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Tumour necrosis factor-α interacts with biglycan and decorin

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Abstract Several interactions of cytokines with extracellular matrix molecules are mediated by proteoglycans, such as biglycan and decorin. Using surface plasmon resonance, we show for the first time that tumour necrosis factor-α (TNF-α) binds to both biglycan and decorin with K_ds of 0.81 μM and 1.23 μM respectively, a binding that was confirmed by Scatchard plots using a solid phase assay. Binding occurs preferentially via the core protein, shown by lower K_ds, 0.26 μM and 0.81 μM for biglycan and decorin respectively. There was also binding to dermatan sulphate, with a K_d of 10.53 μM. The function of this interaction between TNF-α and biglycan and decorin is not known, but we suggest that the differential localisation of the proteoglycans enables the cytokines to be immobilised in different environments.

Key words: Biglycan; Decorin; Interaction; Tumor necrosis factor-α

1. Introduction

Biglycan and decorin are two small proteoglycans, normally substituted at the N-terminal with two and one chondroitin/dermatan sulphate (CS/DS) chain respectively, that are present in the extracellular matrix of connective tissue. They are both known to bind different types of collagens. They also bind to the same binding site on the biglycan/decorin endocytosis receptor [1]. In addition, biglycan is known to bind to the membrane-bound proteoglycan dystroglycan [2], while decorin is known to bind other extracellular matrix components, such as fibronectin and thrombospondin. Most of these interactions are mediated via the core protein, although some interactions can be exerted through the glycosaminoglycans (GAG) chains.

Many proteoglycans are important mediators of cytokine binding and have been shown to modulate the biological activity of a variety of cytokines. A well-known example is the binding of transforming growth factor-β (TGF-β) to decorin, biglycan and fibromodulin [3], where it binds to the core proteins. Some investigators suggest a direct inactivation of TGF-β by decorin. It has been shown that decorin bound to collagen type I fibres is still able to bind TGF-β [4,5] suggesting an immobilisation of TGF-β in the extracellular matrix, keeping it away from its signalling receptors on the cell surface. Immobilised TGF-β can subsequently become activated by partial proteolysis of decorin [6] or collagen after the action of matrix metalloproteinases. A similar function for biglycan is not known, but may well be possible.

Several other cytokines bind matrix molecules, mainly proteoglycans of the heparan sulphate type. A well known example is the binding of fibroblast growth factor-2 to heparan sulphate chains [7]. Similarly, DS has been shown to bind to fibroblast growth factor-2 [8], as well as interferon-γ [9]. Function can be activation, inhibition or activation of subsequent intracellular signalling of respective growth factor receptor.

However, little is known about the binding of tumour necrosis factor-α (TNF-α) to extracellular matrix molecules. TNF-α is a key mediator of inflammatory processes, and is found to act at sites of inflammation. We have previously shown that TNF-α changes the expression of proteoglycans, especially that of biglycan in lung fibroblasts [10], and thereby gives rise to a remodelling of the connective tissue. It has also recently been shown that TNF-α binds to the matrix molecules fibronectin and laminin and complexed in the extracellular matrix functions as a pro-adhesive cytokine augmenting the adhesiveness of infiltrating leukocytes [11,12].

In the present study we have investigated the ability of biglycan and decorin to interact with TNF-α. We show that both biglycan and decorin interact via their core protein to TNF-α, as well as via their DS chains.

2. Materials and methods

2.1. Purification of tissue-derived biglycan, decorin, DS and CS

Biglycan and decorin were purified from bovine sclera as described previously [13]. DS (iduronic acid content of 95%) was purified from porcine skin [14], and CS was purified from horse nasal septum [15], the two being termed DS-18 and CS-4 respectively. ^{125}I radiolabelling of CS and DS was performed as described previously [16].

To remove the GAG chains, proteoglycans were digested with chondroitin ABC lyase (EC 4.2.2.4, Seikagaku Kogyo, Tokyo, Japan).

2.2. Purification of recombinant biglycan and decorin

Recombinant human biglycan and bovine decorin were produced in HeLa cells and Chinese hamster ovary cells, respectively [17]. Purification of proteoglycans was performed by using a Q-Sepharose (Amersham Pharmacia Biotech) column eluting the proteoglycans with a gradient of 0.1–1 M NaCl [18]. For ^{35}S radiolabelling of proteoglycans, cells were grown in the presence of 50 μCi/ml [^{35}S]sulphate and purified as above.

After chondroitin AC-1 lyase (EC 4.2.2.5, Seikagaku Kogyo, Tokyo, Japan) digestion of biglycan and decorin, analysis of the iduronic acid content was carried out on a Bio-Gel P6 column as previously described [19]; the iduronic acid content was calculated [20] to be around 60%.

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Abbreviations: CS, chondroitin sulphate; DS, dermatan sulphate; GAG, glycosaminoglycan; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α

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2.3. Interaction studies using surface plasmon resonance

All measurements were performed with a BIAcore 2000 System. Recombinant human TNF-α and TGF-β (from R&D Systems, Abingdon, UK) were immobilised on sensorchips Pioneer B1 with a 1:1 mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide, as recommended by the manufacturer (BIAcore AB, Uppsala, Sweden). The remaining active groups were blocked with 1 M ethanolamine hydrochloride at pH 8.5. Proteoglycans were diluted in 0.01 M Tris, 0.15 M NaCl, 0.005% (v/v) surfactant P20 (BIAcore AB), which was also used as the running buffer, to a final concentration of 5–250 μg/ml, and injected over the TNF-α surface at 40 μl/min. BIAevaluation 3.1 software was used for affinity analysis according to a 1:1 binding model.

2.4. Solid phase assay of interactions

For the Scatchard analysis, 250 ng/ml recombinant human TNF-α was adsorbed overnight onto microtitre plates (Maxisorb, Nunc, Roskilde, Denmark). Wells were blocked for 2 h with 1% bovine serum albumin in Tris-buffered saline to avoid non-specific interactions. Radiolabelled biglycan (0.5–8 μg) and decorin (0.1–2 μg) were added to the wells in a range of concentrations diluted in blocking buffer supplemented with 0.05% Tween, and incubated overnight. The amount of bound proteoglycans was measured by a LKB Wallac (Turku, Finland) liquid scintillation counter, and the non-specific binding was subtracted.

A similar procedure was used in the additional binding experiments. Recombinant human TNF-α was added to each well in different concentrations (0–500 ng/ml) and the radiolabelled biglycan (2–8 μg) or decorin (0.5–2 μg), DS or CS (1–2 μg) were added.

2.5. Statistical methods

Mean values ± S.E.M. were calculated.

3. Results

3.1. Interactions studied by surface plasmon resonance

Different concentrations (5–250 μg/ml) of tissue-derived biglycan and decorin, as well as DS and CS, were applied at a constant flow to the TNF-α surface. Both biglycan (Fig. 1A) and decorin (Fig. 1B) showed a clear binding to TNF-α, with biglycan binding to a somewhat larger extent, resulting in K_ds of 0.81 (±0.28) μM and 1.23 (±1.07) μM respectively. To determine the specificity of the binding, the core proteins of biglycan and decorin were applied to the TNF-α surface after digestion with chondroitinase ABC. In this case, the core protein of both biglycan (Fig. 1C) and decorin (Fig. 1D) bound to the TNF-α surface to a larger degree than the intact, undigested proteoglycan. Biglycan core bound to a larger degree than decorin core. The K_ds of biglycan and decorin changed

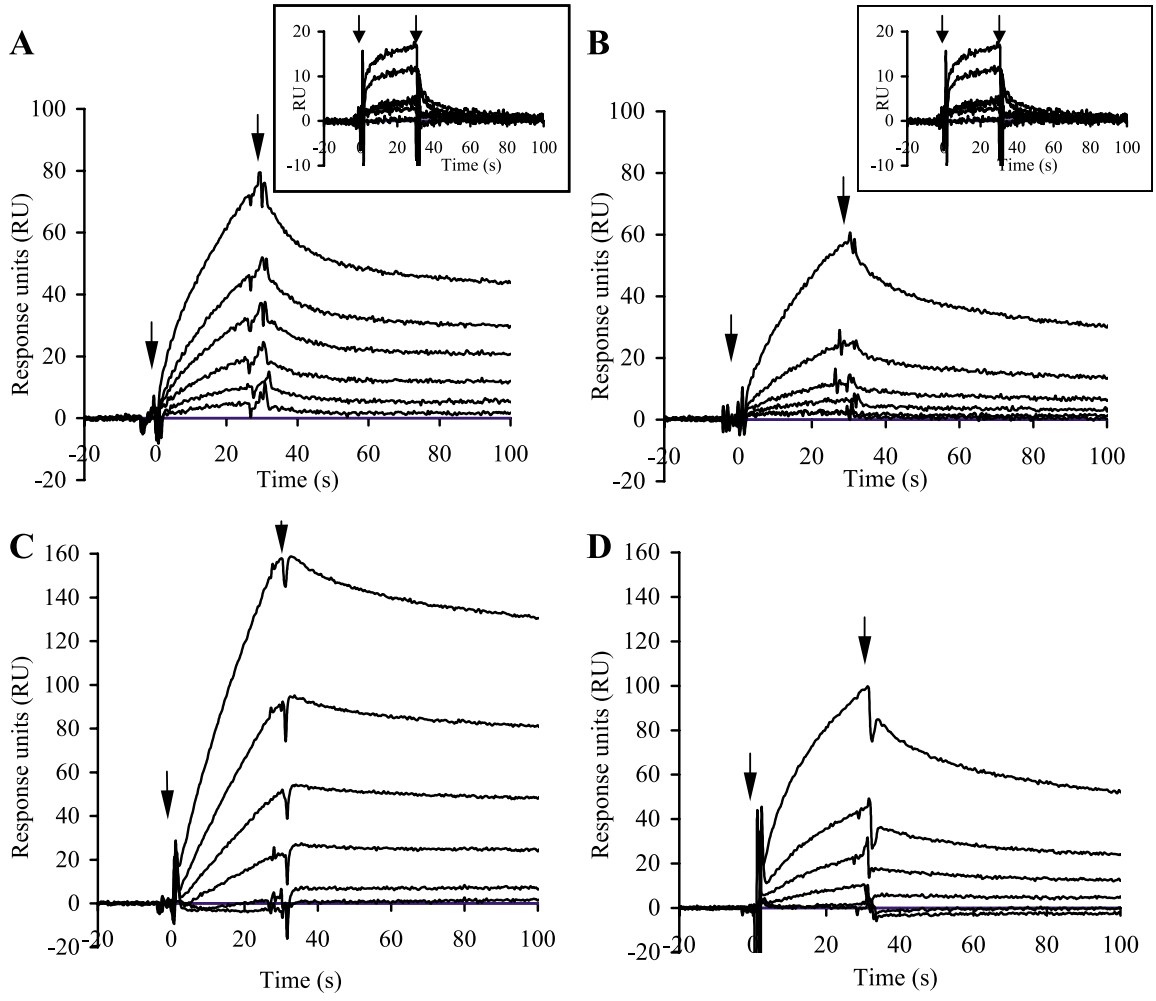

Fig. 1. Interaction of TNF-α to biglycan and decorin by surface plasmon resonance. Tissue-derived biglycan (A) and decorin (B) was injected over a TNF-α surface. Insets in A and B show the flow over a surface with immobilised TGF-β. Biglycan and decorin were also subjected to digestion with chondroitinase ABC prior to injection over the TNF-α surface (C and D respectively). Each chromatogram shows seven different analyte concentrations of 0, 5, 10, 25, 50, 100 and 250 μg/ml. Arrows indicate the injection start and end points. One representative experiment is shown, $n = 3–5$.

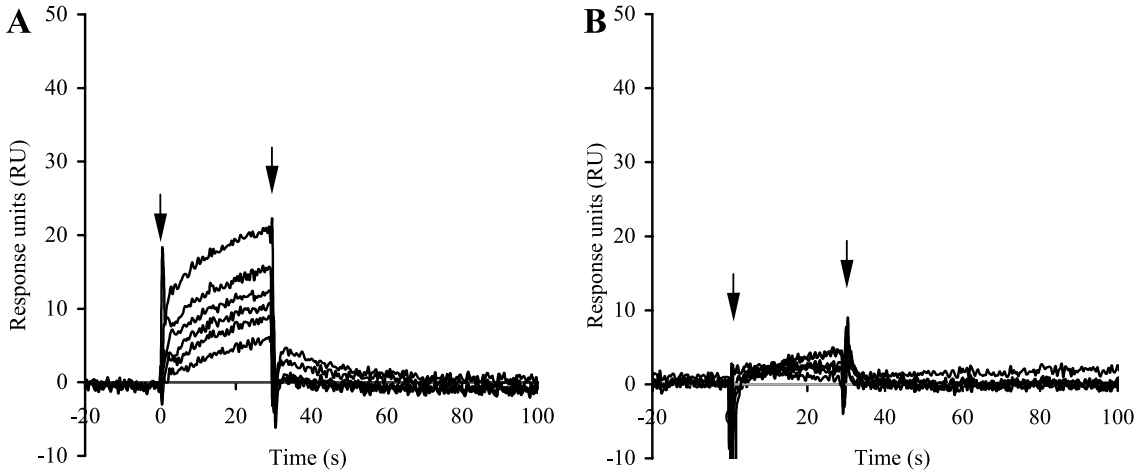

Fig. 2. Interaction of TNF-α to DS and CS by surface plasmon resonance. Tissue-derived DS (A) or CS (B) was injected over a TNF-α surface. Each chromatogram shows seven different analyte concentrations of 0, 5, 10, 25, 50, 100 and 250 μg/ml. Arrows indicate the injection start and end points. One representative experiment is shown, $n = 3–5$.

to 0.26 (±0.08) μM and 0.81 (±0.59) μM respectively, indicating a stronger binding of the core proteins to TNF-α compared to the intact, undigested proteoglycans. Injections with chondroitinase ABC alone gave no signal, verifying the enzyme's lack of binding to TNF-α.

We also observed that there was no difference in binding to TNF-α between the native recombinant and non-native, tissue-derived proteoglycans (data not shown), excluding effects of structural changes during purification.

To further evaluate the binding between TNF-α and biglycan and decorin, different GAG chain compositions, DS and CS, were applied to the TNF-α surface. DS bound to TNF-α in a similar manner as the core protein, albeit to a lesser degree (Fig. 2A) while CS did not bind at all (Fig. 2B). This binding of DS to TNF-α showed a K_d of 10.53 (±5.61) μM. From this we can conclude that both biglycan and decorin bind TNF-α preferentially via their core proteins, but also via their DS chains.

These results were compared to the data published on TGF-β binding to biglycan and decorin [3]. Our experiments showed that both biglycan (inset in Fig. 1A) and decorin (inset in Fig. 1B) bound to TGF-β as expected, with K_ds of 1.57 μM and 0.12 μM respectively, in accordance with the previous study [3].

3.2. Solid phase assay of interactions

The K_d values of the binding of biglycan and decorin to TNF-α were also analysed by Scatchard plots in order to compare the surface plasmon resonance results. The Scatchard plots for the binding of biglycan (Fig. 3A) and decorin (Fig. 3B) to TNF-α gave K_ds of 0.21 (±0.083) μM and 0.054 (±0.016) μM respectively, which is in accordance with K_d values obtained by surface plasmon resonance.

In addition, radiolabelled recombinant biglycan, decorin and tissue-derived DS showed binding to TNF-α that had been added to wells in different concentrations. Although we do not know how much of the added TNF-α was adsorbed, it was possible to confirm the binding results obtained using surface plasmon resonance. The amount of bound proteoglycan, both biglycan and decorin, was shown to be increased with increasing amount of added TNF-α (Fig. 4A,B). Moreover, there was a significant increase in specific binding of DS. CS did not bind at all, showing the importance of iduronic acid content, which was also observed by surface plasmon resonance (Fig. 4C).

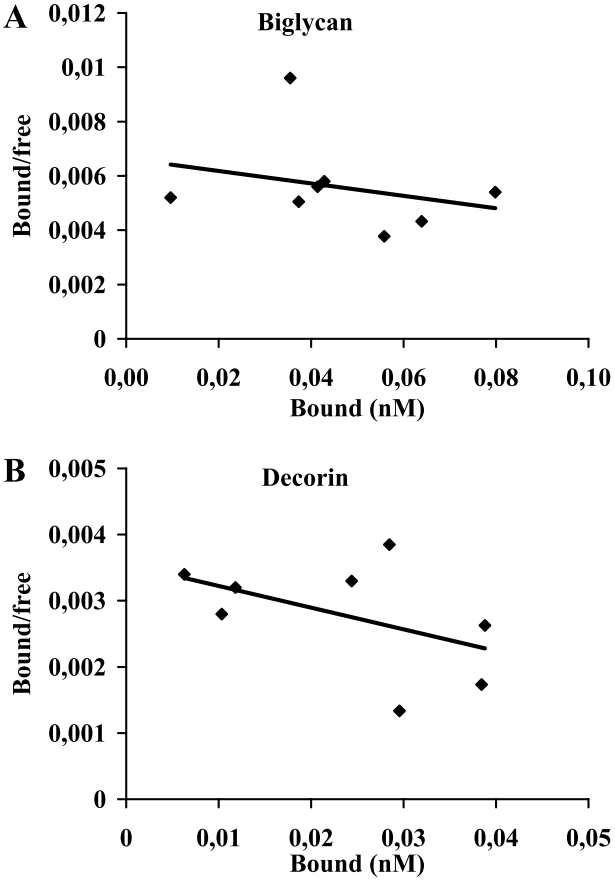

Fig. 3. Scatchard plots of the binding of radiolabelled recombinant biglycan and decorin to TNF-α. Recombinant human TNF-α was added to microtitre plates at a concentration of 250 ng/ml. [^{35}S]Sulphate-labelled recombinant biglycan (A) or decorin (B) was allowed to interact, and bound material was quantified as radioactivity. Bound material was plotted against bound/free material. One representative experiment is shown, $n = 4$.

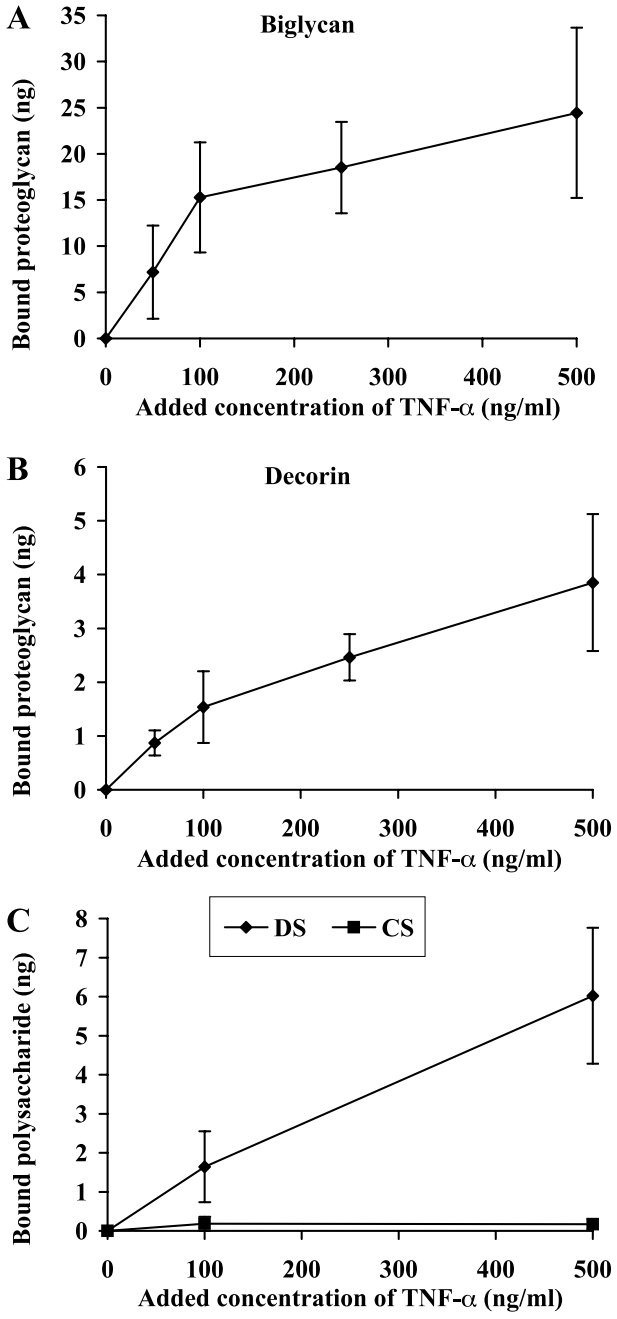

Fig. 4. Binding of radiolabelled recombinant biglycan and decorin to TNF-α in solid phase assay. Recombinant human TNF-α was added to microtitre plates at different concentrations (0–500 ng/ml). [^{35}S]Sulphate-labelled recombinant biglycan (A) and decorin (B) and [^{125}I]iodine-labelled DS and CS (C) were allowed to interact, and bound material was quantified as radioactivity. All values were plotted against added concentration of TNF-α. Data are shown as the mean ± S.E.M., $n = 3$–10.

4. Discussion

The data presented here show for the first time an interaction between the cytokine TNF-α and the small proteoglycans biglycan and decorin. We also demonstrate that TNF-α preferentially uses the core protein of biglycan and decorin as a specific binding site, demonstrated by the stronger affinity of the core protein compared to the intact proteoglycan. In addition, there is also a certain degree of binding of TNF-α to DS chains, although this is weaker than the binding to the core protein. The DS chains display more changes in configuration due to a more mobile structure due to its iduronic acid content compared to the more rigid structure of CS. Thus the waving DS chain has the possibility of first catching the TNF-α molecules and subsequently allowing them to interact more specifically and more strongly to the protein cores.

We also compared binding of tissue-derived proteoglycans, purified under denaturing conditions, to native recombinant proteoglycans. Previously, the importance of secondary structure in some of the interactions of biglycan was demonstrated [21]. We therefore used non-native, tissue-derived biglycan and decorin (fully glycosylated and with an iduronic acid content of 50% compared to glucuronic acid) in comparison with native, recombinant biglycan and decorin (not fully glycosylated with an iduronic acid content of 60% compared to glucuronic acid). In this case, we showed that there was no difference in the binding of TNF-α to either native or non-native biglycan and decorin.

Biglycan and decorin are differently epimerised in different tissues, resulting in a varying iduronic acid content. For example, human lung fibroblasts secrete DS with an iduronic acid content of about 75% compared to glucuronic acid [19], while in human embryonic skin fibroblasts it is only 21% [22]. Different tissues have different levels of iduronic acid and we therefore hypothesise that in some tissues this can give biglycan and decorin a stronger affinity for TNF-α. This in turn may affect the inflammatory activities of the cytokine differently.

This is the first time the interactions of the pro-inflammatory cytokine TNF-α with proteoglycans have been reported. TNF-α is upregulated early in inflammatory processes, as is TGF-β. Similarly, biglycan is upregulated during early inflammation, while decorin is downregulated. In the later stages of inflammation, when fibrotic lesions are formed, biglycan is downregulated and an upregulation of decorin is observed. This has been demonstrated in vivo in several pathological processes such as asthma [23] and systemic sclerosis [24]. It is well known that cytokines influence the expression and turnover of specific matrix molecules. We have previously shown that TNF-α upregulates the expression of biglycan and downregulates the expression of decorin in lung fibroblasts [10]. These matrix molecules are located differently, with biglycan having a pericellular location while decorin is bound to the collagen network [25]. Though both biglycan and decorin bind to TNF-α, the binding could have different functions. One possibility is that decorin functions as a reservoir for sequestering TNF-α out in the extracellular matrix. In inflammation, there is an increase in the expression of TNF-α, as well as a decrease in decorin that reduces this reservoir, resulting in an additional release of TNF-α. Partial proteolysis of decorin after the action of matrix metalloproteinases could be the explanation for releasing immobilised TNF-α [6]. Furthermore, the upregulation of biglycan, which at its pericellular location binds TNF-α, could augment a higher concentration of TNF-α closer to the cell, promoting association of TNF-α to its cell surface receptors.

Furthermore, our data show that there is no significant difference in the binding of TNF-α to biglycan compared to decorin. This is in contrast to the previously reported binding of TGF-β to biglycan and decorin [3], which shows a stronger

binding to the latter proteoglycan. In contrast to our data, where the binding occurs via both the GAG chain and the core protein, the interaction with TGF-β has been shown to be mediated only via the core protein, favouring decorin due to only one GAG chain as steric hindrance. Interestingly, decorin could be used to inhibit TGF-β-induced lung fibrosis in rats while biglycan could not [26]. We conclude that this difference in binding affinity in the interactions between different proteoglycans and cytokines could be of importance in future therapies.

In summary, we conclude that immobilisation of cytokines in the extracellular matrix influences their possibility to reach their cell surface receptors during different pathological conditions.

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