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Published in:
Glycobiology

DOI:
[10.1093/glycob/cwu120](https://doi.org/10.1093/glycob/cwu120)

2015

[Link to publication](#)

Citation for published version (APA):

Andersson Sjöland, A., Hallgren, O., Rolandsson Enes, S., Weitoft, M., Tykesson, E., Larsson Callerfelt, A.-K., Rydell-Törmänen, K., Bjerner, L., Malmström, A., Karlsson, J. C., & Westergren-Thorsson, G. (2015). Versican in inflammation and tissue remodelling: the impact on lung disorders. *Glycobiology*, 25(3), 243-251.
<https://doi.org/10.1093/glycob/cwu120>

Total number of authors:
11

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Versican in inflammation and tissue remodelling: the impact on lung disorders

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Running title: Versican in inflammation and tissue remodelling

Key words: extracellular matrix / lung disorders / remodelling / versican

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Abstract

Versican is a proteoglycan that has many different roles in tissue homeostasis and inflammation. The biochemical structure is comprised of four different types of the core protein with attached glycosaminoglycans that can be sulphated to various extents and has the capacity to regulate differentiation of different cell types, migration, cell adhesion, proliferation, tissue stabilization and inflammation. Versican's regulatory properties are of importance during both homeostasis and changes that lead to disease progression. The glycosaminoglycans that are attached to the core protein are of the chondroitin sulfate/dermatan sulfate type and are known to be important in inflammation through interactions with cytokines and growth factors. For a more complex understanding of versican it is of importance to study the tissue niche, where the wound healing process in both healthy and diseased conditions take place. In previous studies our group has identified changes in the amount of the multifaceted versican in chronic lung disorders such as asthma, chronic obstructive pulmonary disease and bronchiolitis obliterans syndrome, which could be a result of pathologic, transforming growth factor β driven, on-going remodelling processes. Reversely, the context of versican in its niche is of great importance since versican has been reported to have a beneficial role in other contexts *e.g.* emphysema. Here we explore the vast mechanisms of versican in healthy lung and in lung disorders.

Introduction

Versican is, as the name implies, a versatile molecule that plays important roles in cell-matrix interactions during adhesion, migration and inflammatory responses. It is readily expressed by fibroblasts and we have observed that versican is involved in remodelling in inflammatory lung disorders such as asthma, chronic obstructive pulmonary disease (COPD) and bronchiolitis obliterans syndrome (BOS). In this review, we therefore aim to explore the molecular role of versican in lung disorders.

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Remodelling of the extracellular matrix (ECM) is constantly occurring in the body to meet ever-changing demands on stability and flexibility of the matrix. Tissue repair and remodelling are processes in wound healing, but these mechanisms also contribute to the aberrant ECM disposition in several lung disorders such as COPD and Asthma(Dournes, G. and Laurent, F. 2012, Shimizu, K., Hasegawa, M., et al. 2011) -diseases that affect a large population and range from mild to life-threatening. Remodelling and deposition of ECM molecules are also important events in the development of chronic rejection of transplanted lung(Andersson-Sjoland, A., Thiman, L., et al. 2011). The altered matrix along with inflammatory processes contributes to the diminished lung capacity characteristic of these lung disorders.

The tissue-remodelling processes are orchestrated by recruited inflammatory cells, resident cells, cytokines and chemokines (see figure 1). One central player during remodelling in lung disorders is transforming growth factor (TGF)- β (Yang, Y.C., Zhang, N., et al. 2012), which has been reviewed for COPD(Konigshoff, M., Kneidinger, N., et al. 2009), asthma(Duvernelle, C., Freund, V., et al. 2003), pulmonary fibrosis(Khalil, N. and Greenberg, A.H. 1991) and BOS(Andersson-Sjoland, A., Thiman, L., et al. 2011). Alveolar epithelial cells have the capacity to release a host of cytokines and chemokines, and the derangement of epithelial-macrophage interactions induced by injury may result in persistent inflammation

and remodelling(Alber, A., Howie, S.E., et al. 2012). The inflammatory site in the lung triggers homing and activation of both local and bone marrow derived progenitor cells that are important in the healing process(Krause, D.S. 2008).

Remodelling occurs as a result of persistent mechanical stress or hypoxia, but may also be a response to prolonged inflammation since inflammatory processes may affect the tissue. Scar-forming inflammation is thus an important feature of several lung disorders where versican plays an important role. Interestingly, remodelling has also been shown to occur in parallel with inflammation(Rydell-Tormanen, K., Andreasson, K., et al. 2012), often in both airways and pulmonary vessels(Zanini, A., Chetta, A., et al. 2010). During disease progression, the altered blood vessels affect lung structure, and decrease lung function and oxygen saturation(Colombat, M., Mal, H., et al. 2007). The epithelium is also affected and subjected to increased oxidative stress and other triggers, such as pollen, that cause the epithelium to lose its function and differentiate into mesenchymal cells, with increased deposition of ECM and thickening of the gas exchange layer(Gorowiec, M.R., Borthwick, L.A., et al. 2012). Indeed, stiffening of the ECM during remodelling and development of fibrosis affects cell adhesion and migration so that cells migrate towards stiffer ECM(Plotnikov, S.V. and Waterman, C.M. 2013).

Because all these changes contribute to disease progression, it is necessary to study the molecular composition of the ECM niche to understand cell behaviour. Fibroblasts are central during remodelling and major contributors to the increased deposition of proteoglycans (PGs). Versican is one of the deposited PGs and is an important player in COPD(Hallgren, O., Nihlberg, K., et al. 2010), asthma(Westergren-Thorsson, G., Chakir, J., et al. 2002) and BOS(Andersson-Sjolund, A., Erjefalt, J.S., et al. 2009).

PGs consist of a core protein with covalently bound glycosaminoglycans (GAGs), which can be sulfated to varying extent. These molecules play crucial roles in lung

infection, inflammation and tissue repair as major regulators of cell behaviour in the ECM(Gill, S., Wight, T.N., et al. 2010). The reactive GAG chains result in the ability of certain PGs, such as versican, to control the viscoelastic behaviour and stability of the ECM. Different families of PGs contain different GAG side chains; chondroitin sulfate/dermatan sulfate (CS/DS) PGs (lecticans), small leucine-rich repeat PGs (SLRP), and heparan sulfate PGs (HSPGs). Versican belongs to the CS/DS PG gene family along with aggrecan, neurocan, and brevican, each differently distributed: versican in various soft tissues; aggrecan prominent in cartilage; and neurocan and brevican in the central nervous system(Margolis, R.U. and Margolis, R.K. 1994). These PGs are involved in infection and inflammation by interacting with *e.g.* cytokines and growth factors. There are other CS/DS PGs important in remodelling and inflammation events such as the SLRPs biglycan, fibromodulin, and decorin. These PGs are crucial for matrix assembly and regulation of collagen fibrillation. HSPGs are mainly expressed in the alveolar basement membranes and on cell surfaces. The cell surface bound HSPGs are either attached by a glycosylphosphatidylinositol (GPI) anchor (the glypicans) or by a transmembrane part (the syndecans). Also the HSPGs are highly bioactive and implicated in development and disease, including lung emphysema seen in *e.g.* COPD(Smits, N.C., Shworak, N.W., et al. 2010).

Alterations in the expression of PGs directly influences matrix compliance and permeability of vessels, airways and the surrounding tissues. Importantly, the integrity of tissues and stability of the ECM network is supported by interactions between ECM molecules such as versican, and its intimate binding partner hyaluronan. The latter is mainly produced by fibroblasts and binds several proteins. In this respect, hyaluronan, versican and CD44 contribute to the stability of the ECM. In this review, we will explore the current knowledge about the molecular role of versican in the lung niche and how it may be involved in disease.

Structure and function of versican

Versican (VCAN) encoded on human chromosome 5 and spanning over 90 kilo base pairs, shares similar globular (G) structures at the N-terminal (G1 domain) and C-terminal (G3 domain) of the protein core along with the other members of the lectican family. The G1 domain contains an immunoglobulin-like domain (Ig) and a hyaluronan-binding region (HABR), whereas the G3 domain consists of two epidermal growth factor (EGF) repeats, a C-type lectin motif (LC) and a complement-binding protein-like motif (CRP). Between G1 and G3 versican has CS/DS binding domains where GAG side chains attach (see figure 2) (Zimmermann, D.R. and Ruoslahti, E. 1989).

The CS/DS GAG chains are linear anionic polysaccharides consisting of up to around 40 repeating disaccharide units of glucuronic acid (GlcA) and N-acetyl- galactosamine (GalNAc). Some of the GlcA can be epimerized into iduronic acid (IdoA) and the polysaccharide may then be referred to as DS or rather CS/DS, due to the mixed content of GlcA and IdoA. The presence of IdoA confers a more flexible structure of the GAGs and allows for binding of growth factors and cytokines. We have demonstrated that IdoA is of importance during directed migration, and the ablation of dermatan epimerase 1 that is responsible for generation of IdoA led to delayed ability to re-populate wounded areas (Bartolini, B., Thelin, M.A., et al. 2013). Importantly, versican contains only around 10 per cent or less IdoA, and it is not clear whether this content affects cell behaviour such as migration.

Yet another modification affecting cell behaviour is the sulfation of the GAGs to various extents with preference to 4-sulfated GalNAc residues. A substantial amount of 6-sulfated GalNAc is also found. Finally, small amounts of non-sulfated, 2,4-sulfated, 2,6-sulfated and 4,6-sulfated disaccharides have been described (Hitchcock, A.M., Costello, C.E.,

et al. 2006). Importantly, the sulfation pattern is of great importance for cellular events and is crucial for chemokine- and selectin-binding to versican(Kawashima, H., Atarashi, K., et al. 2002).

Adding complexity, versican can be differentially spliced. Alternative splicing of versican mRNA encoding the CS/DS binding domain generates four isoforms of versican, namely V0, V1, V2, and V3, which differ in molecular weight. The splice variants are outlined in figure 2. Recently, an additional isoform, V4, was identified as up-regulated in breast cancer along with the other splice variants(Kischel, P., Waltregny, D., et al. 2010). The versican isoforms differ in length in their CS/DS binding domain and therefore also in the number of GAG side chain attachment sites. The V0 is the largest isoform and contains two GAG binding domains named α -GAG binding domain and β -GAG binding domain. The V1 contains only the β -GAG binding domain, whereas the V2 only has the α -GAG binding domain. The V3 splice form completely lacks GAG attachment sites, and is the smallest of the isoforms (see figure 2).

TGF- β has been shown to induce the expression of proteins that are involved in mRNA splicing and RNA processing in human lung fibroblasts(Hallgren, O., Rolandsson, S., et al. 2012). Importantly, TGF- β 2 and TGF- β 3 increase the expression of splicing variants V0 and V1(Berdiaki, A., Zafiropoulos, A., et al. 2008, Norian, J.M., Malik, M., et al. 2009). The production of these alternative isoforms of versican by fibroblasts primarily, may trigger and perpetuate tissue remodelling and disease progression(Hallgren, O., Rolandsson, S., et al. 2012). Interestingly, different isoforms of versican affect cell behaviour differently, and could be of importance during pathological progression. Alternative splicing leading to increased levels of V1 thus increased proliferation and also resulted in a resistance to apoptosis in fibroblasts. The V2 isoform on the other hand, decreased the proliferation and had no effect on apoptosis(Sheng, W., Wang, G., et al. 2005). Interestingly, the V1 variant had the ability to

induce mesenchymal-epithelial transition in fibroblasts, resulting in an expression-shift from N-cadherin to epithelial specific E-cadherin(Sheng, W., Wang, G., et al. 2006).

The complex structure of the core protein invites many binding partners. For example, integrin β 1, EGF-R, tenascin, fibulin-1 and -2 as well as fibrillin-1 bind to the G3 domain of versican(Wu, Y.J., La Pierre, D.P., et al. 2005). In addition, the negatively charged GAGs create a brush-like structure around the core of versican, and these properties contribute to the long extended shapes of the versican molecules opening up for binding of positively charged molecules such as cytokines, chemokines, growth factors and also selectins and CD44(Wu, Y.J., La Pierre, D.P., et al. 2005). Interestingly, versican can bind specific chemokines such as liver- and activation-regulated chemokine (LARC), and secondary lymphoid-tissue chemokine (SLC), but not others (*e.g.* IL-8 and macrophage inflammatory protein-1 α (MIP-1 α)). The binding occurs through the CS/DS chains of versican, and the binding tends to down-regulate the function of these chemokines(Hirose, J., Kawashima, H., et al. 2001). It is however important to note that versican only displays a few IdoA residues. The DS part of PGs is of great importance during coagulation and also affects wound healing and inflammation(Malmstrom, A., Bartolini, B., et al. 2012).

Taken together, the vast biological diversity of versican highly contributes to its important roles in physiological and pathological events.

Regulation of versican expression

The bio-diversity of versican lies in the different domains of the molecule, and in particular the CS/DS-binding domain contributes highly to the various activities of versican. The specific GAG constitution displayed on the versican molecules is influenced by extracellular signals such as TGF- β , EGF and platelet derived growth factor (PDGF)-BB affecting specific GAG synthetic enzymes involved in the process(Tiedemann, K., Malmstrom, A., et al. 1997).

TGF- β 1 is a key player in regulating ECM production and up-regulates the synthesis of versican in many cells, among those, lung fibroblasts(Westergren-Thorsson, G., Antonsson, P., et al. 1991). The TGF- β signalling pathway is strongly associated with fibrotic and inflammatory lung disorders, and indeed, the binding of TGF- β to its type II receptor in concert with the type I receptor leads to formation of a receptor complex and phosphorylation of the type I receptor. Subsequently, the type I receptor phosphorylates Smad2 or 3, which associates with Smad4 and the whole complex translocates into the nucleus. In the nucleus, the Smad complex associates with transcription factors and the complexes bind to specific binding sites within the promoter of versican, biglycan and many other target genes(Kamato, D., Burch, M.L., et al. 2013). The versican promoter contains a typical TATA box around 16 base pairs upstream of the transcription-starting site. The 5' flanking sequence contains promoter, enhancer and repressor elements allowing for specific regulation of versican in different situations. Several transcription factor binding sites have also been revealed, including cAMP response element-binding protein (CREB), T-cell factor/lymphoid enhancer-binding factor (TCF/LEF), and activator protein 1 (AP-1)(Sotoodehnejadnematalahi, F. and Burke, B. 2013).

Yet another way to transcribe versican is through the canonical wnt pathway, one of the fundamental pathways involved in activities of development and tissue homeostasis(Logan, C.Y. and Nusse, R. 2004), controlling proliferation, differentiation, cell polarity and motility -events of relevance in tissue remodelling. Apart from TGF- β , signaling through the Smads also mediates fibrosis through the wnt signalling pathway(Akhmetshina, A., Palumbo, K., et al. 2012). The key event in the wnt pathway is regulation of the production and stability of β -catenin in the cytosol. In the absence of wnt, β -catenin is phosphorylated by the β -catenin destruction complex and targeted for proteasomal degradation. Wnt proteins stabilize the β -catenins and upon cytosolic 9

β -catenin accumulation, the complex is translocated into the nucleus where it interacts with TCF/LEF(Korinek, V., Barker, N., et al. 1997, Rahmani, M., Carthy, J.M., et al. 2012). This leads to transcription of versican among other wnt target genes(Rahmani, M., Read, J.T., et al. 2005, van Amerongen, R. and Nusse, R. 2009). Interestingly, the wnt pathway may be regulated by integrins, and β 1-integrin can activate the wnt pathway via integrin-linked kinase (ILK)(Maydan, M., McDonald, P.C., et al. 2010). Upon interaction between β 4-integrin and collagen in the ECM, the growth factor bound 2 (GRB2) is recruited; inducing growth factor induced β -catenin accumulation. Recently, aberrant wnt signalling has been proposed as a key pathway in systemic sclerosis pulmonary fibrosis(Beyer, C., Schramm, A., et al. 2012, Lam, A.P., Flozak, A.S., et al. 2011) and studies to inhibit the wnt pathway have unravelled the potential in targeting the tankyrases. The inhibition of tankyrases resulted in reduced nuclear accumulation of β -catenin(Distler, A., Deloch, L., et al. 2012) and inhibited wnt signalling along with reduced bleomycin-induced fibrosis.

Another level of control is through microRNA, which are small non-coding RNA molecules involved in the homeostasis and remodelling events of ECM(Rutnam, Z.J., Wight, T.N., et al. 2013). Interestingly, the 3'UTR of versican can also modulate the function of several microRNAs, signifying the multitude of control mechanisms(Lee, D.Y., Jeyapalan, Z., et al. 2010). In several lung disorders, we have seen an altered phenotype in the lung smooth muscle cell mass. The transcription factor myocardin in smooth muscle cells has been shown to coordinate smooth muscle cell differentiation through the induction of microRNA-143. By specifically binding to the 3' UTR of versican, miRNA-143 attenuates versican expression and subsequently, smooth muscle cell migration(Wang, X., Hu, G., et al. 2010).

Fine-tuning of versican production is thus possible through many levels of control in different tissues and during disease. Importantly, this may lead to therapeutic targeting of versican in a specific way to counteract aberrant wound repair in the lung.

Cellular origins of versican

Versican is expressed by several cell types, and in the lung it is primarily found in elastic fibres in the lamina propria of the central airway wall, predominantly close to the smooth muscle bundles. In the alveolar parenchyma, versican expression is found in irregular and patchy areas in the alveolar septa. Fibroblasts are central producers of the ECM and key regulators of versican during health and even more so during diseases such as COPD(Hallgren, O., Nihlberg, K., et al. 2010), asthma(Westergren-Thorsson, G., Chakir, J., et al. 2002) and BOS(Andersson-Sjoland, A., Thiman, L., et al. 2011). Human embryonic lung fibroblasts express high levels of versican, pointing towards an important role of fibroblasts and versican during lung development(Tufvesson, E. and Westergren-Thorsson, G. 2000). Versican plays a role in cell-ECM binding and in a study on primary lung fibroblasts obtained from lung transplanted patients, the migratory properties of these cells had a tendency to decrease, whereas the production of versican increased(Andersson-Sjoland, A., Thiman, L., et al. 2011). This could be a result of fibroblasts binding to a versican-rich environment. Versican also has a regulatory effect on cell proliferation, being highly expressed in proliferating dermal fibroblasts(Zimmermann, D.R., Dours-Zimmermann, M.T., et al. 1994), as well as muscle cell proliferation during development(Velleman, S.G., Sporer, K.R., et al. 2012). This could be due to the fact that versican is highly regulated by the cytokine/growth factor milieu and, apart from TGF- β , PDGF-AB, has been shown to stimulate the expression of versican core protein in arterial smooth muscle cells(Schönherr, E., Kinsella, M.G., et al. 1997). On the other hand, pro-inflammatory cytokines such as IL-1 β and IFN- γ reduced the expression of versican in arterial smooth muscle cells(Lemire, J.M., Chan, C.K., et al. 2007), pointing towards the complex regulation of versican during inflammation.

The multipotent mesenchymal stromal/stem cells (MSCs) exhibit an increase in versican production during differentiation (Foster, L.J., Zeemann, P.A., et al. 2005) and Murphy *et al.* showed that versican mRNA levels were present already in undifferentiated MSCs, which were maintained during differentiation. Further exploration is required to elucidate the potential role that versican may play in the differentiation of resident lung derived MSC into cell types that may hamper disease progression, or perhaps the opposite.

Fibrocytes are another type of mesenchymal progenitor cells that derive from bone marrow and home to human lung tissue upon tissue damage (Andersson-Sjoland, A., Erjefalt, J.S., et al. 2009). When fibrocytes are recruited from the bone marrow to the tissue they can, in conformity with MSC, differentiate into different cell types plausibly including fibroblasts (Andersson-Sjoland, A., Nihlberg, K., et al. 2011). Previous studies have reported that the collagen and proteoglycan gene expression profiles of fibrocytes and fibroblasts differ, and that fibrocytes express higher mRNA levels of versican than fibroblasts. Also, the production of high levels of versican together with perlecan, hyaluronan and collagen VI support the hypothesis that fibrocytes are involved in tissue stabilization and modulation of inflammatory responses (Bianchetti, L., Barczyk, M., et al. 2012) (see figure 1).

Versican during inflammation

The molecular composition of the niche predisposed by the production of specific ECM molecules such as versican is crucial for a properly mounted inflammatory response. However, in lung disorders, the excessive remodelling processes result in perpetuated inflammation. New insights into how innate immunity influences pathological remodelling are beginning to emerge, revealing interactions between Toll-like receptor (TLRs) and the ECM including damage-associated molecular patterns (DAMPs). A number of endogenous

molecules specifically generated upon tissue injury have been identified to activate TLRs, such as versican, biglycan, and fragments of hyaluronan and HS (Piccinini, A.M. and Midwood, K.S. 2010). Fibroblasts of a different origin and endothelial cells express TLR2 and its co-receptors, thus versican could be a potent trigger for activation of fibroblasts and endothelial cells during inflammation (Wang, W., Xu, G.L., et al. 2009). Binding of DAMPs to TLRs induces the production of proinflammatory cytokines and upregulates co-stimulatory molecules linking matrix remodelling and innate immune responses to the adaptive immunity (Kim, S., Takahashi, H., et al. 2009, Phipps, S., Lam, C.E., et al. 2007, Piccinini, A.M. and Midwood, K.S. 2010, Schaefer, L., Babelova, A., et al. 2005, Tufvesson, E. and Westergren-Thorsson, G. 2003). Thrombospondin-1, which is up-regulated during wound repair and remodelling, binds to the G1 domain of versican resulting in colocalization into microfibrils containing elastin on vascular smooth muscle cells to further the inflammatory niche (Kuznetsova, S.A., Issa, P., et al. 2006) (see figure 2). Hyaluronan is enhanced at sites of inflammation, tumor growth and tissue remodelling, and is thought to modulate cell behaviour through interaction with several receptors among them being CD44. The hyaluronan-versican interaction is important for T cell recruitment into inflamed areas and virus infection-induced hyaluronan synthesis induced the concomitant synthesis of versican. Moreover, CD4+ T-cells cultured on versican-rich ECM were retained in culture, although their migration was inhibited (Evanko, S.P., Potter-Perigo, S., et al. 2012). Studies of lung fibroblasts treated with polyinosinic:polycytidylic acid (poly I:C), which mimics a viral infection, showed an increase in hyaluronan and versican and a related increase in monocyte adhesion to these matrix structures (Evanko, S.P., Potter-Perigo, S., et al. 2009). Versican is thus also important for recruitment of monocytes, which has been demonstrated in a model of myocardial infarction. Here, infiltrating monocytes after stimulation with GM-CSF induced versican expression (Toeda, K., Nakamura, K., et al. 2005) and monocytes have been shown to

be dependent on versican during adhesion(Potter-Perigo, S., Johnson, P.Y., et al. 2010). Indeed, monocytes bind to the ECM during differentiation and have been shown to produce versican along with hyaluronan synthases 2 (HAS) in this process. In addition, TNF-stimulated gene-6 (TSG-6), encoding for the TSG-6 protein that contributes to matrix stability, was also expressed from monocytes during differentiation into macrophages(Chang, M.Y., Chan, C.K., et al. 2012). A recent study showed that versican was up-regulated in monocytes in patients with systemic sclerosis and it is possible that versican contributes to the fibrotic processes through a feedback loop involving versican and chemokines, resulting in influx of monocytes(Masuda, A., Yasuoka, H., et al. 2013). In addition, versican accumulates in tumor stroma and plays an important role in proliferation and metastasis of tumor cells(Du, W.W., Yang, W., et al. 2013). Relevant in inflammatory states, including cancer and the lung disorders discussed here, versican and especially its C-terminus, promotes cell survival and protects cells from H₂O₂-induced apoptotic cell death by enhancing cell-matrix interactions(Du, W.W., Yang, W., et al. 2013).

Versican in lung disease

Versican is expressed in COPD, asthma, and lung-transplanted patients (see figure 3)(Andersson-Sjoland, A., Thiman, L., et al. 2011, Hallgren, O., Nihlberg, K., et al. 2010, Nihlberg, K., Andersson-Sjoland, A., et al. 2010), which indicates that it could be a target for future interventions. Late stage COPD is untreatable and lung transplantation is the only option for these patients and also for other diseases such as cystic fibrosis and IPF. However, lung transplantation is associated with a risk of developing chronic rejection (BOS)- a process involving aberrant wound healing and development of fibrotic plugs in the airways, which leads to insufficient air supply. In a study on lung-transplanted patients, lung fibroblasts

produced 16 times more versican half a year after transplantation compared to healthy volunteers. Histology showed that versican was mainly localized in the alveolar walls and thus may contribute to the plug formation (Andersson-Sjoland, A., Thiman, L., et al. 2011).

Chronic obstructive pulmonary disorder (COPD)

COPD is characterized by loss of elastic fibres from small airways and alveolar walls and the decrease in elastin is associated with increased disease severity. Versican is increased in fibroblasts from distal airways from COPD patients (see figure 3) and indicates that the production is larger than the degradation of versican as seen by immunohistochemistry (Hallgren, O., Nihlberg, K., et al. 2010). Versican in the alveolar wall is also negatively correlated to elastin and elastin-binding protein (EBP) (Merrilees, M.J., Ching, P.S., et al. 2008), a molecular chaperone important in the fibrillization-process of elastin. These molecular parameters are also correlated to lung function (FEV₁) (Black, P.N., Ching, P.S., et al. 2008). Efficient repair by re-synthesis of elastic fibres in alveoli of COPD patients may be hampered by the inhibition of EBP by versican, particularly by its CS/DS chains (Tiedemann, K., Olander, B., et al. 2005). The EBP chaperone escorts tropoelastin from golgi and endosomal compartments to the cell surface. During states of increased versican in the pericellular compartment, the lectin-domain of EBP interacts with galactosamine in CS/DS of versican, and causes a conformational change in the EBP releasing tropoelastin prematurely. However since CS/DS GAG-side chains are very variable in the amount and spatial distribution of iduronic acid it cannot be excluded that it is not the actual amount of versican *per se* but rather the amount of specific CS/DS motifs that regulates the interaction. Normally, following the release of EBP, tropoelastin finds its acceptors, the newly forming microfibrils of elastin. However, during high-versican microenvironment, new formation of elastic fibres is hampered. The relationship between elastic fibre loss and accumulation of

versican has been confirmed in studies showing that modulation of versican influences elastic fibre deposition(Huang, R., Merrilees, M.J., et al. 2006, Hwang, J.Y., Johnson, P.Y., et al. 2008, Merrilees, M.J., Lemire, J.M., et al. 2002). In these settings versican was harmful through inhibition of elastic fibre formation, but in an animal model of emphysema increased proteoglycan and in particular versican was associated with protection of the alveolar walls from rupture(Takahashi, A., Majumdar, A., et al. 2014). In line, in a randomized controlled trial, it was shown that inhaled corticosteroids increased the bronchial expression of versican together with collagen III in COPD patients. This increase in versican was associated with improved lung function. Surprisingly, the smoking status of the patients did not influence versican levels(Kunz, L.I., Strebus, J., et al. 2013) although it may affect tissue remodelling as such through the activation of molecules involved in ECM turnover, such as matrix metalloprotease-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1)(Boue, S., De Leon, H., et al. 2013). However, the up-regulation of versican in COPD lungs is not consistent. Indeed, Annoni *et al.* even showed a decrease in versican expression in alveolar parenchyma in COPD patients compared with healthy non-smokers and may point towards the importance of fine-mapping COPD into subtypes of the disease(Annoni, R., Lancas, T., et al. 2012).

Asthma

Versican is also involved in asthma and in our studies we have shown a heterogeneous pattern of versican distributed throughout the airway tree. Most studies have so far concentrated on central airways, but intriguingly, we have seen a difference in PG production between centrally and distally isolated fibroblasts. Thus distally derived fibroblasts from patients with mild untreated asthma had increased production of versican(see figure 3)(Nihlberg, K., Andersson-Sjolund, A., et al. 2010). Similar results have been obtained from fibroblasts

isolated from the distal airways in patients with COPD(Hallgren, O., Nihlberg, K., et al. 2010) and in fibroblast cultures obtained early after lung transplantation (see figure 3)(Andersson-Sjoland, A., Thiman, L., et al. 2011), emphasizing the importance of studying the distal airways in all lung disorders. Histological analyses of versican in uncontrolled and controlled mild asthmatics and healthy controls showed increased percentage areas of versican in the group of uncontrolled asthmatics in central airways (unpublished data).

Remodelling of the airways contributes to the persistent airway obstruction and decline in lung function in asthmatic patients(Chiappara, G., Gagliardo, R., et al. 2001, Lange, P. 2013). Additionally there is a correlation between PGs deposition in the airway wall and reactivity of provocation by inhaled methacholine (provocative concentration required to decrease FEV₁ by 20% of its baseline value [PC₂₀] less than 4 mg/ml) in patients with mild atopic asthma(Huang, J., Olivenstein, R., et al. 1999). Altered deposition of PGs in the asthmatic lung appears to vary between different asthma phenotypes and severities(de Medeiros Matsushita, M., da Silva, L.F., et al. 2005, K. Nihlberg, M.L., A.-K. Larsson, E. Tufvesson, L. Bjermer, G. Westergren-Thorsson 2011, Pini, L., Hamid, Q., et al. 2007, Roberts, C.R. 1995). Indeed, we have shown that fibroblasts isolated from bronchial biopsies from asthmatic patients with the greatest degree of hyper-responsiveness produced larger amounts of versican(Westergren-Thorsson, G., Chakir, J., et al. 2002). In line, several studies of mild, moderate and fatal asthma have reported increased densities of versican in the tissue(Araujo, B.B., Dolhnikoff, M., et al. 2008, Ludwig, M.S., Ftouhi-Paquin, N., et al. 2004). Patients with fatal asthma had increased versican content in the internal area of large and small airways compared with controls(de Medeiros Matsushita, M., da Silva, L.F., et al. 2005). However, it has not been evaluated if the increased amount of versican and other matrix molecules may have an effect in opposing the contractive properties of increasing smooth muscle layers(Roberts, C.R. 1995). Respiratory viral infections are known

to be a trigger of exacerbation in both asthma and COPD and could be the catalyst that starts an increased deposition of versican and hyaluronan. Furthermore, the versican rich environment is known to have an increased capacity for monocyte infiltration and increased immune response(Potter-Perigo, S., Johnson, P.Y., et al. 2010). It remains to be explored whether this augmentation in versican in asthma is beneficial or may be a target for future therapies.

Conclusion

Versican is clearly involved in disease processes in COPD, asthma and BOS. Taken that the inflammatory response is different in these disorders, it becomes evident that ECM remodelling may be a target for future drugs. The role of the ECM niche during inflammation and remodelling events in lung disorders is to serve as an important scaffold for inflammatory and mesenchymal cells and their fate decision, see figure 1. In this review, we have elucidated versican as an important player in inflammation and remodelling that shows a complex repertoire of cellular actions. An up-regulation of versican in these disorders may perpetuate inflammatory responses and lead to aberrant wound healing processes. The role of versican in innate and adaptive immunity needs further investigations, as does the differential expression of the splice forms. Another structural feature of high potential interest is the function of the CS/DS side chains and its modulation in different inflammatory conditions. We therefore conclude that versican is an interesting target for future research and the dissection of specific roles of its splice variants may be fruitful for finding intervening targets to treat lung disorders such as COPD, asthma, and chronic rejection.

Funding

This work was supported by by the Swedish Medical Research Council (11550), Stockholm Sweden, the Evy and Gunnar Sandberg foundation, Lund, Sweden, the Heart-Lung Foundation, Stockholm, Sweden, Greta and John Kock, Trelleborg, Sweden, the Alfred Österlund Foundation, Malmö, Sweden, the Anna-Greta Crafoord Foundation, Stockholm, Sweden, the Konsul Bergh Foundation, Stockholm, Sweden, the Royal Physiographical Society in Lund, Sweden and the Medical Faculty of Lund University, Sweden.

Acknowledgement

We thank Lena Thiman and Marie Wildt for skillful technical assistance and Alexander Doyle for proof-reading.

Figure 1. Events involving versican in the development of lung disease (*e.g.* COPD). Panel A: As a response to tissue damage and the cytokine/chemokine milieu (dots), mesenchymal (fibroblasts) and inflammatory (monocytes) cells migrate towards injury, and progenitor cells (MSC and fibrocytes) are recruited locally or from the circulation. Panel B: As a result, among other ECM molecules, versican is deposited in the lung tissue, increasing the reserve of cytokines/chemokines that perpetuate the recruitment of inflammatory cells. Panel C: Versican is involved in the differentiation of cells as depicted colour coded (monocyte to macrophage, MSC to fibroblast and fibrocyte to myofibroblast. Panel D: Immunohistochemistry visualizes the deposition of versican (brown colours, arrow heads) in lung parenchyma from COPD patients.

Figure 2. Structure of versican and its splice variants. Versican has globular domains at the N-terminal (G1) and C-terminal (G3). The G1 contains an immunoglobulin-like domain (Ig), and a hyaluronan-binding region (HABR); the G3 contains two epidermal growth factor repeats (EGF); a C-type lectin motif (LC); and a complement-binding protein-like motif (CRP). Between G1 and G3, CS/DS binding sites attach GAG side chains to a various extent depending on splice variant (V0, V1, V2, and V3).

Figure 3. Graph shows that versican production is increased in lung-transplanted patients, asthma (controlled), and COPD compared to healthy controls. ** $p < 0.01$, *** $p < 0.005$ compared to controls.

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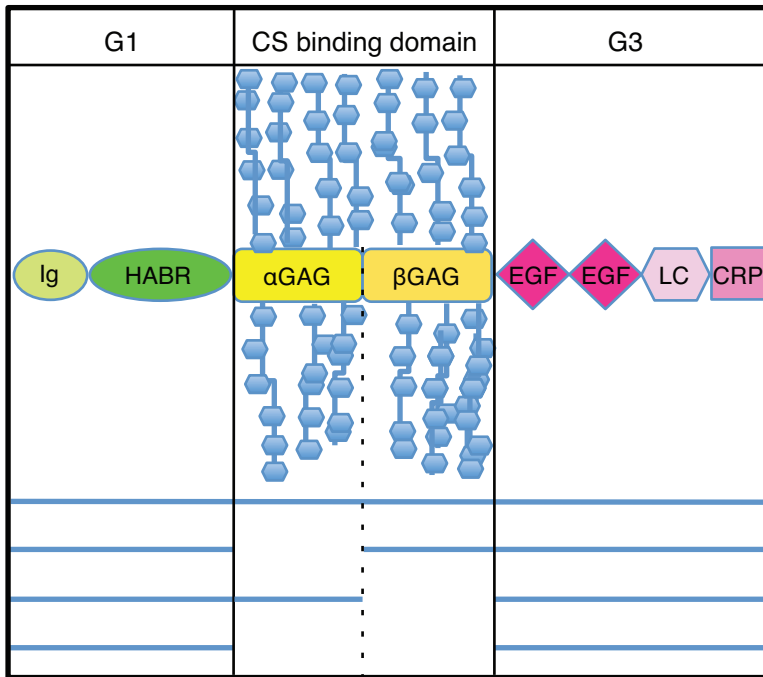
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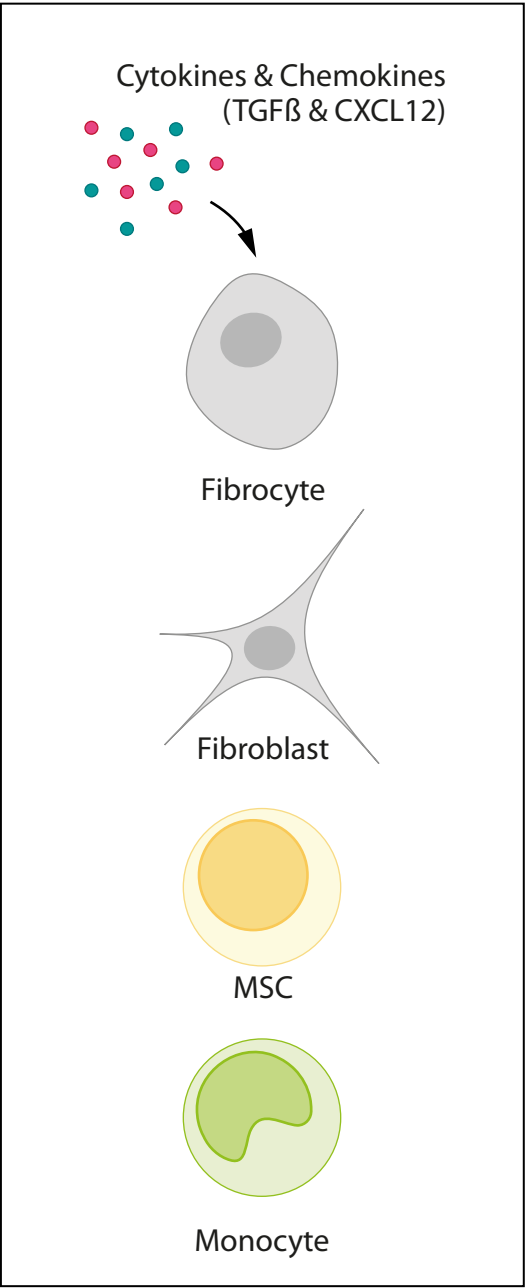
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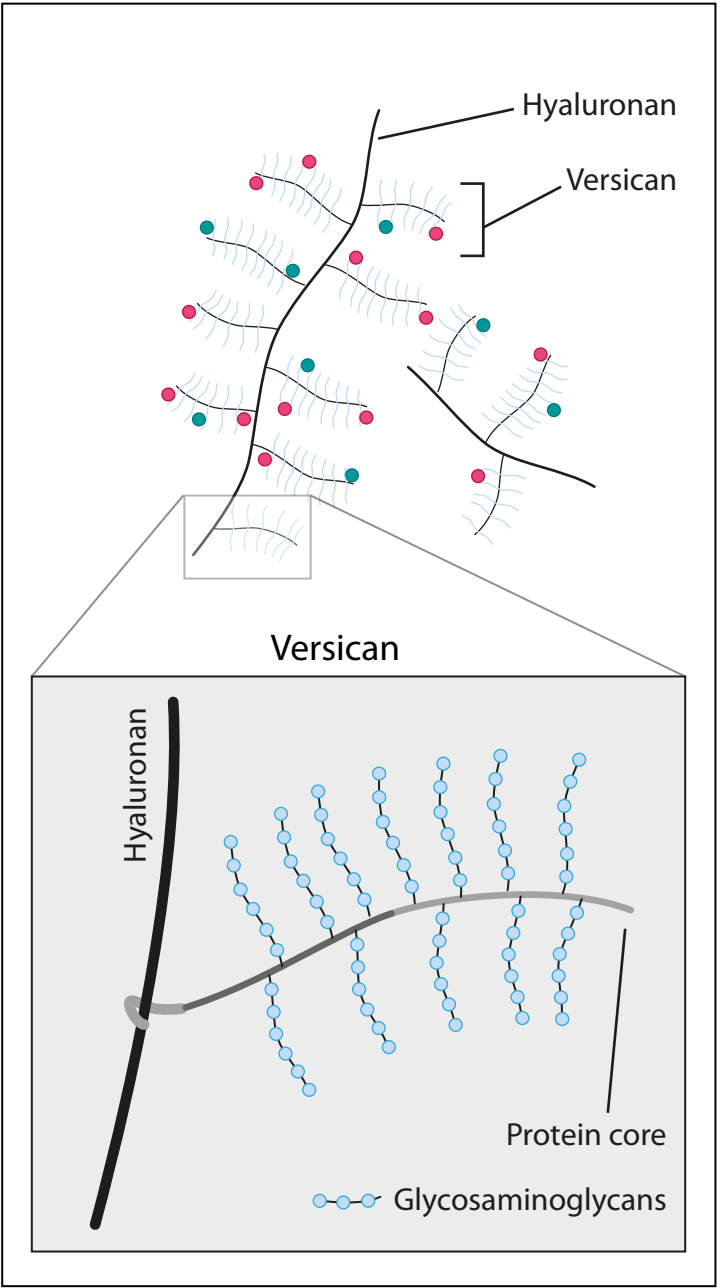
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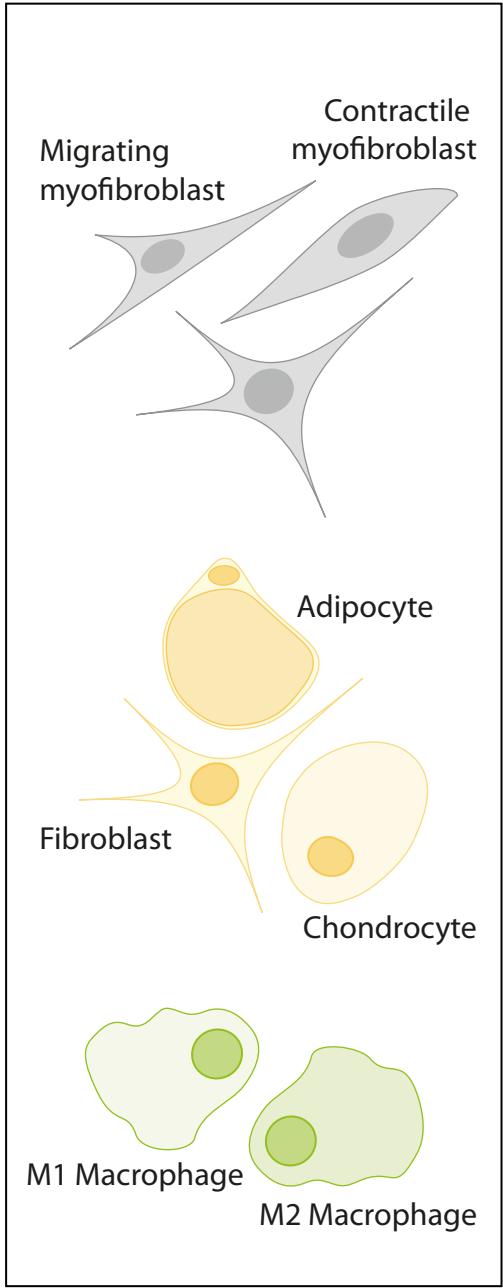
A. Tissue entry
Activation of resident cells



B. Versican deposition



C. Differentiation



D. Tissue remodeling
Disease

