Chapter

Fibroblasts in Sjögren's Syndrome

Kerstin Klein

Abstract

The Sjögren's syndrome is an autoimmune disease characterized by chronic inflammation of the exocrine glands, leading to dryness of mucosal surfaces, and often to severe systemic manifestations. Here, the immunomodulatory function of fibroblasts derived from salivary glands, a primary site affected by the Sjögren's syndrome, is discussed. Specific subsets of these fibroblasts drive the formation of tertiary lymphoid structures, which are associated with severe disease and which constitute a risk factor for the development of lymphoma in Sjögren's syndrome. Single cell RNA-sequencing has provided new insights into subsets of fibroblasts in inflamed salivary glands and has provided evidence for the existence of shared inflammation-associated fibroblasts across chronically inflamed tissues. These findings support the concept of targeting the fibroblast compartment in Sjögren's syndrome and other chronic inflammatory diseases. In addition to the immunomodulatory role of fibroblasts, the interaction of the epithelium with fibroblasts is essential for salivary gland homeostasis. Fibroblasts provide essential signals for the regeneration of salivary gland epithelial cells, which is disturbed in Sjögren's syndrome, and leading to the loss of saliva secreting cells and subsequent hyposalivation.

Keywords: Sjögren's syndrome, autoimmunity, inflammation, tertiary lymphoid structure, salivary gland

1. Introduction

1.1 Sjögren's syndrome

The Sjögren's Syndrome (SjS) is a systemic autoimmune disease, most commonly presenting between the fourth and six decades of life. It affects predominantly women, with an estimated female to male ratio of 9:1 [1]. With a prevalence of 0.3 to 1 per 1000 people [1], the SjS represents the second most common rheumatic autoimmune condition after rheumatoid arthritis (RA). The SjS can either occur as single disease, often termed as primary SjS, or is associated with other autoimmune diseases, such as RA, systemic lupus erythematosus (SLE), systemic sclerosis (SSc), or dermatomyositis [2]. Up to now, no disease modifying therapies for SjS have been approved and treatment is mainly symptomatic [3].

The hallmark of SjS is a hypofunction of exocrine glands, in particular salivary and lacrimal glands [3]. Dryness of mouth (xerostomia) and eyes (xerophthalmia), alongside fatigue and pain are the major symptoms affecting more than 80% of

patients with SjS [2]. The majority of patients with SjS present with glandular symptoms, which are often present over many years before diagnosis [4]. Whilst often considered as "benign features", these symptoms underpin great patient-reported disability. Other signs of systemic dryness, with scant information regarding the etiology and affecting patients' quality of life, involve the skin, the nose, the throat, the trachea and the vagina [5].

A major classification criterion for SjS is the infiltration of salivary glands with lymphocytes (focus score ≥1, in minor labial salivary gland biopsy), a condition called sialadenitis. The second major classification criterion is the presence of anti-SSA/Ro auto-antibodies, which is mandatory in patients with a lack of sialadenitis [3]. In 30 to 40% of patients, systemic epithelial and extra-epithelial manifestations occur that can affect the joints, skin, lungs, kidneys and nervous system [2].

People with SjS have increased morbidity and mortality compared to the general population [4]. Although being a rare event, the risk of B-cell lymphomas is 15 to 20 times higher in patients with SjS as the general population and accounts for the leading cause with an impact on patients's survival [2, 4]. The most common type of lymphomas in patients with SjS are mucosa-associated lymphoid tissue (MALT) lymphomas. Chronic activation of B cells at the primary sites affected by SjS, such as the salivary glands, was attributed to the development of lymphoma. Several risk factors have been defined for the development of lymphoma in patients with SjS; among them is the presence of ectopic germinal centers in tertiary lymphoid structures (TLS) [2].

1.2 Epithelial cell activation is central in the pathogenesis in SjS

The SjS develops in genetically predisposed individuals upon exposure to stress factors. Hormones as well as infectious agents, and in particular viruses, are assumed to play key roles in the pathogenesis of the SjS. Activated epithelial cells in salivary glands are the central cell type in the current concept underlying the pathogenesis. They are on the one hand drivers of the ongoing inflammation and on the other hand, due to the excess of apoptosis of epithelial cells in salivary glands, a source of auto-antigens for infiltrating lymphocytes [6]. Epithelial cells respond to and produce pro-inflammatory cytokines and chemokines and thus, promote inflammation. They produce MHC-II and co-stimulatory molecules, enabling them to directly interact with and activate T-cells. Furthermore, they produce B-cell activating factor (BAFF), inducing the activation and survival of B cells [7]. Many of these characteristics have been previously described for fibroblasts, e.g. in the synovium of rheumatoid arthritis patients, and analogies of synovial fibroblasts with salivary gland-derived fibroblasts have been recognized [7, 8]. However, the ability of salivary gland-derived fibroblasts to exert similar functions is only at the beginning to be characterized in detail.

2. Fibroblasts in tertiary lymphoid structures

2.1 Tertiary lymphoid structures are often present in autoimmune diseases

TLS, or ectopic lymphoid organs (ELS), often develop at sites of inflammation in target tissues. Their formation has been associated with chronic inflammation, autoimmune disease, cancer, and transplant rejection [9]. TLS are sites of ectopic

autoantibody production and expansion of potential autoreactive B cell clones [7, 10]. The formation of TLS in salivary glands is an established model for studying TLS formation in autoimmunity in general. The frequency of the presence of TLS varies among different autoimmune diseases, with a high frequency in autoimmune thyroiditis and low presence in systemic lupus erythematosus [10]. Approximately 30–40% of patients with SjS exhibit TLS in their salivary glands, the primary sites of the disease [11, 12]. A similar percentage of TLS is found in patients with rheumatoid arthritis, in which TLS are associated with a lympho-myeloid pathotype that represents a distinct disease entity as the diffuse myeloid and pauci-immune fibroid pathotpyes [13]. Hence, the presence and absence of TLS in salivary glands of patients with SjS might underlie different pathophysiological processes in different, not yet characterized, disease subsets. The formation of TLS is across autoimmune diseases associated with more severe disease and poor prognosis [7].

TLS often share several typical structural characteristics with secondary lymphoid tissues (lymph nodes, tonsils, spleen, Peyer's patches, mucosa-associated lymphoid tissues), including highly organized lymphocytic aggregates, with T and B cell segregation, the development of high endothelial venules, and follicular dendritic cell networks. In contrast to secondary lymphoid tissues, the lymphocytic aggregates found in TLS can range from a simple aggregates to highly ordered structures with bona fide germinal centers that support the production of autoreactive plasma cells [9, 10]. TLS formation and secondary lymphoid tissue development follow numerous overlapping signaling pathways, however, the cellular sources of signaling molecules differ [10]. In contrast to secondary lymphoid structures whose development is initiated at the embryonic stage, TLS develop postnatally in response to inflammatory signals, where they provide a specialized pro-inflammatory environment that plays a key role in perpetuating disease progression in autoimmune conditions [7, 14]. Podoplanin (pdpn)-expressing fibroblastic reticular cells in secondary lymphoid organs and $pdpn^+$ stromal fibroblasts in TLS provide signals and the scaffold structure that foster the interaction of T cells with dendritic cells, and hence drive innate and adaptive immune responses [15, 16].

2.2 Tertiary lymphoid structures are associated with severe disease in Sjögren's syndrome

The routine histopathological examination of minor salivary gland biopsies carries a substantial prognostic value regarding disease severity and outcome [17]. Higher inflammatory scores, and the presence of germinal center-like structures in particular, in salivary glands of patients with SjS were associated with more severe disease, illustrated by elevated titers of rheumatoid factor, anti-Ro/SSA and anti-Ro/SSB auto-antibodies, enhanced levels of local and systemic pro-inflammatory mediators and a reduced saliva secretion [11, 17, 18]. Germinal center-like structures have been identified in approximately 25% of patients with SjS [17, 19]. Their presence at time of diagnosis, or sole high lymphocytic scores, were shown to account as independent risk factors for the development of Non-Hodgkin's lymphomas in patients with SjS [17, 19].

Given the pivotal role of TLS in SjS, the identification of factors and pathological mechanisms triggering and regulating their formation and those of germinal center-like structures is of high interest in order to identify potential targets for drug development.

2.3 Adenoviral infection using retrograde excretory duct cannulation is a model of SjS

Studying the chronology of TLS formation and associated cell types in animal models, together with complementary evaluation of human salivary gland specimens, provided new insights into pathomechanisms associcated with sialadenitis in SjS, and unraveled the analogy of TLS formation to the development of secondary lymphoid organs. A model that proved to be of particular value for studying salivary gland inflammation in SjS, and the formation of TLS in autoimmune processes in general, is the selective submandibular gland administration of a replication-defective adenovirus 5 (AdV5) through retrograde excretory duct cannulation in wild-type C57/Bl76 mice. These mice were shown to resemble several hallmarks of SjS, including lymphocytic infiltration of salivary glands, TLS formation, anti-nuclear autoantibody (ANA) formation, and reduction in salivary flow indicative of excretory gland dysfunction [20]. Cannulated mice developed SjS-like periductal lymphoid aggregates within two weeks after AdV5 delivery. Within three weeks, the inducible TLS acquired progressively hallmarks of functional germinal centers, with segregated B and T cell areas, high endothelial venules in T-cell rich areas, and follicular dendritic cell networks in up to 70% of the lymphocytic aggregates. Local expression of activation-induced cytidine deaminase (AID), the enzyme required for Ig somatic hypermutation and class-switch recombination, pointed to the functional activation of B cells in TLS [20].

2.4 Fibroblasts drive the formation of tertiary lymphoid structures

During secondary lymphoid organ development in embryogenesis, mesenchymal precursor cell maturate into intercellular adhesion molecule-1 (ICAM-1)^{high}, vascular cell adhesion molecule-1 (VCAM-1)^{high} organizer cells, in a process that is dependent on lymphoid tissue inducer cells and lymphotoxin β receptor (LTβR) signaling. This leads to a sustained stromal cell production of interleukin 7 (IL7), C-X-C motif chemokine ligand 13 (CXCL13) and to a lesser extent C-C motif chemokine ligand 21 (CCL21) [21]. The subsequent migration of lymphocytes into the anlagen is responsible for the full differentiation of fibroblastic reticular cells within distinct areas of the secondary lymphoid organ [22–24].

In TLS of salivary glands of patients with SjS, a network of pdpn$^+$ and fibroblast activating protein (FAP)$^+$ fibroblasts were identified that support the formation of TLS [24]. The same markers have been previously identified on fibroblast reticular cells in lymph nodes [15, 25], which provide, by the secretion of CCL19 and CCL21, the key factors for the migration and retention of T cells in secondary lymphoid organs [16, 25, 26]. Among the pdpn$^+$ fibroblasts in TLS, two functionally distinct populations have been identified in human salivary glands that provide the signals for lymphocyte survival and organization within TLS, respectively (**Figure 1**) [24]. The first cluster of pdpn$^+$ fibroblasts, was characterized by high expression of FAP, ICAM-1, VCAM-1 and CD34 [24]. Pdpn+CD34+ fibroblasts in TLS produced IL7 and BAFF, underlying their function in supporting lymphocyte survival and homeostasis [24]. The second cluster of pdpn$^+$ CD34$^-$ fibroblasts was characterized by high expression levels of CXCL13, CCL19 and CCL21. Expansion of a similar network of pdpn+ fibroblasts has been observed upon salivary gland infection of mice with Ad5V. Of note, this expansion occurred before lymphocyte infiltration, suggesting a pivotal, early role for fibroblasts in SjS. Fibroblasts in TLS of cannulated mouse salivary

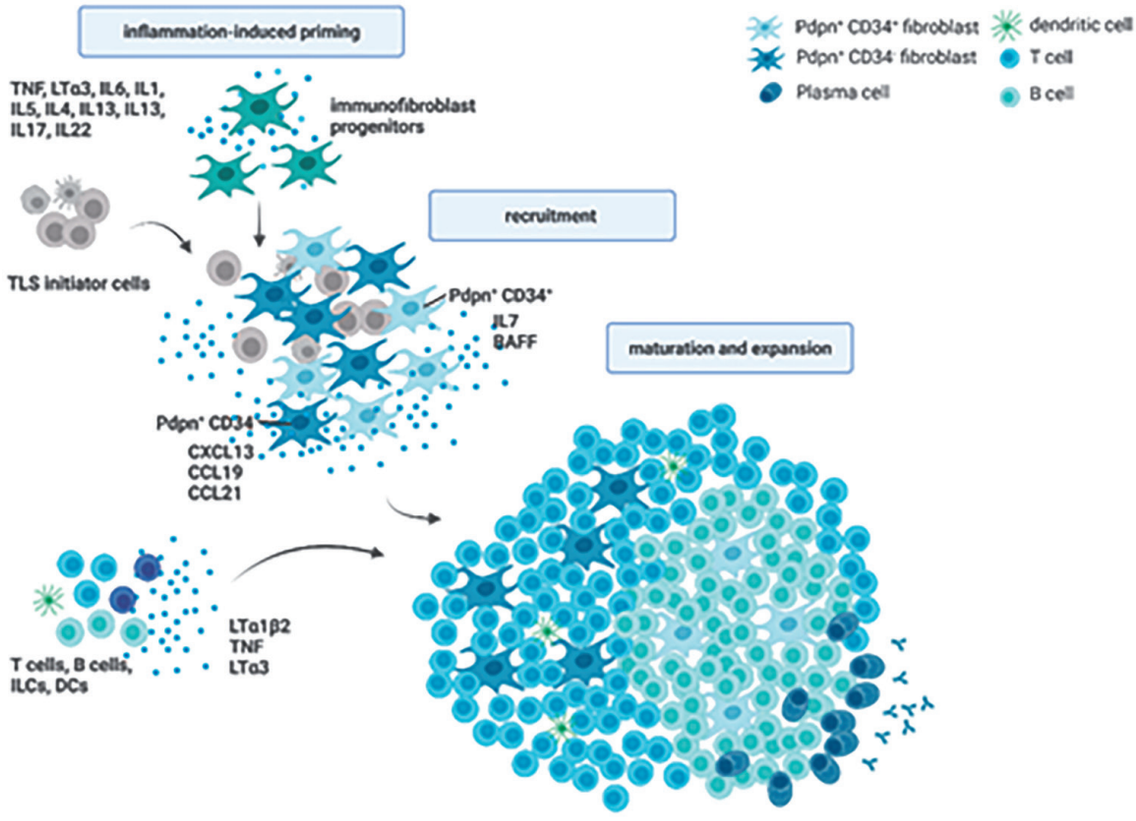

Figure 1.
Stromal fibroblast populations contribute to the formation of tertiary lymphoid structures (TLS). Created by BioRender.com. DCs, dendritic cells; ILCs, innate lymphoid cells.

glands expressed CXCL13, CCL19, BAFF, IL7 and LTβR, with an increased expression of lymphoid chemokines specifically in the ICAM-1$^+$ VCAM-1$^+$ subpopulation of pdpn$^+$ fibroblasts [24].

2.5 IL13 and IL22 induce the formation of tertiary lymphoid structures

Pdnp+ fibroblasts in human TLS expressed receptors for and responded to stimulation with IL13, IL4, IL22, TNF, and LTα1β2 [24, 27]. Elevated levels of IL13 have been detected in serum of patients with SjS, where they correlated with titers of anti-Ro/SSA auto-antibodies [28], and in Id3 knockout mice, a model for T cell mediated SjS [29, 30]. Also, high levels of IL22 in sera of patients with SjS have been shown to correlate with clinically relevant parameters, such as reduced salivary flow, hypergammaglobulinemia, as well as serum titers of rheumatoid factor, anti-Ro/SSA and anti-Ro/SSB auto-antibodies [31]. Together these data suggested a potential link between increased levels of IL13 and IL22 with the auto-antibody production in SjS.

IL13 stimulation of cultured human salivary gland fibroblasts, in synergy with TNF and LTα1β2, induced the expression of VCAM-1, ICAM-1 and pdpn *in vitro* (**Table 1**). This function was confirmed *in vivo*. Innate lymphoid cells, fibroblasts and epithelial cells have been identified as the source of IL-13 in the developing TLS. IL13 expression was induced rapidly within a few hours upon AdV5 administration in mice, leading to the priming of immunofibroblast progenitors by activation of IL4 receptor signaling, and the subsequent expression of pdpn, ICAM-1 and VCAM-1. AdV5 administration in IL4R$^{-/-}$ mice led to a disturbed TLS assembly, and salivary glands were characterized by reduced expression levels of CXCL13 and

Chemokines	Producing cells	Receptor	Function on target cells
IL13	Innate lymphoid cells Fibroblasts Epithelial cells	IL4R	Fibroblast priming, induces expression of pdpn, VCAM1-, ICAM-1
IL22	T cells Innate lymphoid cells Natural killer cells	IL22Rα	Proliferation of fibroblasts, expansion of the fibroblast network
LTα1β2	Lymphoid tissue inducer cells	LTβR	Final differentiation of fibroblasts in lymphoid structures
CXCL19	Fibroblasts	CXCR7	T cells and dendritic cells chemotaxis
CXCL21	Fibroblasts	CXCR7	Natural killer cells, dendritic cells, T cells chemotaxis
CXCL12	Epithelial cells	CXCR4	Lymphocyte retention inside the lymphoid structures
CXCL13	Fibroblasts	CXCR5	B cells, T cells chemotaxis
BAFF	Fibroblasts Epithelial cells	BAFFR	B cell activation and survival

Table 1.
Chemokines involved in the formation of tertiary lymphoid structures in salivary glands of patients with Sjögren's syndrome.

CCL21, smaller inflammatory foci and an abolished autoantibody production. The early priming of immunofibroblasts by IL13 was independent of the presence of lymphocytes [24].

In contrast to IL13, IL-22 stimulation of cultured human salivary gland fibroblasts induced proliferation but did not induce the expression of pdpn, ICAM-1 and VCAM-1 *in vitro* [24]. Studies in cannulated mice revealed that the induction of IL22 was, similarly to the induction of IL13, an early event after AdV5 delivery in TLS formation. The main sources of IL22 in the developing TLS were T cells, along with innate lymphoid cells and natural killer cells. IL22 induced the expression of CXCL13 in stromal fibroblasts and the expression of CXCL12 in epithelial cells, leading to the subsequent B cell aggregation and auto-antibody production [27]. Together these data demonstrated that IL13 mediates the early priming of fibroblasts in the developing TLS, and IL22 is responsible for the expansion of the fibroblastic network. These early steps in TLS development were independent of LTα1β2 and RORγ^{+} lymphoid tissue inducer cells [24], which regulate the final maturation of fibroblastic reticular cells in the development of secondary lymphoid organs [21]. However, studies in LTβr$^{-/-}$ and RORγ$^{-/-}$ mice have underlined their critical role in the final differentiation and stabilization of the functional phenotype of immunofibroblasts at later stages of the TLS assembly [24].

3. Overlap of fibroblasts from patients with SjS and other inflammatory diseases

3.1 Single cell RNA sequencing identifies fibroblast clusters across diseases

The asset of single cell RNA sequencing (scRNA-seq) technologies has enabled the identification of different fibroblasts populations associated with chronic inflammation across different diseases and anatomical sites [32–39]. Given the

pro-inflammatory role of fibroblasts and their ability to carry a certain degree of inflammatory memory [40], interfering with fibroblasts has become a new potential therapeutic strategy in chronic inflammatory diseases.

In a recent study, scRNA-seq data sets derived from four different inflammatory diseases, namely rheumatoid arthritis, interstitial lung disease, ulcerative colitis and the SjS were integrated, with the aim to provide a stromal cell atlas to identify pathogenic fibroblast subsets shared across diseases [8]. For each inflamed tissue, non-inflamed control tissues were included in the analysis. With respect to the SjS, biopsies derived from minor salivary gland biopsies of patients with SjS were compared to those from patients with sicca symptoms, characterized as non-autoimmune dryness, and who did not fulfill the classification criteria for SjS. Given the lack of a universal fibroblast marker, fibroblasts in this study were characterized by the expression of collagen (COL) 1A1 and defined as non-epithelial, non-immune, non-endothelial, and non-mural cells based on the respective specific markers for those cell types. By pooling the scRNA-seq data sets from salivary glands, lungs, the synovium and the gut, 14 clusters of fibroblasts have been identified, each of them consisting of genes that were shared across different tissues in addition to tissue-specific genes (**Table 2**). Among these clusters, two of them expanded across tissues in inflamed versus respective non-inflamed controls.

Cluster	Shared markers	Other markers, localization, and characteristics	Shared function
0	n.d.	SG: n.d. Lung: n.d. Synovium: $PRG4^+$ lining SF Gut: $WNT5B^+$ villus-associated GF	n.d.
1	n.d.	SG: n.d. Lung: n.d. Synovium: $THY1^+$ sublining Gut: $WNT2B^+$ crypt-associated GF	n.d.
2	n.d.	SG: n.d. Lung: n.d. Synovium: $THY1^+$ sublining Gut: $WNT2B^+$ crypt-associated GF	n.d.
3	n.d.	SG: n.d. Lung: n.d. Synovium: $THY1^+$ sublining Gut: $WNT2B^+$ crypt-associated GF	n.d.
4	$SPARC^+$ $COL3A1^+$	SG: $CD34^+$ Lung: myofibroblasts Synovium: split between $DKK3^+$ and $THY1^+$ sublining SF, $CD90^{hi}$ NOTCH3-activated, perivascular Gut: split between inflammatory and myofibroblasts	crosstalk with endothelial cells
5	$FBLN1^+$	SG: n.d. Lung: $HAS1^+PLIN2^+$ Synovium: $CD34^+THY1^+$ Gut: $RSPO3^+$	n.d.
6	n.d.	SG: n.d. Lung: n.d. Synovium: $PRG4^+$ lining SF Gut: $WNT5B^+$ villus-associated GF	n.d.

Cluster	Shared markers	Other markers, localization, and characteristics	Shared function
7	n.d.	SG: n.d. Lung: n.d. Synovium: n.d. Gut: n.d.	n.d.
8	PTGS2$^+$ SEMA4A$^+$	SG: n.d. Lung: n.d. Synovium: THY1$^+$ sublining Gut: WNT2B$^+$ crypt-associated GF	n.d.
9	CD34$^+$ MFAP5$^+$	SG: n.d. Lung: HAS1$^+$PLIN2$^+$ Synovium: CD34$^+$THY1$^+$ Gut: RSPO3$^+$	n.d.
10		SG: n.d. Lung: n.d. Synovium: PRG4$^+$ lining SF Gut: WNT5B$^+$ villus-associated GF	n.d.
11	CXCL10$^+$ CCL19$^+$	SG: CCL19$^+$PDPN$^+$ Lung: n.d. Synovium: THY1$^+$ sublining, HLA-DRA^{hi} SF Gut: RSPO3$^+$, WNT2B$^+$Fos^{hi}	interaction with immune cells
12	n.d.	SG: n.d. Lung: n.d. Synovium: PRG4$^+$ lining SF Gut: WNT5B$^+$ villus-associated GF	n.d.
13	MYH11$^+$	SG: n.d. Lung: myofibroblasts Synovium: n.d. Gut: myofibroblasts	n.d.

GF, gut fibroblast; n.d., not defined; SF, synovial fibroblast.

Table 2.
Shared fibroblasts clusters between salivary gland (SG), lung, synovium, and gut, as defined by Korsynsky et al. [8].

3.2 CXCL10$^+$ CCL19$^+$ fibroblasts: Interactors with immune cells

The first of these shared clusters is characterized by the marker genes CXCL10 and CCL19. Based on a gene set enrichment and pathway analysis, CXCL10$^+$ CCL19$^+$ fibroblasts were identified as a subset that potentially directly interacts with immune cells. Among the enriched pathways were "lymphocyte chemotaxis", "antigen presentation", and "positive regulation of T cell proliferation". Furthermore, scRNA-seq data suggested that CXCL10$^+$ CCL19$^+$ fibroblasts respond to key pro-inflammatory cytokines, including interferon (IFN) γ and IFNα, TNF, IL1, and IL1. The responsiveness to IFNγ and IFNα was specific to CXCL10$^+$ CCL19$^+$ fibroblasts [8]. This might be of high relevance in the context of the SjS, given the pronounced role of type I and II interferon signatures detected in SjS, and their association with more severe disease [41, 42]. CXCL10$^+$ CCL19$^+$ fibroblasts functionally resembled the pdpn$^+$ CD34$^-$ CCL19 expressing fibroblasts that have been described to be involved in the formation of TLS in salivary glands of patients with SjS [24].

3.3 SPARC+ COL3A1+ fibroblasts: a vascular-interacting population

The second shared cluster of fibroblasts that was identified to be expanded across inflamed tissues was characterized by the expression of secreted protein acidic and cysteine rich (SPARC) and COL3A1. $SPARC^+$ $COL3A1^+$ fibroblasts resembled a potentially endothelium-driven activated fibroblast state, characterized by the enrichment of pathways associated with extracellular matrix binding and remodeling. In addition, key developmental and morphogen signaling pathways were enriched, including hedgehog, transforming growth factor (TGF) β, WNT, bone morphogenic protein (BMP) and Notch signaling. By comparing these shared human fibroblast clusters to the temporal activation of fibroblast clusters in the mouse model of dextran sulfate sodium (DSS)-induced colitis, the expansion of $SPARC^+$ $COL3A1^+$ fibroblasts was identified as an early event in the inflammatory process, in which vascular remodeling preceded leukocyte infiltration [8].

4. Fibroblasts in the regeneration of salivary gland epithelial cells

A key process in gland development is the epithelial-mesenchymal interaction [43]. The stromal-derived extracellular matrix is essential for the growth, morphogenesis and differentiation of salivary gland tissues [44]. Extracellular matrix remodeling and fibrosis are pathological features found in minor salivary gland biopsies of patients with SjS, that are associated with salivary gland inflammation, reduced stimulated salivary flow but not with age [44–46]. Point mutations in people with hypohidrotic ectodermal dysplasia (HED) lead to a disturbed signaling between the salivary epithelium and mesenchymal fibroblasts, affecting their gland development. Salivary and sweat glands have the same embryonic origin and people with HED present with defects in salivary glands, sweat glands, teeth and hair [47].

Several studies have pointed out that the correlation of salivary flow with the degree of inflammation in salivary glands of patients with SjS is low [48–51], suggesting that other mechanisms than inflammation underlie hyposalivation in SjS. Salivary gland epithelial cells of patients with SjS are more prone to anoikis, a detachment-induced apoptosis, after activation of Toll-like receptor 3 signaling [52]. In healthy salivary glands, salivary gland stem cells reside in ducts of salivary glands and differentiate into saliva secreting acinar cells to maintain homeostasis. In SjS, salivary gland stem cells are fewer in numbers and exhibit an aged phenotype, with a reduced capacity to self-renew and proliferate [53]. This suggests that saliva production in patients with SjS might not be restored solely be the use of anti-inflammatory drugs. In regenerative medicine approaches, the co-culturing of stem cells together with fibroblasts is essential for engineering secreting salivary epithelial cells [54, 55]. Hence, fibroblasts might also be involved in the disturbed regeneration of salivary gland epithelial cells in SjS and are likely to have functions beyond promoting inflammation.

5. The potential role of fibroblasts in extra-glandular manifestations of the SjS

Extra-glandular manifestations are found in 30–40% of patients with SjS, and can be divided into epithelial and extra-epithelial manifestations that can affect the central

and peripheral nervous system, the lungs, lymph nodes, kidneys, joints, the skin and the muscles [2, 4]. A role of fibroblasts in extra-glandular manifestations has not been studied yet, maybe due to limited assess of available tissue samples from affected sites.

Articular manifestations, such as arthralgias and synovitis, are the most common extra-glandular manifestations and affect 30–60% of patients with SjS [2, 56]. Arthritis in patients with SjS is often classified as non-erosive [57]. However, recent more sensitive methods such as ultrasound and magnetic resonance imaging (MRI) have detected erosions in more than one third of SjS patients with joint pain and no previous diagnosis of arthritis [57, 58]. In patients with SjS, arthritis most frequently occurs in proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints and wrists [57], a pattern that is overlapping with the one found in hands of RA patients [59]. Synovial fibroblasts are the major stroma cells of the joint and play a pivotal role in the pathogenesis of rheumatoid arthritis by promoting the ongoing inflammation and cartilage degradation [60]. The existence of shared fibroblast clusters in salivary glands of SjS patients and synovial tissues of rheumatoid arthritis patients, suggests a role of fibroblasts also in articular manifestations of the SjS. However, this potential role of fibroblasts remains to be proven.

6. Conclusions

The Sjögren's syndrome is a chronic inflammatory autoimmune disease with huge unmet needs for patients and clinicians. No therapies for the treatment of the SjS have been approved so far. The pathogenic processes in the exocrine glands have only partially unraveled. Whereas the contribution of salivary gland epithelial cells has been studied in detail, the functional role of fibroblasts in maintaining epithelial cell function, as well as their role in the regulation of the inflammatory process has only recently been recognized. The potential of targeting the fibroblast compartment in salivary glands of patients with SjS has been underscored by studies characterizing their role in the establishment of TLS as well as by scRNA-seq of minor salivary gland tissues. Together these studies pointed to an early, to a large extent lymphocyte-independent, role of fibroblasts in the pathogenesis of the SjS.

Integration of the scRNA-seq data sets across inflamed human tissues, including minor salivary gland tissues from patients with SjS, together with scRNA-seq data sets from mouse models, have suggested a two stage mechanism for fibroblast activation and fibroblast-mediated regulation of inflammation. In this model, the expansion of $SPARC^{+}$ $COL3A1^{+}$ vascular-associated fibroblasts initiates vascular remodeling and subsequent leukocyte infiltration and precedes the expansion of $CXCL10^{+}$ $CCL19^{+}$ immune-interacting fibroblasts.

$CXCL10^{+}$ $CCL19^{+}$ immune-interacting fibroblasts functionally resemble $pdpn^{+}$ $CD34^{-}$ CCL19 expressing fibroblasts that are critically involved in TLS assembly. Formation of TLS is initiated after experimental salivary gland infection by IL13 and IL22 that prime immunofibroblast progenitors and induce the expansion of the fibroblast network, respectively. This supports the concept of fibroblast-targeting strategies to treat TLS-associated autoimmune diseases such as the SjS.

Conflict of interest

The author declares no conflict of interest.

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Author details

Kerstin Klein[1,2]

1 Department of BioMedical Research, University of Bern, Bern, Switzerland

2 Department of Rheumatology and Immunology, University Hospital Bern, Bern, Switzerland

*Address all correspondence to: kerstin.klein@dbmr.unibe.ch; kerstin.klein@insel.ch

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