Analysis of syndecan-1 and TGF-β expression in the nasal mucosa and nasal polyps

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Abstract

Objective: Increased understanding of cytokines and their associated proteoglycans will contribute to the investigation of the formation of nasal polyps. Recently, some studies have suggested that syndecan-1 ectodomains are shed in response to low respiratory infection, but no studies regarding nasal and paranasal diseases have been reported. Transforming growth factor-beta (TGF-β) is involved in the regulation of nasal polyps, especially in processes crucial to the initiation, maintenance, and resolution of inflammatory responses. In the nasal mucosa and nasal polyps, we analyzed the expression of syndecan-1, which readily promotes infection, and TGF-β, which plays a role in syndecan-1 activity.

Methods: Fifteen patients who underwent turbinectomy for the treatment of nasal obstruction and seventeen patients with nasal polyps who underwent nasal endoscopic sinus surgery were included in this study. The localization of syndecan-1 and TGF-β in the nasal mucosa and nasal polyps was investigated by immunohistochemistry, and mRNA transcript levels of syndecan-1 and TGF-β were examined using quantitative real-time PCR.

Results: Immunohistochemical staining revealed that the expression of syndecan-1 in the nasal mucosa and nasal polyps was co-localized with TGF-β. The mean mRNA expression values for syndecan-1 and TGF-β were higher in nasal polyps compared to the nasal mucosa.

Conclusions: This is the first report showing expression of syndecan-1 in the nasal mucosa and nasal polyps. In nasal polyps, syndecan-1 expression may be increased by an unknown mechanism, permitting infection and inducing larger nasal polyps. We hypothesize that the accumulation of TGF-β, which is involved in the pathophysiological development of nasal polyps, may result in a change in the binding properties of syndecan-1 at inflammatory sites.

Keywords: Rhinosinusitis; Syndecan-1; Immunohistochemistry; Nasal mucosa; Heparin sulphate

1. Introduction

Chronic rhinosinusitis is one of the most frequent chronic diseases in humans, and nasal polyps are almost always present in conjunction with chronic rhinosinusitis [1]. Although present in the nose and paranasal sinuses, the presence of nasal polyps is a clinically and pathophysiologically distinct chronic inflammatory disease [2] causing nasal obstruction, rhinorrhea, and loss of smell. Nasal polyps are characterized by proliferation of the epithelial layer, glandular hyperplasia, thickening of the basement membrane, edema, focal fibrosis, and cellular infiltration of the stroma layer [3]. The molecular changes required for nasal polyp development have been recently investigated, and have been mainly described as changes in the expression of cytokines, chemokines, and eicosanoids, which are induced by several types of inflammation.

Recently, heparan sulphate proteoglycans (HSPGs) present in the inflammatory microenvironment have been shown to induce several cellular responses by binding to several specific surface heparin-binding proteins [4].

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Heparan sulfate (HS) and heparin are members of the glycosaminoglycan group of complex polysaccharides [5]. The biological roles of heparin/HS are highly diverse, and include the well-known anticoagulant action, association with various biological processes of growth factors and cytokines, as well as cell adhesion, recognition, migration, and regulation of various enzymatic activities [6]. Syndecans, a member of the group of HSPGs, are involved in regulation of cell proliferation and control of cell behavior in association with cytoskeletal organization [7]. Syndecans are a family of integral membrane proteoglycans with conserved membrane-spanning and intracellular domains, but with structurally distinct extracellular domains (ectodomains). Syndecans function as HS co-receptors in fibroblast growth factor signaling and link cells directly to the extracellular matrix (ECM). Four different syndecans, designated syndecans 1–4 (syndecan, fibroglycan, N-syndecan, and amphioglycan, respectively), are known. Syndecan-1 and syndecan-3 interact with midkine, a growth and differentiation factor [8]. Syndecan-2 is highly expressed in areas of high morphogenetic activity, such as epithelial–mesenchymal interfaces and prechondrogenic and preosteogenic mesenchymal condensations [9]. Syndecan-4 functions cooperatively with integrins in the processes of cell spreading and focal adhesion assembly [10]. Syndecan-1 is the major cell surface HSPG of epithelial cells, and it can serve as a primary receptor for some ligands [11]. However, syndecan-1 often functions as a co-receptor by acting as a cell surface scaffold that catalyzes the binding between ligands and their respective signaling receptors. Syndecan-1 also functions as a soluble HSPG when proteolytically cleaved in the juxtamembrane region and released into the extracellular environment in a process known as ectodomain shedding [11]. In mice, syndecan-1 ectodomains are shed in response to lung infection by Pseudomonas aeruginosa, where they bind and inhibit host defense factors (e.g., antimicrobials) to enhance bacterial virulence [12], indicating that syndecan-1 exacerbates infectious inflammation.

Some reports [13–16] have shown that certain cytokines are involved in syndecan-1 activity. For example, transforming growth factor-beta (TGF-β) alters the binding properties of syndecan-1 [13], and may be involved in the pathophysiological development of nasal polyps [17–19]. In addition, we suggest that TGF-β enhances syndecan-1 function, which promotes formation and growth of nasal polyps. In the lung, syndecan-1 interferes with host defense by inhibiting the activity of antimicrobial peptides [12]. Similarly, syndecan-1 may inhibit antimicrobial peptides in the nasal mucosa, promoting infectious inflammation of the nasal mucosa, and formation and growth of nasal polyps.

Thus, we hypothesized that overexpression of syndecan family members, especially syndecan-1, may be a factor involved in increasing the size of nasal polyps, and that TGF-β may be associated with polyp formation by altering syndecan-1 activity. For the first time, we investigated the expression of syndecan-1 in the nasal mucosa and nasal polyps, and examined whether syndecan-1 expression is correlated with TGF-β expression.

2. Materials and methods

2.1. Patients

Biopsies of the inferior turbinate mucosa were obtained from 15 patients (7 males and 8 females; age range, 29–71 years).
years) who underwent turbinectomy for the treatment of nasal obstruction. All patients had a nasal obstruction due to irreversible changes in the inferior turbinate or a deflected nasal septum. Patients with allergic rhinitis and chronic rhinosinusitis were excluded. Biopsies of nasal polyps were obtained from 17 patients (9 males and 8 females; age range, 24–76 years) who were diagnosed with chronic rhinosinusitis and who underwent nasal endoscopic sinus surgery. None of the patients had a history of allergic symptoms, and all had a negative radioallergosorbent test. No patients had received oral corticosteroids or antibiotics for 1 month prior to surgery. The Institutional Review Board of the University of Hiroshima School of Medicine approved the study.

2.2. Immunohistochemistry

Tissue specimens were fixed in Carnoy’s fluid, dehydrated, frozen in OCT mounting medium (Sakura Finetechical Co., Ltd., Tokyo, Japan), serially sectioned at 5 μm, and mounted on glass slides. For antigen retrieval, sections were immersed in HistVT One (Nacalai Tesque, Kyoto, Japan) at 70 °C for 40 min. For peroxidase staining, sections were immersed in 0.3% H2O2 for 30 min at room temperature to block endogenous peroxidase activity. After pretreatment with blocking serum, the specimens were incubated with a goat polyclonal antibody to syndecan-1 (1:40, RD Systems, Minneapolis, MN, USA) or a rabbit polyclonal antibody to TGF-β (1:200, Torrey Pines Biolabs, East Orange, NJ, USA) at 4 °C overnight. Immunostaining was performed using the streptavidin-biotin amplification technique (ChemMate kit; Dako, Glostrup, Denmark). Peroxidase activity was visualized by immersing tissue sections in diaminobenzidine (DAKO, Glostrup, Denmark), resulting in a brown reaction product. Sections were counterstained with Mayer’s haematoxylin for 10 min. Control specimens were incubated with PBS instead of the primary antibodies.

Fig. 2. Double staining for syndecan-1 (red) and TGF-β (green) in the nasal mucosa. Syndecan-1 showed extensive co-localization with TGF-β in the epithelium (A), as well as the nasal glands and inflammatory cells in the subepithelial region (B) (original magnification 200×). Control specimens were incubated with PBS instead of primary antibodies. Staining was negative. (C) The epithelium and (D) the glands (original magnification 200×).
2.3. Double-staining study

For the double-staining studies, the sections were first labeled with syndecan-1 antibody (1:40) at 4 °C overnight, followed by labeling with Alexa Fluor 555 rabbit anti-goat secondary antibody (1:200, Invitrogen, Tokyo, Japan) at room temperature for 1 h. Sections were then labeled with primary antibodies to TGF-β (1:200) at 4 °C overnight, followed by labeling with Alexa Fluor 488 goat anti-rabbit secondary antibody (1:200, Invitrogen, Tokyo, Japan) at room temperature for 1 h. The specimens were viewed with a confocal laser scanning microscope equipped with an appropriate filter set. Control specimens were incubated with PBS instead of the primary antibodies.

2.4. Real-time PCR

Tissue specimens were minced with scissors as soon as possible after retrieval, treated in RNAlater (Ambion, Austin, TX, USA) at once, and stored at −40 °C. TaqMan Gene Expression Assays for syndecan-1 (Hs00896423_m1) and GAPDH (Hs99999905_m1) were purchased from Applied Biosystems (Foster City, CA, USA), and mRNA levels were quantified in triplicate using the Applied Biosystems 7300 Real-Time PCR system according to the supplier’s recommendations. The absolute values for syndecan-1 were normalized to the values of GAPDH, and the relative value from the vehicle-treated control group was designated one arbitrary unit.

2.5. Statistical analysis

The data were assumed to not be normally distributed, and therefore, comparisons between clinical groups for gene expression levels were made using the non-parametric Mann–Whitney U-test. A P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Immunohistochemistry

3.1.1. Nasal mucosa

Nearly all of the mucosa was composed of epithelial mucosa and submucosa, including the nasal gland.

3.1.2. Syndecan-1 (Fig. 1A and B)

The nasal glands and inflammatory cells were positive for syndecan-1, and the blood vessels and fibroblasts were negative. In the epithelium, ciliary cells were strongly stained. The basal membrane was negative.

Fig. 3. Double staining for syndecan-1 (red) and neutrophil elastase (green) in the nasal mucosa. Syndecan-1 showed extensive co-localization with neutrophil elastase in the subepithelial region (original magnification 400×).
3.1. TGF-β (Fig. 1C and D)

The blood vessels, nasal gland, and inflammatory cells were positive for TGF-β, and fibroblasts were negative. In the epithelium, ciliary cells were positively stained. The basal membrane was negative.

Staining of control specimens was negative (Fig. 1E and F).

In the double-staining study (Fig. 2A and B), syndecan-1 was extensively co-localized with TGF-β in the epithelium and the nasal glands.

Staining of control specimens was negative (Fig. 2C and D).

To identify the type of inflammatory cells that were positive for syndecan-1 in the nasal mucosa, a double-staining study was performed. Syndecan-1 showed extensive co-localization with neutrophil elastase (Fig. 3) and eosinophil major basic protein (MBP) (Fig. 4) in the subepithelial region. This observation suggested that syndecan-1 is expressed in neutrophils and eosinophils, and may play a role in their function.

3.2. Nasal polyps

Nearly all polyps were composed of epithelial mucosa and submucosa, including the seromucous glands and blood vessels.

3.2.1. Syndecan-1 (Fig. 5A and B)

Almost the same epithelial staining pattern as described for the nasal mucosa was found. The seromucous glands were positive. The basal membrane and fibroblasts were negative. Endothelial cells in the blood vessels were positive for syndecan-1. Syndecan-1-positive inflammatory cells were observed in the subepithelial region. Pseudocyst formation, when present, was positively stained.

Staining of control specimens was negative (Fig. 5E and F).

In the double-staining study (Fig. 6A and B), syndecan-1 was extensively co-localized with TGF-β in the epithelium, the seromucous glands, and inflammatory cells in the subepithelial region.

Staining of control specimens was negative (Fig. 6C and D).

Fig. 4. Double staining for syndecan-1 (red) and MBP (green) in the nasal mucosa. Syndecan-1 showed extensive co-localization with MBP in the subepithelial region (original magnification 400×).
3.3. Real-time PCR

Quantitative real-time PCR was conducted to determine syndecan-1 and TGF-β mRNA transcript levels. We normalized the values of all target genes to GAPDH in all tissues, and compared them to the expression levels of syndecan-1 and TGF-β in the nasal mucosa and nasal polyps.

3.4. Syndecan-1

The mean mRNA expression values for syndecan-1 were significantly higher in nasal polyps (NP) compared to the nasal mucosa of the inferior turbinate (IT; Fig. 7A; \( p < 0.01 \)).

3.5. TGF-β

The mean mRNA expression values for TGF-β were significantly higher in nasal polyps (NP) compared to the nasal mucosa of the inferior turbinate (IT; Fig. 7B; \( p < 0.05 \)).

4. Discussion

In this study, we describe the expression of syndecan-1 in the nasal mucosa and in nasal polyps. Cell surface HSPGs are ubiquitous and abundant receptors/co-receptors for extracellular ligands \([13,20]\), including many microbes \([21,22]\). These receptors transmit signals into the cell and often use cell surface HS to recognize their ligands or to regulate their activation. Cell surface HS provides cells with a mechanism to bind a wide variety of extracellular effectors without requiring multiple novel binding proteins. HS is synthesized on a variety of cell surface proteins but is found most abundantly on members of two major families of membrane-bound proteoglycans: the syndecans and the glypicans \([11]\).

The prototypic cell surface HSPG syndecan-1 was first identified as a developmentally regulated type-I transmembrane protein that bound ECM components to epithelial cells \([23,24]\). Recent studies \([13–16]\) have suggested that syndecan-1 ectodomains may play a proinflammatory role by regulating the activities of various inflammatory mediators. In mice, syndecan-1 ectodomains are shed in response to lung infection by \(P. aeruginosa\), where they bind and inhibit host defense factors (e.g., antimicrobials), resulting in enhanced bacterial virulence \([12]\). In a mouse model of acute lung injury, syndecan-1 shedding promoted airway inflammation by inducing the generation of a CXC chemokine gradient that guides the transepithelial migration of neutrophils into the alveolar spaces \([25]\). Thus, syndecan-1 is involved in infectious inflammation in the lung. Similarly, syndecan-1 in the nasal mucosa may be involved in rhinosinusitis and nasal polyps. We observed that the mean mRNA expression values for syndecan-1 were higher in nasal polyps compared to the nasal mucosa using real-time PCR. In nasal polyps, the expression of syndecan-1 may be increased by an unknown mechanism that promotes infection and increases the size of nasal polyps. Similar to the lung, we suggest that one possible mechanism for syndecan-1 to promote infection in nasal polyps is to inhibit antimicrobial peptides in the nasal mucosa, which promotes infectious inflammation of the nasal mucosa, and formation and growth of nasal polyps.

Cytokines are diffusible, soluble factors with pleiotropic actions. HSPGs present on either the cell surface or in the ECM enhance the function of heparin-binding cytokines and chemokines by immobilizing and presenting them to their receptors on target cells. HSPGs also: (1) increase the concentration of cytokines by binding them at the
appropriate location where they encounter their target cells; (2) protect cytokines from chemical and physiological stimuli; and (3) induce conformation-dependent association of cell surface molecules by binding to them. Furthermore, since many interactions between HSPGs and cytokines are highly specific, HSPGs promote the assembly of appropriate molecular complexes and initiate signal transduction [26]. Variation in the biochemical composition of HSPGs determines the specificity of binding of cytokines. A number of studies [13–16] have identified interactions between syndecan-1 and certain cytokines or chemokines. Most of the functions of syndecan-1 are related to its ability to recognize extracellular effector molecules. Syndecan-1 interacts with several ECM components and various factors associated with cellular growth control [15]. Syndecan-1 ectodomains bind and inhibit CC chemokines that potentiate the accumulation of Th2 cells in the lung [27].

The TGF-β family is one of the largest families of secreted multifunctional peptides that exerts an array of biological effects in many cell types. TGF-β is involved in the regulation of processes crucial to the initiation, maintenance, and resolution of inflammatory responses.

Fig. 6. Double staining for syndecan-1 (red) and TGF-β (green) in nasal polyps. Syndecan-1 showed extensive co-localization with TGF-β in the epithelium (A), as well as the seromucous glands and inflammatory cells in the subepithelial region (B) (original magnification 200×). Control specimens were incubated with PBS instead of primary antibodies. Staining was negative. (C) The epithelium and (D) the glands (original magnification 200×).
The effects of TGF-β and related molecules include inhibition of proliferation of many cell types [30], regulation of immune responses, stimulation of ECM formation [31], and chemotaxis of inflammatory cells [32,33]. TGF-β also plays a role in epithelial cell regeneration, inflammation, and tissue repair [34]. TGF-β can act as a chemoattractant and activator of inflammatory cells during the early immune response, but also is known to promote downregulation of inflammation through the inhibition of cell activation and promotion of apoptosis [35].

In contrast, many reports have suggested that TGF-β promotes formation and growth of nasal polyps. Production of eotaxin-1, which is an eosinophil migration factor, was markedly increased when the combination of TGF-β1 and IL-13 was added fibroblasts [18], suggesting that TGF-β may promote eosinophil infiltration into nasal polyps. Myofibroblast accumulation induced by TGF-β is involved in the pathogenesis of nasal polyps, and TGF-β is a potent inducer of myofibroblasts [19]. Thus, TGF-β that is released from inflammatory cells and promotes eosinophil infiltration may induce ECM accumulation through myofibroblasts, increasing the size of nasal polyps.

In this study, we showed co-localization of syndecan-1 with TGF-β, which suggests an interaction between these two molecules. Because TGF-β affects the glycosaminoglycan composition of syndecan-1 [13], it may also influence the binding properties of syndecan-1 with molecules such as growth factors. We observed that the mean mRNA expression levels of syndecan-1 and TGF-β were higher in nasal polyps compared to the nasal mucosa. These results suggest that syndecan-1 may be intimately associated with TGF-β, consistent with a previous report [13]. We hypothesize that accumulation of TGF-β may result in a change in the binding properties of syndecan-1 in nasal polyps, which activates the inhibition level of antimicrobial peptides in the mucosa of nasal polyps. Therefore, we also hypothesize that these mechanisms may lead to the development of additional nasal polyps.

Syndecan-1 is involved not only in infectious inflammation, but also in allergic inflammation. A recent report [27] showed that deletion of syndecan-1 results in exacerbated allergic lung disease after an airway allergen challenge. However, when purified, intact syndecan-1 ectodomains containing heparin are coapplied with allergens, allergic lung disease is significantly suppressed [27]. Shedding of syndecan-1 ectodomains is an endogenous mechanism that has evolved in the airway epithelium to protect the host from tissue damage associated with excessive allergic inflammation [27]. It will be important to examine the possible involvement of syndecan-1 in allergic rhinitis in future studies.

5. Conclusion

This study shows for the first time that the nasal mucosa and nasal polyps express syndecan-1. Intact syndecan-1 ectodomains and heparin may control allergic or infectious inflammation, which is caused by syndecan-1–cytokine and syndecan-1–chemokine interactions that also involve TGF-β. The function of syndecan-1 in rhinology is not well understood. Examination of syndecan-1 expression in
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References
