REVIEW ARTICLE

Structure, Function and Regulation of Versican: The Most Abundant Type of Proteoglycan in the Extracellular Matrix

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Abstract- One of the main members of the large aggregating proteoglycans (PGs) family is versican which is able to bind to hyaluronate. Versican is a chondroitin sulfate proteoglycan and is a key ingredient of the extracellular matrix. Due to its widespread expression in the body, versican is involved in cell adhesion, proliferation and migration. Induced expression of versican is often observed in tissues such as breast, brain, ovary, gastrointestinal tract, prostate, and melanoma. In addition, versican has important role in development. For example, versican conducts the embryonic cell migration which is essential in the formation of the heart and outlining the path for neural crest cell migration. Several studies in the past decade up to now have shown that versican produced by mononuclear cells has an important role in wound healing and blood vessel formation and suggested that it promotes tumorigenesis and angiogenesis. In this mini-review, we summarise and discuss the role of versican in healthy and pathological tissues and suggest the possible function of transcription factors and signalling pathway in regulation of versican.

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Keywords: Extracellular matrix; Proteoglycans; Versican

Introduction

The extracellular matrix (ECM) which provides structural support for organs and tissues (1) is also forming the basement membranes for the cell layers (2) and cell migration (3). ECM is composed of collagens and elastic fibres which are embedded in a viscoelastic gel that comprises proteoglycans (*e.g.* versican and hyaluronan), glycoproteins and water (4,5). ECM forms a complex, three-dimensional network among the cells of different tissues in an organ-specific manner (6) and plays vital roles in the differentiation, proliferation and survival of cells (7-9).

Proteoglycans are the main components of ECM, and are characterised by a protein portion (core protein) and one or more unbranched, long and negatively charged polysaccharide chains called glycosaminoglycans (GAG) which are covalently attached to the core protein (10,11). Depending upon the nature of the GAG chains, proteoglycans can be categorised as heparan sulphate proteoglycans, chondroitin sulphate proteoglycans (CSPGs) and dermatan sulphate proteoglycans, or keratan sulphate proteoglycans (12,13). Of these, the CSPGs such as versican are the most abundant type of proteoglycan in the ECM of mammalian tissues (14,15).

Versican structure

Versican is a large chondroitin sulphate proteoglycan which is a major component of the ECM (16). Versican is transcribed from a single gene which is localized on chromosome 5q 12–14 in the human genome and extends over 90 kb (17) which is divided into 15 exons which range in size from 76 to 5262 bp (16). The alternative mRNA-splicing of these exons gives rise to four different versican isoforms which are distinguished by different core-middle regions (18). Versican is comprised of three domains. The amino terminal G1 domain interacts with a GAG called hyaluronan present in the extracellular matrix (14). The carboxyl terminal domain of versican is called the G3 domain and it contains a C-type lectin binding domain, two epidermal growth factor repeats and a complement regulatory

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region. The versican core protein contains the GAG attachment region and the chondroitin sulphate chains extend from this region of the protein (Figure 1) (14,19,20).

Versican isoforms

The central area of versican is encoded by two exons that specify chondroitin sulphate attachment regions (14). RNA splicing of these two exons generates four isoforms of versican named V0, V1, V2 and V3 core protein molecular weight of about 370 kDa, 263 kDa, 180 kDa, and 74 kDa, respectively (18). V0, the largest versican isoform, is encoded by exons 7 and 8 and contains the GAG-α and β domains. The V1 isoform contains GAG-β attachment domain which is encoded by exon 8 (lacking exon 7) whereas the V2 isoform contains a GAG-α domain which is encoded by exon 7 (lacking exon 8) (22). V3 does not include either exon 7 or 8 and consequently has no GAG attachment sites (Figure 2) (19,22).

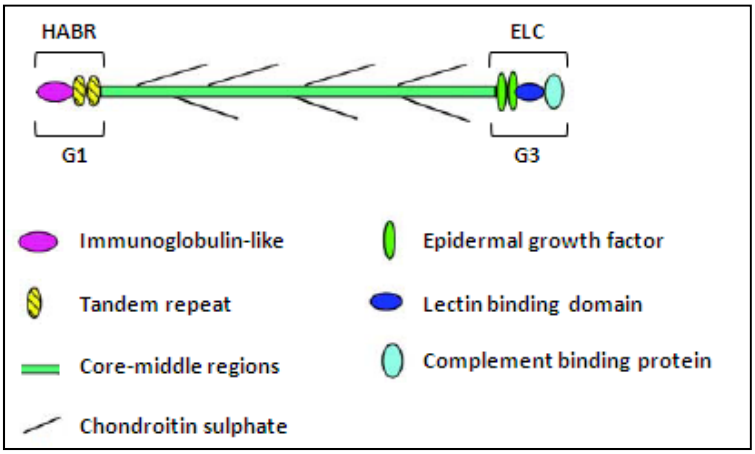

Figure 1. Schematic model of versican structure. Versican contains globular domains at the amino terminus (G1) and carboxyl terminus (G3). The G1 domain is composed of an immunoglobulin-like motif, followed by two proteoglycan tandem repeats which bind hyaluronan (HABR; hyaluronan binding region). The G3 domain contains two epidermal growth factor-like repeats, a carbohydrate recognition domain (a lectin-binding domain) and complement binding protein (ELC). The core-middle region of versican contains GAG attachment regions that are encoded by exons 7 and 8 which give rise to four different versican isoforms. GAG chondroitin sulphate chains extend from the core protein (21).

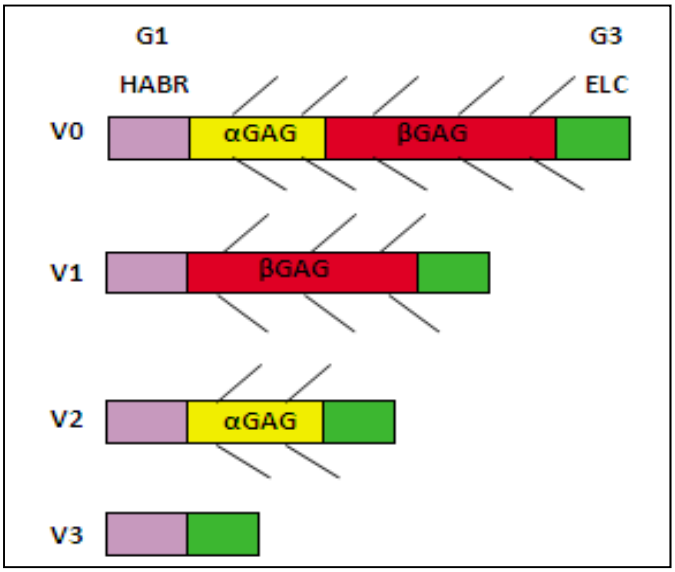

Figure 2. Cartoon of versican isoforms generated by alternative splicing of the mRNA transcript. Different colours show specific domains in the gene. G1 and G3 are shown at the amino and carboxyl termini respectively. Purple = hyaluronan binding region (HABR); yellow = α GAG exon; red = β GAG exon and green = epidermal growth factor repeats (E), a lectin binding domain (L) and a complement regulatory region (C). The glycosaminoglycan chondroitin sulphate chains are shown as (/)

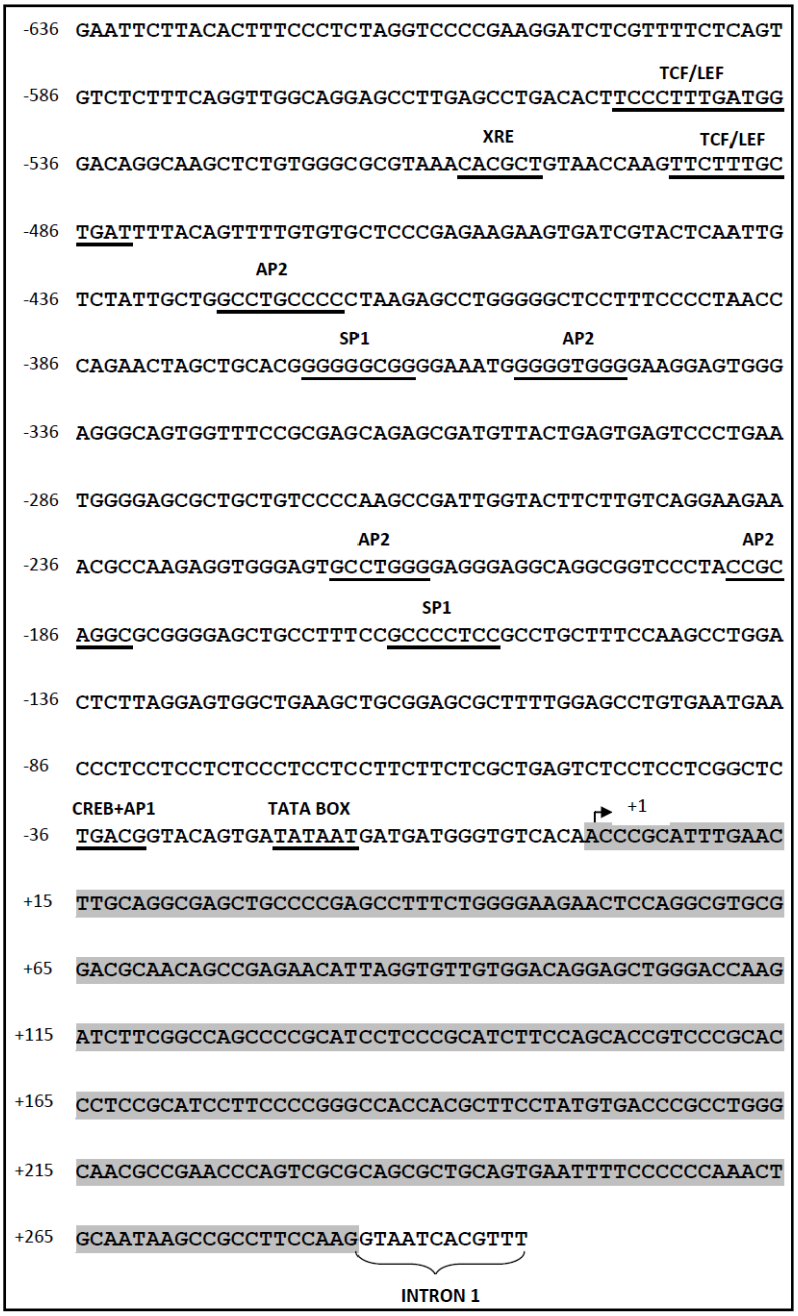

Figure 3. Sequence analysis of versican gene promoter and exon 1. Naso *et al*., revealed a typical TATA box located approximately 16 bp upstream of the transcription start site and binding sites for a number of transcription regulatory factors including CREB at position -36 bp, SP-1 at position -370 bp, XRE at position -509 bp and AP-2 at positions -426 bp, -355 bp, -218 bp and -190bp respectively (16). Rahmani *et al.,* showed two binding sites for TCF/LEF at positions -546 bp and -492 bp respectively (29). Also further investigation by Domenzain *et al.,* revealed one binding site for SP-1 at position -166 bp and one binding site for AP-1 at position -36 bp. Positions of all transcription factors are relative to the transcription start site (30). The sequence of exon 1 is shaded. CREB; cAMP response element-binding, AP-1& 2; (activator protein 1&2), XRE; xenobiotic responsive element, TCF/LEF; T-cell factor/lymphoid enhancer factor.

An interesting study on human adult tissues showed that all four versican isoforms are transcribed in more than 50% of tissues, although, intriguingly, only the V1 isoform is expressed in liver and spleen (23). Another study suggested that V1 versican enhances cell proliferation and protects mouse fibroblast cell lines

from apoptosis whereas the V2 isoform exhibits opposite activities by inhibiting cell proliferation (24).

In addition, it has been reported that V0 and V1 are the predominantly expressed isoforms in tumours suggesting that these isoforms are mainly involved in tumour development (25,26). In contrast, over-expression of V3 (the smallest versican isoform which consists of only the G1 and G3 domains) markedly reduced cell growth in melanoma cancer cells, suggesting a role for V3 versican as an inhibitor of tumour growth (27,28).

Versican promoter

The transcription start site for the versican gene was first identified by Naso *et al.,* in 1994 (16). This study reported that the versican promoter contains a typical TATA box located approximately 16 base pairs upstream of the transcription start site (Figure 3). Transient transfection of a reporter construct driven by an 876-bp (-632/+240) fragment of the versican promoter showed significant expression in HeLa cells and IMR-90 embryonic lung fibroblasts. Furthermore, deletion constructs of the 876-bp confirmed that the human versican 5'-flanking sequence contains promoter, enhancer and repressor elements which are able to drive the expression of the versican reporter gene in different cells (14). In addition, sequence analysis has revealed potential binding sites for several transcription factors in the 876 bp versican promoter region including CREB (14), T-cell factor/ lymphoid enhancer-binding factor (TCF/LEF) (29), AP-1 and SP1 (30) (Figure 3).

Versican function

Versican is a main component of the ECM where its hygroscopic properties create a loose and hydrated matrix which is necessary to support key events in development (31,32). Versican is found in a variety of tissues including the brain (33) and skin (34). Increased expression of versican is also observed in sites of tissue injury (35) and in cancers including breast (36), ovarian (37), gastrointestinal tract (38), prostate (39), brain (40), cervical (41) and melanoma (42). Several reports have also highlighted the role of versican in wound healing (23), angiogenesis, tumour growth (43) and in vascular diseases, especially atherosclerosis (44,45). It has been demonstrated that versican binds low-density lipoprotein (LDL) particles and it is believed that accumulation of versican in blood vessels promotes extracellular lipoprotein retention, suggesting roles in lipid accumulation, inflammation and thrombosis (46,47). Due to versican's structural composition and its widespread expression in the body it is able to regulate cell adhesion and survival, cell proliferation, cell migration and ECM assembly (48) that the key studies in these areas are reviewed below.

Cell adhesion

Early studies reported that most chondroitin sulphate proteoglycans may be considered as anti-adhesion molecules for the regulation of cell adhesion to the substratum, which is essential for various cell and tissue functions (49,50). Different studies presented evidence suggesting that this inhibitory activity could be to due to the G1 domain of versican (49,51). They showed that selective exclusion of versican from podosomes of cultured human osteosarcoma cells suppresses the malignant cell-adhesive phenotype, suggesting that versican can act as an anti-adhesive molecule (52). However there is evidence that the carboxy-terminal domain of versican interacts with the β1 integrin of brain tumour cells leading to the activation of focal adhesion kinase (FAK), promoting cell adhesion and protecting the cell from apoptosis (53,54).

Interaction of versican with selectins and chemokines has been studied. It has been shown that versican binds to selectins, adhesion molecules on the surface of activated endothelial cells, through its chondroitin sulphate chains (55). In addition, versican has been shown to bind secondary lymphoid tissue chemokine (SLC) through chondroitin sulphate chains and this binding tends to down-regulate chemokine function for recruitment of lymphocytes (56). Taken together the data suggest that versican, which is induced in inflammatory conditions such as arthritis (57), asthma and lung disease (58,59), may regulate inflammation by regulating interaction with selectins and chemokines (56).

Cell proliferation

Abundant expression of versican in fast growing tissues and cells suggested a key role for versican in cell proliferation (60,61). For example high expression of versican is detected in the loose connective tissue of various organs including the central and peripheral nervous system (60), blood vessels (62), dermis and in the proliferative zone of the epidermis (34).

Versican is also involved in the proliferation of smooth muscle cells (SMC) (63,64). Several studies have reported proteins such as platelet-derived growth factor (PDGF) and transforming growth factor-β 1 (TFG-β1) increase versican synthesis in arterial smooth muscle cells (ASMCs) (63,65). It

was demonstrated that increases in versican and the associated protein hyaluronan in response to PDGF and TFG-β1 cause increases in the pericellular matrix of the cells and expansion of the ECM that is required for the proliferation and migration of these cells (66). In addition it was shown that proliferation of ASMCs treated with PDGF is blocked by inhibition of the formation of versican-hyaluronan complex which serves as an important mechanism for controlling cell shape and cell division (67).

Other studies have suggested a role for versican in cell proliferation through its two epidermal growth factor sequences in the G3 domain of the molecule (68, 69). These studies showed that expression of the G3 domain of versican promotes proliferation in NIH3T3 fibroblasts cells whereas this effect can be inhibited by removing EGF motifs in the versican G3 domain (68). These results suggested that the EGF-like motifs in the versican G3 domain may promote cell proliferation through direct or indirect interaction with the EGF receptor (EGFR) (70).

Cell migration

Controversial studies have demonstrated that versican is widely expressed at both mRNA and protein level in neural crest pathways and influences neural cell migration (71) whereas a number of other studies have shown that versican prevents migration of these cells (35,72). These contradictory findings are believed to be due to different versican isoforms which differ in the core-middle region (73). Some studies have investigated the role of versican in the nervous system and axonal outgrowth (74-76). These studies showed that chondroitin sulphate (CS) chains of versican isotype V2 are involved in inhibiting axonal outgrowth and migration of the mature nervous system. The role of versican in axonal migration was investigated by Asher *et al.*, in 2002 (77) who showed up-regulation of versican following central nervous system (CNS) injury and suggested these changes in versican regulation are associated with the failure of nerves to regenerate.

As mentioned earlier, the G3 domain of versican can interact with integrin β1 which is able to form clusters with EGF receptors (78). Growing evidence indicates that interaction of integrins with EGF receptors induces down-stream signal to extracellular regulated kinase (ERK) which is crucial in regulating a range of cell activities, such as migration (79-81). Also, the role of versican in the migration of embryonic cells in the development of the heart has been studied (82, 83). It has been reported that versican mRNA and protein is strongly expressed during the development of mouse heart suggesting a key role for versican in cardiac development (82).

Furthermore other studies have reported that the G3 domain of versican directly interacts with fibronectin, another extracellular matrix glycoprotein (84) and showed that formation of a complex of versican G3 domain and fibronectin with VEGF can enhance endothelial cell migration which this process was reversed by removal of the complex (43). This study and other investigation by Wijelath *et al.*, in 2002 (85) indicated that expression of versican G3 enhanced brain tumour growth, suggesting the role of versican G3 fragment on promoting angiogenesis and tumour growth that it suggests targeting versican G3 fragments may help to develop a new approach for anticancer and anti-angiogenic therapies.

Extracellular matrix assembly

Versican interacts with different ECM molecules and has been reported to have an important role during ECM assembly (21). Possibly the best known is a specific interaction between the G1 domain of versican and hyaluronan (86). Hyaluronan is a large polysaccharide in the ECM and is able to create a lattice structure which may regulate cell adhesion and migration (87). Versican binds hyaluronan and this binding requires the double tandem repeat present in the G1 domain of versican (88,89).

Versican regulation

Signal transduction pathways

The signalling pathways which modulate versican synthesis are not fully understood although studies on the intracellular pathways have reported that PDGF-stimulated versican expression in arterial smooth muscle cells (SMC) is regulated by endogenous tyrosine kinase activity of the PDGF receptor which up-regulates versican synthesis at both mRNA and protein levels in vascular smooth muscle cells (63,90). In addition, another study has suggested the role of protein kinase C (PKC) and ERK in the PDGF stimulated versican gene expression in non-human ASMC (91).

An interesting study by Rahmani *et al.,* in 2005 (29) demonstrated the role of the PI3-K/ PKB (Protein kinase B) pathway in versican expression in SMC. They suggested that phosphorylation and inactivation of glycogen synthase kinase-3β (GSK-3β), a downstream

effector of PI3K/PKB (92), leads to activation and nuclear accumulation of β-catenin which binds to the TCF/LEF transcription factors in the versican promoter and then increases versican transcription in SMC (Figure 4) (29). Further investigation by this group using a specific inhibitor of the PI3-Kinase pathway, LY294002, showed inhibited activation of downstream PKB and resulted in significant inhibition of versican promoter reporter activity and versican mRNA expression in SMC. A similar mechanism has been observed for other genes such as MMP-7 (93) and VEGF (94), which have also been shown to be targeted by PI3-K/PKB and up-regulated by formation of a β-catenin TCF/LEF complex.

Transcription factors

Analysis of the versican promoter by Rahmani *et al*., in 2005 (29) revealed two putative binding sites for TCF/LEF transcriptions factors at positions -546 and -492 bp relative to the transcription start site (Figure 3). They showed that site-directed mutagenesis of the TCF sites in the versican promoter markedly diminished reporter luciferase activity in SMC. Furthermore, electrophoretic mobility shift assay (EMSA) and supershift assays revealed that the β-catenin/ TCF transcription factor complex is essential for versican expression in SMC (29).

A recent study by Domenzain *et al.,* identified several other transcriptional regulatory elements including AP1, SP1 and AP2 on a 620 bp (-618/+2 relative to the transcriptional start site) in a proximal versican promoter reporter construct (30). This study demonstrated that mutagenesis of the AP-1 site at position -36 bp completely abolished versican promoter activity in human melanoma cell lines. Also further investigation by EMSA confirmed the importance of the AP-1 binding site for versican promoter transcription in these cell lines. In addition, versican promoter activity in a TCF/LEF mutated construct was reduced by half, suggesting that versican expression is also up-regulated via the β-catenin/TCF pathway in human melanoma cell lines (30).

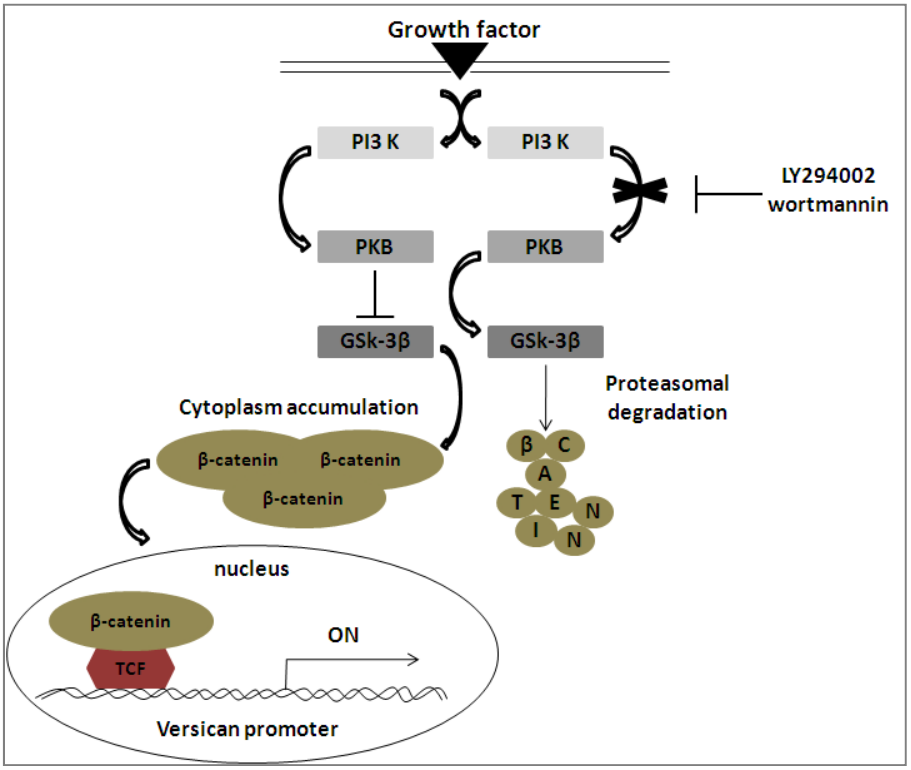

Figure 4. Schematic model of versican promoter regulation via PI3/PKB signalling and β-catenin TCF transcription factor complex suggested by Rahmani *et al*. (29). Activation of PI3K signalling by growth factors leads to phosphorylation and inactivation of GSK-3β which results in β-catenin cytoplasmic accumulation and subsequent translocation to the nucleus. Nuclear accumulation of β-catenin leads to complex formation with TCF/LEF transcription factors and transactivation of TCF/LEF target genes. Specific inhibitors of the PI3K signalling pathway such as LY294002 and wortmannin lead to activation of GSK-3β and subsequently to β-catenin degradation.

Yoon *et al.,* investigated the role of the transcription factor p53 in versican gene expression in a broad range of human carcinoma cell lines (95). P53 is a transcription factor involved in important cellular processes such as cell cycle checkpoint regulation, DNA damage and apoptosis (96,97). Oligonucleotide-array gene expression analysis of human carcinoma cell lines by Yoon *et al.,* demonstrated high expression of the versican gene in wild type p53 (p53 +/+) cells but lower expression in p53 -/- cells, suggesting that versican is a direct target of p53. Further investigation using wild type p53 over-expression in p53-null cells transfected with versican promoter reporter constructs showed 200-fold increases in luciferase activity in comparison with the control plasmid. EMSA and super-shift assays confirmed the interaction of p53 protein and the versican p53 binding site (95). In conclusion, in the present review, I provided evidence about versican structure, regulation and its role in normal tissue and tumour growth. In addition, this review discussed the function of versican as a key factor in inflammation through interactions with adhesion molecules on the surfaces of inflammatory leukocytes and interactions with chemokines that are involved in recruiting inflammatory cells. A better understanding of how versican is regulated will hopefully be helpful for the development of future therapies for a range of different disease such as vascular disorders, including atherosclerosis, where versican accumulation plays a key role.

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