bed by increasing the density of intratumoral Batf3-DC and depleting the cDC2 (CD11c+ CD11b+) subset. Our findings highlight an unappreciated facet of immune regulation of the tumor microenvironment through matrix proteolytic fragments ("matrikines") (Papadas et al., unpublished data accepted for presentation at the American Society of Hematology, 2019). Interestingly intense versican proteolysis in the bone marrow of patients who underwent autologous stem cell transplantation for myeloma correlated with adverse outcomes despite robust CD8+ infiltration [19]. Versican accumulation in this context is likely to produce an intensely immunosuppressive micro-

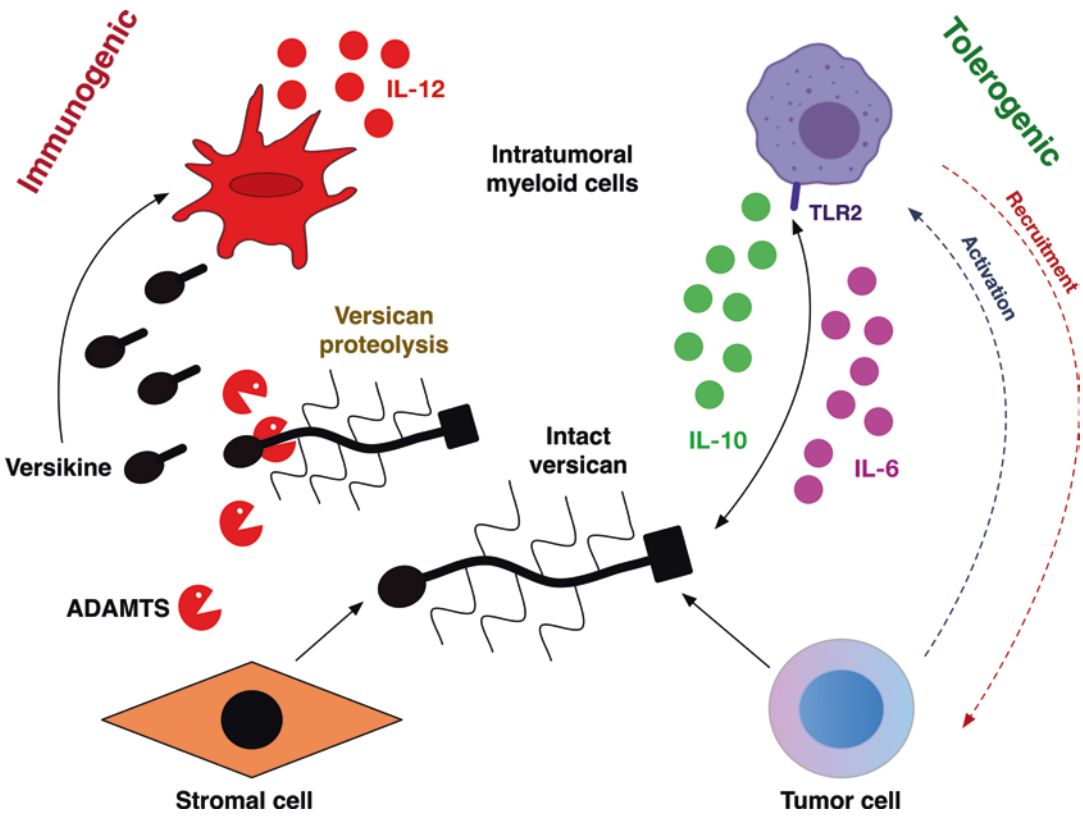

Fig. 4.3 Complex coordinated actions of versican and its proteolytic product, versikine, in the tumor microenvironment

environment that leads to effector dysfunction and impaired antitumor responses, despite the potential moderating effects of versikine signaling.

4.6 Versican: Potential for Cancer Biomarker Discovery

Versican expression correlates with poor prognosis, disease progression, metastasis, and drug resistance in cancer. The prognostic role of versican expression is tissue-specific. Versican is considered an independent and adverse prognostic marker in oral squamous cell cancer: high stromal versican expression correlates with both increased risk for disease recurrence and shortened survival for this cancer [86]. On the other hand, versican expression in the primary tumor is not an independent prognostic factor in pharyngeal squamous cell carcinoma (PSCC), although versican is more strongly expressed in the stroma of local metastases and in the earlier stages of disease in PSCC [87]. In hepatocellular carcinoma (HCC), versican expression correlates with poor prognosis, increased intratumoral macrophage infiltration, poor tumor differentiation, and a higher tumor-grade metastasis (TNM stage) [106, 125]. In colon cancer, versican expression by RT-PCR is significantly upregulated (threefold) compared to normal tissues [103]. High stromal versican expression is associated with reduced 5-year survival rates of ovarian cancer patients (44% versus 32%) [113]. Versican is upregulated in chemoresistant ovarian cancer compared to chemosensitive ovarian cancer [82]. In multiple myeloma, we recently presented the first set of data ascribing prognostic significance to the

versican proteolysis immunoregulatory pathway. We observed the somewhat paradoxical association between intense versican proteolysis and high CD8+ T cell infiltration with poor post-autologous stem cell transplant (ASCT) survival. Patients with low versican proteolysis compared to moderate/high versican proteolysis had better 2-year PFS (72% vs. 29%, $p = 0.018$) and 2-year OS (83% vs. 35%, $p = 0.006$) [19]. Thus, versican expression and/or proteolysis detection may generate powerful prognostic and in certain cases predictive (e.g., association of versican proteolysis with CD8+ T cell infiltration) cancer biomarkers [74].

4.7 Concluding Remarks and Future Directions

The versatile roles of versican in regulating cell behavior are critical in tumor development and progression. Key pathogenetic processes such as tumor proliferation, tumor cell adhesion, tumor cell survival, and apoptosis have been found to be regulated by versican. Versican supports tumor vasculature formation, tissue invasion, metastasis, and chemoresistance. Versican could act either in a cell-autonomous fashion, by having an impact on the cancer cell phenotype (proliferation, migration, and metastasis), or in non-cell-autonomous manners by influencing the tumor microenvironment, with particular bearing on tumor-associated immune cells. Versican proteolysis generates matrikines that engage in cross-talk with signaling emanating from their parent macromolecule, intact versican. Our work on multiple myeloma and relevant studies on solid tumors from other groups have provided a rationale for testing versican and versican proteolysis as potential biomarkers to predict patient outcomes. A fuller understanding of the wide array of regulatory mechanisms controlled by versican and versican-derived matrikines will strengthen the rational basis for further clinical development of tumor matrix-targeting therapies.

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