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Chapter

Fibroblast-Like Synovial Cell Subsets in Rheumatoid Arthritis

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Abstract

Fibroblasts like synoviocytes (FLS) play several significant roles in rheumatoid arthritis (RA) pathophysiology. This chapter will describe known roles of FLS in disease initiation, joint inflammation, disease persistence and joint destruction. It will describe the newly characterized subsets of FLS based on single cell RNA sequencing studies, and their association to specific aspects of the disease. Finally, we will discuss the future of targeting FLS in the treatment of RA. The FLS in the synovial lining layer are identified by surface complement decay-accelerating factor (CD55) along with lubricin and metallopeptidase expression. Pathological activation of this lining layer subset result in bone and cartilage damage in mice. FLS of the sublining layer are often characterized by THY1 expression, but recent studies have highlighted a heterogeneity where several distinct subsets are identified by additional markers. Sublining FLS expressing human leukocyte antigen-DRA (HLA-DRA) produce C-X-C motif chemokine 12 (CXCL12) and receptor activator of nuclear factor- κ B ligand (RANKL) and seems to constitute a pro-inflammatory subset that is associated with inflammation and tertiary lymphoid structures. Another subset of FLS characterized by CD34 expression may discriminate a common progenitor fibroblast subset. Taken together, studies isolating and characterizing gene expression in synovial FLS report both associations of unknown importance and markers that may impose protective or destructive features. This supports evidence of FLS as active players in RA pathology capable of cellular recruitment, local cellular crosstalk and promotion of joint destruction. These discoveries may serve as an atlas for synovial activation in RA and have identified several potential fibroblast markers for the development of targeted treatment.

Keywords: Fibroblast like synoviocytes, Rheumatoid arthritis, Inflammation, Autoimmunity, Tertiary lymphoid structures, Fibroblast activation protein, Fibroblast targeted treatment

1. Introduction

In normal resting conditions the synovial membrane is a thin layer of well-ordered cells historically called type A and B synoviocytes. These cells form a barrier between the articular cavity and a sublining layer, the latter being heterogeneous and composed of several cell lineages. Fibroblasts, immune cells and mature

vasculature (capillaries, arterioles and venules) made up of pericytes and endothelia are some of the various cell types constituting this layer [1–3].

2. Rheumatoid arthritis

Rheumatoid arthritis (RA) has a multifactorial etiology and is one among the most common systemic autoimmune diseases [4, 5]. The factors that mediate the initiation of RA is yet to be unraveled. However, the pathology of RA involves abnormalities in both the innate and the adaptive immune system, and both of these systems are implicated with the progression and persistence of the disease [6, 7]. The synovial membrane is the primary site of pathology during the synovitis stage of the disease and characterized by proliferation of tissue resident, synovial cells and the infiltration of inflammatory cells from the blood. RA is a chronic, progressive disease leading to degradation of articular cartilage and bone along with several systemic manifestations [8].

In RA, the inflamed synovial membrane undergoes hyperplasia and transforms into less structured lining layer and sublining tissues both rich in fibroblasts like synovio-cytes (FLS) [9, 10]. This inflamed synovial membrane eventually begins to invade the cartilage surfaces and the underlying bone, commonly referred to as pannus [11, 12].

Present day treatment strategies for RA primarily focuses on suppression of cytokine signaling and T- and B-cell activity. These therapies have highlighted the importance of immune response in driving the progression of RA. However, they also clearly demonstrate that in a large proportion of patients these treatments are incapable of inducing disease remission [8, 13]. Synovial phenotyping of RA patients based on histology has highlighted a fibroblast dominated synovial pathotype [14]. This pathotype is believed to include a large proportion of the non-responders to conventional and biologic disease modifying anti-rheumatic drugs [15–17]. This is supported *in vitro* where anti-tumor necrosis factor alpha (TNF α) treatments were ineffective in cultures dominated by FLS [18]. Furthermore, a recently published, biopsy driven clinical trial in RA patients with inadequate response to anti-TNF α treatment, showed significantly higher response rates when patients with B-cell poor synovium were treated with IL-6 receptor inhibitor tocilizumab compared to the B-cell depleting agent rituximab [19].

In the following sections, we will first describe RA FLS in general before the era of single cell RNA sequencing (scRNA-seq). We will summarize the known and proposed roles of FLS in RA initiation, joint inflammation, disease persistence and joint destruction. Finally, we will describe the newly characterized subsets of FLS based on scRNA-seq studies their connection to specific aspects of clinical disease, future outlooks in the context of RA diagnosis, RA tissue phenotyping and therapy targeting FLS.

3. Fibroblast like synovial cells in rheumatoid arthritis

3.1 Disease initiation

The central role of FLS in RA pathology is highlighted in murine studies demonstrating that activation of FLS is sufficient to initiate local joint inflammation leading to persistent arthritis [20, 21].

Furthermore, FLS greatly contribute to the transformation of the thin synovial membrane into a multi-layered invasive hyperplastic pannus [22]. This expansion of FLS in the inflamed synovium is likely a result of at least one of the following processes. First, pathological subsets of FLS seem to proliferate to some extent and develop a local resistance to apoptosis [23–25]. Secondly, pluripotent mesenchymal stem cells may

migrate into the synovium from the circulation, where they differentiate into mature pathological subsets of FLS [26]. Lastly, a local mesenchymal progenitor cell population may undergo activation and differentiation into distinct phenotypes of FLS [27]. Collectively, this leads to a local increase in pathological FLS in the RA synovium.

3.2 Joint inflammation

Pathogenic FLS constitute the majority of cells found in the inflamed synovial tissues, and play an important role in the inflammatory cascade, linking innate and adaptive immunity [6, 10]. FLS are capable of significantly affecting the local inflamed environment through production of cytokines and chemokines leading to recruitment and activation of immune cells [9, 28]. Specifically, pathogenic FLS are able to provide an adequate survival signal for synovial T-cells [29], a signal that is superior to the one produced by non-inflammatory fibroblasts [30]. This interaction between FLS and lymphocytes can inhibit the resolution of local inflammation [30, 31] through both paracrine and direct cell–cell interactions [32]. This pathogenic role of the FLS is facilitated by the up-regulation of several adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) [6, 33]. In addition to recruitment and co-activation of T-cells in the inflamed joint, FLS have been shown to be able to present antigens on class II major histocompatibility complex (MHC-II) to CD4+ T-cells [34].

Furthermore, FLS are involved in the formation of tertiary lymphoid structures (TLS) in the RA synovium. Stromal cell populations such as the fibroblastic reticular cell support organization of these lymphocyte aggregates similarly to that of secondary lymphoid organs with distinct T- and B-cell niches [35]. Thymocyte differentiation antigen 1 (THY1, also known as CD90) and podoplanin (PDPN) positive fibroblast associated with TLS in RA (**Table 1**) produce several chemokines such as C-X-C motif ligand (CXCL) 13 and C-C motif ligand (CCL)21 implicated with lymphocyte recruitment and organization [47, 48]. Another marker associated with the TLS associated fibroblast is the receptor activator of nuclear factor kappa- β ligand (RANKL), which is important in both bone homeostasis and lymph node development [35, 49].

Collectively, FLS may be involved in both the pro-inflammatory initiation in the synovium, lymphocyte recruitment and the organization of TLS. A fibroblast driven RA phenotype resulting in persistent inflammation and a lymphoid rich synovium similar to what have been shown by histology.

3.3 Disease persistence

The highly proliferating and pathogenic RA FLS are very different from their quiescent state during non-inflamed conditions where FLS control the structural integrity of the joint lining and sublining layer [22]. The immunological events initiating a pathogenic state of RA FLS is still not fully understood, but proliferation and transformation of the FLS may occur prior to immune infiltration [50].

In RA, subsets of FLS can differentiate to become inflammatory, migratory, and invasive, thus collectively fostering disease aggravation in various animal models of RA [45, 51, 52]. Constitutive activation is a hallmark of RA FLS and leads to production of several inflammatory cytokines, such as interleukin (IL)-1 β , TNF α and IL-6 and chemokines such as monocyte chemoattractant protein 1 (MCP-1/CLL2) [9] and CXCL12 [53]. Even though the activation of RA FLS is greatly affected by pro-inflammatory factors in the local environment, epigenetic changes are also important [54]. Epigenetic changes lead to constitutive activation even when the cells are removed from the inflamed environment and remain without addition of proinflammatory stimuli [52]. Moreover, a

Classical synovial subsets in RA	Markers		
Fibroblast like synoviocytes [36–38]	Vimentin, THY1, prolyl-5-hydroxylase, CDH11, CD45, HLA-DR, α -SMA, CD55		
Macrophage like synoviocytes [36, 39–41]	CD14, CD68, RFD7, CD163, CD206, HLA-DR, CD97		
Tertiary lymphoid structure associated fibroblast [35]	PDPN, THY1, FAP, CXCL13, CCL21, RANKL, CD21		
Fibrocyte [42]	CD34, CD45, CD14, CD11, MHC-II		
Single cell analysis of synovial fibroblast subsets in RA			
Published studies and subsets	Cluster markers	Associated transcription profile	
Stephenson et al. 2018 [43]	Fibroblast sorting strategy: CD45 - Propidium iodide - PDPN+		
Sublining fibroblast	THY1+		
Lining fibroblast	CD55+	HAS1	
Mizoguchi et al. 2018 [44]	Fibroblast sorting strategy: CD45- CD31- CD235a- CD146- PDPN+		
Perivascular fibroblast	THY1+ CD34-	RANKL ^{high} , OPG ^{low}	Migration factors: CTHRC1, TWIST1,
Sublining fibroblast	CD34+	IL6, CXCL12, CCL2, OPG	POSTN, LOXL2, PDGFBB, MMP14
Lining fibroblast	THY1- CD34-	CD55, PRG4, HAS1, MMP1, MMP3	
Zhang et al. 2019 [10]	Fibroblast sorting strategy: CD45- CD31- PDPN+		
SC-F1 (sublining)	THY1+ CD34+	C3, FOS	
SC-F2 (sublining)	THY1+ HLA-DRA ^{high}	IL6, CXCL12	
SC-F3 (sublining)	THY1+ DKK3+	CADM1, COL8A2	
SC-F4 (lining)	THY1- CD55+	PRG4, HBEGF, CLIC5	
Croft et al. 2019 [45]	Reanalysis of human data from Zhang et al. [10].		
F1 (sublining)	THY1+	DKK3, OGN, CD9,	
F2 (sublining)	THY1+	MDK, COL8A1, AEBP1	
F3 (sublining)	THY1+	IRF1, EGR1, JUNB	
F4 (lining)	THY1-	CLIC5, CD55, HBEGF	
F5 (sublining)	CD34+	C3, APOD	
Single cell analysis of circulating mesenchymal cells in RA:			
Published study and subset	Associated transcription profile		
Orange et al. 2020 [46]	Fibroblast sorting strategy: CD45- CD31- PDPN+		
AC3 (sublining fibroblast phenotype)	CD34, HLA-DR, DKK3, FAP α , CDH11		

The table contains a list of surface and transcriptional profiles of fibroblast subsets, fibroblast like cell subsets and macrophage subsets (pre-scRNA-seq) related to rheumatoid arthritis. For scRNA-seq studies, fibroblast subset names refer to the original articles. “+” and “-” shows whether the cells of interest are positive or negative for the cellular markers. The cellular markers which are discussed in the text are also listed under abbreviations.

Table 1.
Surface and transcriptional profiles of FLS subsets (and related cellular subsets) in rheumatoid arthritis.

recent study suggests a link between epigenetic-driven positional identity of FLS (e.g. small versus large joints and proximal versus distal joints) and clinical disease patterns [55]. This link is further supported by the finding of oncogenes at sites of tissue destruction [56, 57] together with a highly activated nuclear factor κ beta pathway in RA FLS [58].

Altered metabolic activity with increased glycolysis is another hallmark of RA FLS [59]. Metabolic reprogramming of FLS were recently connected to complement

C3 and C3a receptor-activation. Here repeated inflammatory challenges resulted in a distinct pro-inflammatory phenotypic priming of FLS in mice models of arthritis [60].

On the opposite side, several factors attempt to facilitate remission of proinflammatory FLS. One such potential immune regulator is the MerTK expressing synovial macrophage which *in vitro* reduce matrix metalloproteinase (MMP) production by lining layer FLS [61].

Thus, even though FLS are responsive to their inflammatory context they may possess a distinct positional identity which enables a cytokine-independent intrinsic activation contributing to disease persistence in RA.

3.4 Joint destruction

The severe joint destruction of late-stage RA is in part attributed to the pannus tissue which is rich in FLS. RA FLS are identified as invaders of the joint cartilage *in vivo* [62, 63], an invasive behavior that has been confirmed *in vitro* [64] and in mice [52]. FLS mediate cartilage degradation which is attributed to a combination of facilitating adhesion factors and production of proteases, here among several well-known matrix metalloproteinases (MMPs) [9, 52, 64]. Cartilage degradation is ameliorated when fibroblast activation protein (FAP) deficiency is induced in the human TNF α transgenic mice model of arthritis [65]. The invasiveness of pathological RA FLS is further emphasized by human FLS migrating to other joints in mouse models of RA and degrading the implanted human cartilage [51]. Migration that may be facilitated by specific anticitrulinated protein antibodies [66]. Notably, the *ex vivo* invasiveness of FLS correlates with joint erosions [67].

Increased osteoclastic activity leading to bone erosions in RA is another major factor in joint destruction. Here FLS produce CXCL12, RANKL, dickkopf related protein (DKK) 1, etc. which may increase both osteoclast migration, differentiation, proliferation/activation and inhibit osteoblast function [53, 68, 69].

4. Single cell analysis of synovial fibroblast subsets in rheumatoid arthritis

4.1 Phenotyping of fibroblast like synovial cells

Increasing spatial and molecular resolution in present day cellular analysis are changing our view of the synovial membrane in RA. Most notable is the identification of different fibroblast subsets within the inflamed synovial membrane. Recent work and ongoing studies are utilizing scRNA-seq, CyTOF and flow cytometry cell sorting to further investigate and distinguish these subsets and their role in disease pathology.

Recent scRNA-seq studies have identified several distinct disease-associated subsets in the inflamed synovial membrane, often grouped as lining layer or sublining layer FLS [10, 43, 44], **Figure 1**. The present studies utilize flow cytometry assisted cell sorting and transcriptomic clustering strategies based on exclusion of hematopoietic lineage cells (CD45), endothelial cells (CD31), red blood cells (CD235a), and pericytes (CD146) while using PDPN or collagen production as a positive marker (**Table 1**).

4.2 Lining layer fibroblasts

A common finding in scRNA-seq studies confirms the presence of complement decay-accelerating factor (CD55) and absence of THY1 expression in FLS of the

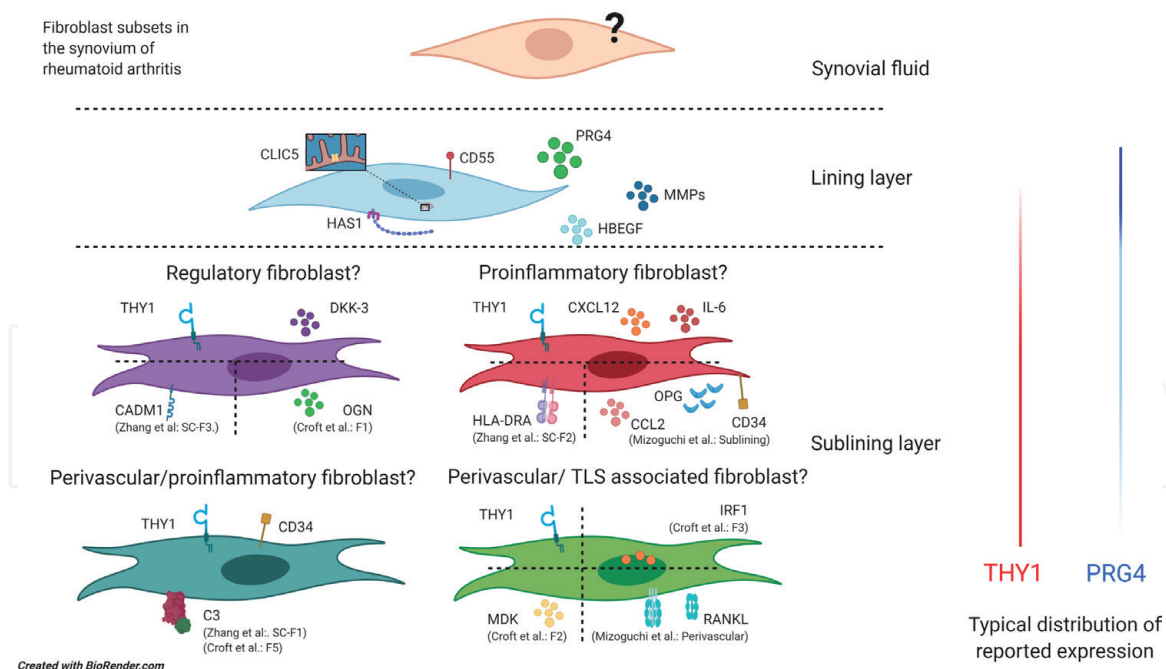


Figure 1.

The figure is a schematic presentation of fibroblast subsets identified by scRNA-seq studies of synovial tissue from patients with rheumatoid arthritis. The subsets have been divided into lining layer FLS and sublining layer FLS. No scRNA-seq studies yet have examined fibroblast subsets from the synovial fluid. Based on grouping markers and transcription profiles listed in **Table 1**, we propose 4 sublining phenotypes. Cells have been divided by dashed lines when the cellular markers were not listed in all the original studies. THY-1 and PRG4 expression gradients from the lining layer to the sublining layer is shown by the color density of the red and blue bars. The cellular markers are discussed in the text and listed under abbreviations. TLS: Tertiary lymphoid structures.

lining layer (**Table 1**). Of note, Mizoguchi et al. [44] did not report histological data of CD55 distribution, but a high level of CD55 gene expression in CD34- THY1- lining layer fibroblasts. All scRNA-seq studies (**Table 1**) of joint tissue reported lubricin (PRG4) expression in the lining layer subset [10, 43–45]. All present studies showed similar patterns of gene expression pertaining to the potential markers of FLS presented in the following section.

Zhang et al. [10] and the reanalysis of the same human data by Croft et al. [45] both reported a distinct lining fibroblast subset, SC-F4 and F4 respectively. This lining fibroblast subset was associated with expression of chloride intracellular ion channel 5 (CLIC5) and heparin binding epidermal growth factor-like growth factor (HBEGF). Mizoguchi et al. [44] and Stephenson et al. [43] also reported increased hyaluronan synthase 1 (HAS1) and metalloproteinase expression.

HAS1 is important for hyaluronan production and is a response to pro-inflammatory stimuli in RA synoviocytes. This activation results in hyaluronan cell coating, leukocyte/monocyte recruitment and facilitation of fibroblast-monocytes binding [70].

CD55, a C3 convertase inhibitor, has received increasing interest in cancer, where CD55/CD97 binding is associated with several oncogenic properties such as invasion and migration [71]. In RA, CD55 positive FLS are exclusive to the lining layer and in proximity to CD97 positive macrophages, suggesting a possible mechanism of crosstalk [36]. CD55 is not exclusive to RA [72], but it has been suggested as a protective factor in a mice model of immune complex mediated arthritis [73].

In the context of joint tissue, the mucin-like glycoprotein, PRG4, has been proposed as having a dual role comprising of well-known lubricating property and as a moderator of inflammation via NF- κ B pathways through interaction with both CD44 and toll-like receptors [74].

CLIC5 is present in several intracellular organelles, but predominantly located at the mitochondrial inner membrane, where it has been associated with modulation

of reactive oxygen species [75]. However, no functional studies have been published regarding CLIC5 in RA.

The epidermal growth factor family member, HBEGF, is present and involved in several physiological processes such as wound healing, tumor formation and angiogenesis. One common topic is its association with cell migration, as seen in keratinocyte/fibroblast models and in enterocytes in necrotizing enterocolitis [76]. In RA, HBEGF positive macrophages have recently been shown to increase synovial fibroblast invasiveness in an *in vitro* model [77].

Several matrix metalloproteinases, MMP1, MMP3 and MMP14 was connected to a specific subset of FLS by Mizoguchi et al. [44]. These destructive enzymes have previously been connected to cartilage degradation in RA, but MMP14 was also noted by Mizoguchi et al. as a migratory factor [44].

Taken together, studies isolating and characterizing gene expression in lining layer fibroblasts report both associations of unknown importance and markers that may impose protective and destructive features. This suggests that the lining layer fibroblast subset is an active subset in RA pathology capable of cellular recruitment and significant local cellular crosstalk.

4.3 Sublining layer fibroblasts

The scRNA-seq studies have reported several distinct sublining subsets presented in **Table 1**. The initial study by Stephenson et al. [43] identified THY1 as a marker of sublining fibroblasts and the subsequent scRNA-seq studies confirmed THY1 as a specific, albeit not universal marker of sublining fibroblasts [10, 44, 45].

Zhang et al. characterized this heterogeneity of the sublining layer fibroblasts and defined three THY1+ groups with additional subset markers; CD34 defined the SC-F1 cluster, human leukocyte antigen (HLA)-DRA^{high} defined the SC-F2 cluster and DKK-3 defined the SC-F3 cluster. The SC-F2 in particular was significantly increased in leukocyte-rich RA synovium compared to leukocyte-poor RA synovium and osteoarthritis (OA) synovium [10], suggesting these to encompass TLS-associated fibroblast subsets. Reanalysis of these human data by Croft et al. [45] enabled the distinction of four sublining layer fibroblast groups (F1–3,-5, **Table 1**).

As with the lining layer, large sets of multiomics data are available. Several markers connected to joint inflammation and destruction have been identified in these subsets. However, the markers most consistently reported are THY1, HLA-DRA, CD34, DKK3.

THY1 is a glycoprotein present on the membrane of several different cells including endothelial and mesenchymal cells [78]. Among the functions associated with THY1 expression is cellular contact, CD97 binding, integrin binding, trans-endothelial migration and MMP-9 and CXCL8 secretion after binding to neutrophils [78].

As with THY1, CD34 is an established marker in different cell types including several stromal cells, epi/endothelial cells and fibrocytes [79]. Its function is largely unknown but has been linked to proliferation, adhesion, differentiation and is proposed as a marker of progenitor subsets in both mesenchymal, epithelial and endothelial cells [79].

MHC molecule (both class I and II) functions are typically attributed to antigen presentation. Several MHC molecules have been associated with autoimmune disease. Examples are the association of the MHC-I molecule HLA-B27 with ankylosing spondylitis, reactive arthritis and juvenile idiopathic arthritis subsets [80], and the association of MHC-II molecules HLA-DR1 and DR4 association with RA [81]. The specific function of HLA-DRA in RA FLS is yet to be investigated.

The DKK family of glycoproteins are well known modulators of WNT pathways connected to embryogenesis, bone formation and eye and skin development [82]. DKK-1 has been extensively described in fibroblasts from RA patients and is a key player in joint remodeling [69]. DKK-3 has been reported as a chondroprotective factor in OA [83] and suggested as a B-cell modulator whose absence aggravates autoimmune symptoms in a murine systemic lupus erythematosus model [84] and a CD8 T-cell modulator involved in antigen tolerance [85].

Enrichment of several genes related to pro-inflammatory cytokines and proteins related to bone metabolism in RA have been reported in sublining fibroblasts including IL-6, MCP-1/CCL2, CXCL12 and RANKL. Two proteins not mentioned above is the RANKL decoy receptor osteoprotegerin (OPG) which inhibit osteoclastogenesis in synovial macrophages [86] and the relatively new osteoglycin (OGN) that may both be part of the vascular system and may affect osteoblast differentiation [87].

The interferon regulatory factor 1 (IRF1) is a significant component of the interferon signature/inflammation pathway, through which TNF induces production of CXCL9–11 and in its absence diminishes B-cell activating factor expression [88].

The heparin-binding growth factor midkine (MDK) is less investigated than the above-mentioned cytokines but has been identified in human synoviocytes and associated with leukocyte migration to the synovium and osteoclastogenesis in mice [89].

C3, a unifying step for all three complement activating pathways has previously been located around microvasculature in the sublining of the RA synovium [90].

The cellular adhesion molecule 1 (CADM1) is a transmembrane member of the immunoglobulin superfamily with no known relation to RA. It has been identified as a tumor suppressor gene in solid cancers such as squamous cell carcinoma a, but may contribute to infiltration in adult T-cell leukemia/lymphoma [91].

To summarize, the sublining layer is a heterogeneous compartment of the inflamed RA synovium, regarding both cell lineages and especially fibroblast subsets (**Table 1** and **Figure 1**). Several distinct fibroblast subsets have been identified, but recurring markers such as HLA-DRA, CD34 and DKK-3 are relatively unknown in the RA context. Results from scRNA-seq studies propose that the sublining layer fibroblast subsets are significantly involved in cellular crosstalk, leukocyte recruitment, para- and autocrine pro-inflammatory stimulation, and joint tissue destruction. Notably, some distinguishing factors such as DKK-3 may be enriched to form a regulatory anti-inflammatory and pro self-tolerance subset with similar chondroprotective effects and immune modulation of antigen tolerance mentioned in the previous section. An HLA-DRA^{high}/CXCL12/RANKL^{high} associated subset may constitute the pro-inflammatory TLS associated fibroblast subsets and CD34 may discriminate a common progenitor fibroblast subset. Together, the presence of both pro-inflammatory subsets and potential anti-inflammatory and progenitor subsets suggests an ongoing cellular balancing throughout the sublining layer, which may open avenues for new research in treatment strategies targeting FLS.

4.4 Fibroblast like synoviocytes in rheumatoid arthritis compared to other arthritides

In RA, synovial division into lining/sublining layers suggests differentiated roles of subsets of FLS regarding cytokine production, joint destruction, and possible regulatory mechanisms.

The expansion of these distinct subsets is different in RA compared with OA. Mizoguchi et al. reported a greater fraction of the THY1⁺ CD34⁻ (perivascular) subset but less of the THY1⁻ CD34⁻ (lining) subset in RA compared with OA [44]. Notably, here the proportion of THY1⁺ CD34⁻ (perivascular) FLS correlated with leukocyte infiltration and ultrasonic and histological synovitis [44].

Similarly, Zhang et al. reported an overabundance of the THY1⁺ CD34⁻ HLA-DRA^{high} (SC-F2) subset with upregulated expression of CXCL12 and IL-6 and a THY1⁺ CD34⁺ (SC-F1) subset in RA. In contrast, lining FLS (SC-F4) were more abundant in OA [10].

The causal link between distinct subsets and RA pathogenesis was investigated in mice by Croft et al. Here the mouse thy1⁻ subset homologous to human lining FLS (F4) were correlated to joint damage and mouse thy1⁺ sublining FLS correlated to inflammation [45]. Notably, the elimination of FAP expressing subsets reduced pannus formation and joint destruction [45]. This suggests that FAP is a marker of pathologically active FLS in RA [45, 92, 93].

Comparison of subsets of FLS in RA and psoriatic arthritis are underway [94] and may potentially assist in discriminating these arthritides.

5. Fibroblasts derived from synovial fluid versus synovial tissue

Arthrocentesis is a common therapeutic procedure in treatment of RA. Fibroblast cultured from synovial fluid aspirates initially express similar phenotypical traits compared to tissue derived synovial fibroblast cultures [95, 96]. Despite these similarities, synovial fluid derived fibroblasts are likely a proxy regarding changes in the synovium and results must be interpreted as such. In both research and clinical settings synovial biopsies are both economical and well tolerated [97–100]. However, synovial fluid analysis of both cellular and soluble components is very useful in clinical settings where the length of consultations/sterile procedural environments/analytic facilities may limit the use of synovial biopsies. To the authors knowledge, no studies have yet reported scRNA-seq analysis of synovial fluid fibroblasts.

6. Circulating mesenchymal fibroblast like cells in rheumatoid arthritis

In excess to tissue resident FLS, Orange et al. recently highlighted the presence of circulating fibroblast-like cells in the blood of RA patients shortly before symptomatic disease flare [46]. Interestingly these pre inflammatory mesenchymal (PRIME) cells show enrichment of previously reported markers of distinct sublining subsets of FLS e.g., DKK-3, CD34 and HLA-DR. This suggests that PRIME cells may constitute a heterogeneous pool of circulating FLS-like cells with distinct functions. Subsets of FLS migrating from the RA affected synovium, or a common homogeneous pool of circulating progenitor FLS awaiting recruitment signals from local sites of inflammation could potentially be the origin of these cells, although this remains to be investigated. Regardless, PRIME cells may not only be a useful marker predicting disease flares in RA, but also potentially explain how synovitis is transmitted from joint to joint [51].

7. Future therapeutic perspectives

The insights recently generated through high resolution scRNA-seq have revolutionized our understanding of specific subsets of FLS in RA and their involvement in driving different aspects of RA pathobiology. This understanding has also provided the basis for generating specific targetable markers of pathological subsets of FLS in RA. Targeting strategies that could be used as either monotherapy or as an add-on treatment to present day cytokine or lymphocyte inhibitors [101].

FLS could be targeted by drugs used in fibrotic conditions such as nintedanib or pirfenidone [102]. However, these drugs are likely affecting a completely different aspect of fibroblast functions. Therefore, new drugs are needed. An example is the addition of the cyclin-dependent kinase inhibitor, Seliciclib, which is currently being evaluated [103].

The well-known FAP marker of activated stromal cells has a diagnostic and prognostic potential through precise and low background positron emission tomography tracers developed in cancer-immunology [104]. The recent development of specific quinoline-based positron emission tomography tracers that act as FAP inhibitors have demonstrated promising results both preclinically and clinically in different cancers but could also be promising as diagnostic and prognostic markers of RA [105]. Further, the clinical potential of targeting FAP expressing FLS would be a targeted treatment eliminating pathologically activated RA FLS, in both the lining and the sublining layer [45, 93].

Among other interesting targets, NOTCH3 is one of the most recently *in vivo* validated pathological targets. NOTCH3 is expressed on the surface of RA FLS and linked with THY1 expression. NOTCH3 may also be a useful target in a therapeutic senescence strategy through selective activation of the g-protein coupled receptor melanocontin type 1 receptor [106]. Furthermore, in an animal model of RA injection of NOTCH3-neutralizing monoclonal antibody attenuated the severity of arthritis. Taken together, the *in vitro* studies on NOTCH3, including its connection to spatial distribution of FLS and the above-mentioned animal study underline NOTCH3 as a promising therapeutical target in RA [106, 107]. Targeting the complement C3 - C3a receptor axis may serve as another preventive or complementary strategy, where metabolic priming of FLS can be avoided or reduced [60]. Another possible strategy of targeting FLS is drug delivery via the extra domain A fibronectin splice variant identified in OA and RA [108, 109] and utilized in cancer [110].

Several other reagents targeting FLS are currently being tested ranging from metabolite modulators to treatments targeting intracellular signal transduction or epigenetic changes [111].

Collectively, these therapies targeting subsets of FLS are emerging as promising diagnostic and therapeutic tools. Tools for optimized and stratified treatments in RA based on which cellular mechanisms and which fibroblast subsets are pathologically activated in the individual patient.

8. Conclusions

Collectively, pathological FLS presented in this chapter are deeply connected to the RA pathophysiology of disease initiation, joint inflammation, disease persistence and joint tissue destruction.

Recent scRNA-seq studies have identified several distinct subsets of FLS causally linked to major elements of RA pathogenesis e.g., inflammation and joint destruction, while other subsets may present regulatory, pro-inflammatory TLS associated or common progenitor FLS.

These first steps in a scRNA-seq era of RA research warrants both rejoice and due diligence. Due diligence because we henceforth must appreciate the cellular diversity and the complex cellular crosstalk of the RA synovium. Like FLS, monocytes/macrophages and lymphocytes exhibit distinct subsets in RA, which may be as important in understanding the spectrum of RA disease, e.g., lymphocyte dominated vs. lymphocyte poor synovium and erosive vs. non-erosive disease. Furthermore, we must appreciate the heterogeneity of FLS and cellular organization (here among TLS formation) of the sublining layer.

Rejoice because the recent subset studies have produced a language and knowledge and a novel nomenclature for FLS in future research. A breakthrough that might enable clinicians in the future to modulate specific aspects of RA through fibroblast subset targeted treatment.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

RA	Rheumatoid arthritis
FLS	Fibroblast like synoviocytes
TNF α	Tumor necrosis factor alpha
scRNA-seq	Single cell RNA sequencing
ICAM-1	Intercellular adhesion molecule 1
VCAM-1	Vascular cell adhesion molecule 1
MHC-II	Type 2 major histocompatibility complex
TLS	Tertiary lymphoid structures
THY1	Thymocyte differentiation antigen 1
PDPN	Podoplanin
CXCL	C-X-C motif ligand
CCL	C-C motif ligand
RANKL	Receptor activator of nuclear factor kappa- β ligand
IL	Interleukin
MCP-1/CLL2	Monocyte chemoattractant protein 1
MMP	Matrix metalloproteinases
FAP	Fibroblast activation protein
DKK	Dickkopf related protein
CD55	Complement decay-accelerating factor
PRG4	Lubricin
CLIC5	Chloride intracellular ion channel 5
HBEGF	Heparin binding epidermal growth factor-like growth factor
HLA	Human leukocyte antigen
OA	Osteoarthritis
OPG	Osteoprotegerin
OGN	Osteoglycin
IRF1	Interferon regulatory factor 1
MDK	Midkine
CADM1	Cellular adhesion molecule 1

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References

- [1] Castor CW. The microscopic structure of normal human synovial tissue. *Arthritis and rheumatism*. 1960;3:140-51.
- [2] Kennedy A, Ng CT, Biniecka M, Saber T, Taylor C, O'Sullivan J, et al. Angiogenesis and blood vessel stability in inflammatory arthritis. *Arthritis and rheumatism*. 2010;62(3):711-21.
- [3] Culemann S, Grüneboom A, Nicolás-Ávila J, Weidner D, Lämmle KF, Rothe T, et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature*. 2019;572(7771):670-5.
- [4] Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. *Autoimmun Rev*. 2003;2(3):119-25.
- [5] Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis research & therapy*. 2009;11(3):229.
- [6] Cooles FA, Isaacs JD. Pathophysiology of rheumatoid arthritis. *Current opinion in rheumatology*. 2011;23(3):233-40.
- [7] Burmester GR, Feist E, Dörner T. Emerging cell and cytokine targets in rheumatoid arthritis. *Nature reviews Rheumatology*. 2014;10(2):77-88.
- [8] McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet*. 2017;389(10086):2328-37.
- [9] Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev*. 2010;233(1):233-55.
- [10] Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S, et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nature immunology*. 2019;20(7):928-42.
- [11] Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature*. 2003;423(6937):356-61.
- [12] Shiozawa S, Shiozawa K, Fujita T. Morphologic observations in the early phase of the cartilage-pannus junction. Light and electron microscopic studies of active cellular pannus. *Arthritis and rheumatism*. 1983;26(4):472-8.
- [13] Atzeni F, Sarzi-Puttini P, Gorla R, Marchesoni A, Caporali R. Switching rheumatoid arthritis treatments: an update. *Autoimmun Rev*. 2011;10(7):397-403.
- [14] Dennis G, Jr., Holweg CT, Kummerfeld SK, Choy DF, Setiadi AF, Hackney JA, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis research & therapy*. 2014;16(2):R90.
- [15] Pitzalis C, Kelly S, Humby F. New learnings on the pathophysiology of RA from synovial biopsies. *Current opinion in rheumatology*. 2013;25(3):334-44.
- [16] Townsend MJ. Molecular and cellular heterogeneity in the Rheumatoid Arthritis synovium: clinical correlates of synovitis. *Best practice & research Clinical rheumatology*. 2014;28(4):539-49.
- [17] Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Annals of the rheumatic diseases*. 2019;78(6):761-72.

- [18] Nielsen MA, Lomholt S, Mellekjær A, Andersen MN, Buckley CD, Kragstrup TW. Responses to Cytokine Inhibitors Associated with Cellular Composition in Models of Immune-Mediated Inflammatory Arthritis. *ACR Open Rheumatol.* 2020;2(1):3-10.
- [19] Humby F, Durez P, Buch MH, Lewis MJ, Rizvi H, Rivellese F, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *Lancet.* 2021;397(10271):305-17.
- [20] Armaka M, Ospelt C, Pasparakis M, Kollias G. The p55TNFR-*IKK2*-*Ripk3* axis orchestrates arthritis by regulating death and inflammatory pathways in synovial fibroblasts. *Nature communications.* 2018;9(1):618.
- [21] Armaka M, Apostolaki M, Jacques P, Kontoyiannis DL, Elewaut D, Kollias G. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. *The Journal of experimental medicine.* 2008;205(2):331-7.
- [22] Bottini N, Firestein GS. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. *Nature reviews Rheumatology.* 2013;9(1):24-33.
- [23] Kato M, Ospelt C, Gay RE, Gay S, Klein K. Dual role of autophagy in stress-induced cell death in rheumatoid arthritis synovial fibroblasts. *Arthritis & rheumatology (Hoboken, NJ).* 2014;66(1):40-8.
- [24] Shin YJ, Han SH, Kim DS, Lee GH, Yoo WH, Kang YM, et al. Autophagy induction and CHOP under-expression promotes survival of fibroblasts from rheumatoid arthritis patients under endoplasmic reticulum stress. *Arthritis research & therapy.* 2010;12(1):R19.
- [25] Matsumoto S, Müller-Ladner U, Gay RE, Nishioka K, Gay S. Ultrastructural demonstration of apoptosis, Fas and Bcl-2 expression of rheumatoid synovial fibroblasts. *The Journal of rheumatology.* 1996;23(8):1345-52.
- [26] Marinova-Mutafchieva L, Williams RO, Funa K, Maini RN, Zvaifler NJ. Inflammation is preceded by tumor necrosis factor-dependent infiltration of mesenchymal cells in experimental arthritis. *Arthritis and rheumatism.* 2002;46(2):507-13.
- [27] Roelofs AJ, Zupan J, Riemen AHK, Kania K, Ansboro S, White N, et al. Joint morphogenetic cells in the adult mammalian synovium. *Nature communications.* 2017;8:15040.
- [28] Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *The American journal of pathology.* 1997;151(2):317-22.
- [29] Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, et al. Inhibition of T cell apoptosis in the rheumatoid synovium. *The Journal of clinical investigation.* 1997;99(3):439-46.
- [30] Filer A, Parsonage G, Smith E, Osborne C, Thomas AM, Curnow SJ, et al. Differential survival of leukocyte subsets mediated by synovial, bone marrow, and skin fibroblasts: site-specific versus activation-dependent survival of T cells and neutrophils. *Arthritis and rheumatism.* 2006;54(7):2096-108.
- [31] Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol.* 2001;22(4):199-204.
- [32] Fox DA, Gizinski A, Morgan R, Lundy SK. Cell-cell interactions in

- rheumatoid arthritis synovium. *Rheumatic diseases clinics of North America*. 2010;36(2):311-23.
- [33] Kyburz D, Rethage J, Seibl R, Lauener R, Gay RE, Carson DA, et al. Bacterial peptidoglycans but not CpG oligodeoxynucleotides activate synovial fibroblasts by toll-like receptor signaling. *Arthritis and rheumatism*. 2003;48(3):642-50.
- [34] Tran CN, Davis MJ, Tesmer LA, Endres JL, Motyl CD, Smuda C, et al. Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis and rheumatism*. 2007;56(5):1497-506.
- [35] Barone F, Gardner DH, Nayar S, Steinthal N, Buckley CD, Luther SA. Stromal Fibroblasts in Tertiary Lymphoid Structures: A Novel Target in Chronic Inflammation. *Front Immunol*. 2016;7:477.
- [36] Hamann J, Wishaupt JO, van Lier RA, Smeets TJ, Breedveld FC, Tak PP. Expression of the activation antigen CD97 and its ligand CD55 in rheumatoid synovial tissue. *Arthritis and rheumatism*. 1999;42(4):650-8.
- [37] Müller-Ladner U, Ospelt C, Gay S, Distler O, Pap T. Cells of the synovium in rheumatoid arthritis. Synovial fibroblasts. *Arthritis research & therapy*. 2007;9(6):223.
- [38] Kiener HP, Niederreiter B, Lee DM, Jimenez-Boj E, Smolen JS, Brenner MB. Cadherin 11 promotes invasive behavior of fibroblast-like synoviocytes. *Arthritis and rheumatism*. 2009;60(5):1305-10.
- [39] Udalova IA, Mantovani A, Feldmann M. Macrophage heterogeneity in the context of rheumatoid arthritis. *Nature reviews Rheumatology*. 2016;12(8):472-85.
- [40] Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. *Arthritis and rheumatism*. 1996;39(1):115-24.
- [41] Salisbury AK, Duke O, Poulter LW. Macrophage-like cells of the pannus area in rheumatoid arthritic joints. *Scand J Rheumatol*. 1987;16(4):263-72.
- [42] Chong SG, Sato S, Kolb M, Gauldie J. Fibrocytes and fibroblasts-Where are we now. *The international journal of biochemistry & cell biology*. 2019;116:105595.
- [43] Stephenson W, Donlin LT, Butler A, Roza C, Bracken B, Rashidfarrokhi A, et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. *Nature communications*. 2018;9(1):791.
- [44] Mizoguchi F, Slowikowski K, Wei K, Marshall JL, Rao DA, Chang SK, et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nature communications*. 2018;9(1):789.
- [45] Croft AP, Campos J, Jansen K, Turner JD, Marshall J, Attar M, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature*. 2019;570(7760):246-51.
- [46] Orange DE, Yao V, Sawicka K, Fak J, Frank MO, Parveen S, et al. RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares. *The New England journal of medicine*. 2020;383(3):218-28.
- [47] Manzo A, Bugatti S, Caporali R, Prevo R, Jackson DG, Ugucioni M, et al. CCL21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis. *The American journal of pathology*. 2007;171(5):1549-62.
- [48] Manzo A, Paoletti S, Carulli M, Blades MC, Barone F, Yanni G, et al.

- Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis. *Eur J Immunol.* 2005;35(5):1347-59.
- [49] Mueller CG, Hess E. Emerging Functions of RANKL in Lymphoid Tissues. *Front Immunol.* 2012;3:261.
- [50] Caulfield JP, Hein A, Dynesius-Trentham R, Trentham DE. Morphologic demonstration of two stages in the development of type II collagen-induced arthritis. *Lab Invest.* 1982;46(3):321-43.
- [51] Lefèvre S, Knedla A, Tennie C, Kampmann A, Wunrau C, Dinser R, et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nature medicine.* 2009;15(12):1414-20.
- [52] Müller-Ladner U, Kriegsmann J, Franklin BN, Matsumoto S, Geiler T, Gay RE, et al. Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *The American journal of pathology.* 1996;149(5):1607-15.
- [53] Janssens R, Struyf S, Proost P. Pathological roles of the homeostatic chemokine CXCL12. *Cytokine Growth Factor Rev.* 2018;44:51-68.
- [54] Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet.* 2016;17(8):487-500.
- [55] Frank-Bertoncelj M, Trenkmann M, Klein K, Karouzakis E, Rehrauer H, Bratus A, et al. Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nature communications.* 2017;8:14852.
- [56] Pap T, Nawrath M, Heinrich J, Bosse M, Baier A, Hummel KM, et al. Cooperation of Ras- and c-Myc-dependent pathways in regulating the growth and invasiveness of synovial fibroblasts in rheumatoid arthritis. *Arthritis and rheumatism.* 2004;50(9):2794-802.
- [57] Pap T, Aupperle KR, Gay S, Firestein GS, Gay RE. Invasiveness of synovial fibroblasts is regulated by p53 in the SCID mouse in vivo model of cartilage invasion. *Arthritis and rheumatism.* 2001;44(3):676-81.
- [58] Miagkov AV, Kovalenko DV, Brown CE, Didsbury JR, Cogswell JP, Stimpson SA, et al. NF-kappaB activation provides the potential link between inflammation and hyperplasia in the arthritic joint. *Proceedings of the National Academy of Sciences of the United States of America.* 1998;95(23):13859-64.
- [59] Ahn JK, Kim S, Hwang J, Kim J, Kim KH, Cha HS. GC/TOF-MS-based metabolomic profiling in cultured fibroblast-like synoviocytes from rheumatoid arthritis. *Joint, bone, spine : revue du rhumatisme.* 2016;83(6):707-13.
- [60] Frišćić J, Böttcher M, Reinwald C, Bruns H, Wirth B, Popp S-J, et al. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity.* 2021.
- [61] Alivernini S, MacDonald L, Elmesmari A, Finlay S, Toluoso B, Gigante MR, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nature medicine.* 2020;26(8):1295-306.
- [62] Bromley M, Woolley DE. Histopathology of the rheumatoid lesion. Identification of cell types at sites of cartilage erosion. *Arthritis and rheumatism.* 1984;27(8):857-63.
- [63] Fassbender HG. Histomorphological basis of articular cartilage destruction in

rheumatoid arthritis. *Coll Relat Res.* 1983;3(2):141-55.

[64] Tolboom TC, Pieterman E, van der Laan WH, Toes RE, Huidekoper AL, Nelissen RG, et al. Invasive properties of fibroblast-like synoviocytes: correlation with growth characteristics and expression of MMP-1, MMP-3, and MMP-10. *Annals of the rheumatic diseases.* 2002;61(11):975-80.

[65] Wäldele S, Koers-Wunrau C, Beckmann D, Korb-Pap A, Wehmeyer C, Pap T, et al. Deficiency of fibroblast activation protein alpha ameliorates cartilage destruction in inflammatory destructive arthritis. *Arthritis research & therapy.* 2015;17(1):12.

[66] Sun M, Rethi B, Krishnamurthy A, Joshua V, Circiumaru A, Hensvold AH, et al. Anticitrullinated protein antibodies facilitate migration of synovial tissue-derived fibroblasts. *Annals of the rheumatic diseases.* 2019;78(12):1621-31.

[67] Tolboom TC, van der Helm-Van Mil AH, Nelissen RG, Breedveld FC, Toes RE, Huizinga TW. Invasiveness of fibroblast-like synoviocytes is an individual patient characteristic associated with the rate of joint destruction in patients with rheumatoid arthritis. *Arthritis and rheumatism.* 2005;52(7):1999-2002.

[68] Pettit AR, Walsh NC, Manning C, Goldring SR, Gravalles EM. RANKL protein is expressed at the pannus-bone interface at sites of articular bone erosion in rheumatoid arthritis. *Rheumatology.* 2006;45(9):1068-76.

[69] Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nature medicine.* 2007;13(2):156-63.

[70] Siiskonen H, Oikari S, Pasonen-Seppänen S, Rilla K.

Hyaluronan synthase 1: a mysterious enzyme with unexpected functions. *Front Immunol.* 2015;6:43.

[71] Dho SH, Lim JC, Kim LK. Beyond the Role of CD55 as a Complement Component. *Immune Netw.* 2018;18(1):e11.

[72] Choi IY, Karpus ON, Turner JD, Hardie D, Marshall JL, de Hair MJH, et al. Stromal cell markers are differentially expressed in the synovial tissue of patients with early arthritis. *PloS one.* 2017;12(8):e0182751.

[73] Karpus ON, Kiener HP, Niederreiter B, Yilmaz-Elis AS, van der Kaa J, Ramaglia V, et al. CD55 deposited on synovial collagen fibers protects from immune complex-mediated arthritis. *Arthritis research & therapy.* 2015;17(1):6.

[74] Das N, Schmidt TA, Krawetz RJ, Dufour A. Proteoglycan 4: From Mere Lubricant to Regulator of Tissue Homeostasis and Inflammation: Does proteoglycan 4 have the ability to buffer the inflammatory response? *Bioessays.* 2019;41(1):e1800166.

[75] Gururaja Rao S, Patel NJ, Singh H. Intracellular Chloride Channels: Novel Biomarkers in Diseases. *Front Physiol.* 2020;11:96.

[76] Taylor SR, Markesbery MG, Harding PA. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) and proteolytic processing by a disintegrin and metalloproteinases (ADAM): a regulator of several pathways. *Semin Cell Dev Biol.* 2014;28:22-30.

[77] Kuo D, Ding J, Cohn IS, Zhang F, Wei K, Rao DA, et al. HBEGF(+) macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci Transl Med.* 2019;11(491).

[78] Leyton L, Díaz J, Martínez S, Palacios E, Pérez LA, Pérez RD.

- Thy-1/CD90 a Bidirectional and Lateral Signaling Scaffold. *Front Cell Dev Biol.* 2019;7:132.
- [79] Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. *Stem cells.* 2014;32(6):1380-9.
- [80] Bowness P. HLA-B27. *Annual review of immunology.* 2015;33:29-48.
- [81] Kurkó J, Besenyi T, Laki J, Glant TT, Mikecz K, Szekanecz Z. Genetics of rheumatoid arthritis - a comprehensive review. *Clin Rev Allergy Immunol.* 2013;45(2):170-9.
- [82] Niehrs C. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene.* 2006;25(57):7469-81.
- [83] Snelling SJ, Davidson RK, Swingler TE, Le LT, Barter MJ, Culley KL, et al. Dickkopf-3 is upregulated in osteoarthritis and has a chondroprotective role. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society.* 2016;24(5):883-91.
- [84] Ludwig J, Federico G, Prokosch S, Küblbeck G, Schmitt S, Klevenz A, et al. Dickkopf-3 acts as a modulator of B cell fate and function. *Journal of immunology.* 2015;194(6):2624-34.
- [85] Papatriantafyllou M, Moldenhauer G, Ludwig J, Tafuri A, Garbi N, Hollmann G, et al. Dickkopf-3, an immune modulator in peripheral CD8 T-cell tolerance. *Proceedings of the National Academy of Sciences of the United States of America.* 2012;109(5):1631-6.
- [86] Itonaga I, Fujikawa Y, Sabokbar A, Murray DW, Athanasou NA. Rheumatoid arthritis synovial macrophage-osteoclast differentiation is osteoprotegerin ligand-dependent. *J Pathol.* 2000;192(1):97-104.
- [87] Starup-Linde J, Viggers R, Handberg A. Osteoglycin and Bone-a Systematic Review. *Current osteoporosis reports.* 2019;17(5):250-5.
- [88] Bonelli M, Dalwigk K, Platzer A, Olmos Calvo I, Hayer S, Niederreiter B, et al. IRF1 is critical for the TNF-driven interferon response in rheumatoid fibroblast-like synoviocytes : JAKinibs suppress the interferon response in RA-FLSs. *Experimental & molecular medicine.* 2019;51(7):75.
- [89] Maruyama K, Muramatsu H, Ishiguro N, Muramatsu T. Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis. *Arthritis and rheumatism.* 2004;50(5):1420-9.
- [90] Neumann E, Barnum SR, Turner IH, Echols J, Fleck M, Judex M, et al. Local production of complement proteins in rheumatoid arthritis synovium. *Arthritis and rheumatism.* 2002;46(4):934-45.
- [91] Sawada Y, Mashima E, Saito-Sasaki N, Nakamura M. The Role of Cell Adhesion Molecule 1 (CADM1) in Cutaneous Malignancies. *International journal of molecular sciences.* 2020;21(24).
- [92] van der Geest T, Laverman P, Gerrits D, Walgreen B, Helsen MM, Klein C, et al. Liposomal Treatment of Experimental Arthritis Can Be Monitored Noninvasively with a Radiolabeled Anti-Fibroblast Activation Protein Antibody. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2017;58(1):151-5.
- [93] van der Geest T, Roeleveld DM, Walgreen B, Helsen MM, Nayak TK, Klein C, et al. Imaging fibroblast activation protein to monitor therapeutic effects of neutralizing interleukin-22 in collagen-induced

arthritis. *Rheumatology*.
2018;57(4):737-47.

[94] Cunningham C AS, Wade S, Low C, Mullan R, Veale D, Fearon U. Characterisation of Rheumatoid and Psoriatic Arthritis Synovial Fibroblasts [abstract]. *Arthritis & rheumatology* (Hoboken, NJ). 2020;72.

[95] Stebulis JA, Rossetti RG, Atez FJ, Zurier RB. Fibroblast-like synovial cells derived from synovial fluid. *The Journal of rheumatology*. 2005;32(2):301-6.

[96] Ahn JK, Kim H, Lee J, Bae EK, Cha HS, Koh EM. Phenotypic characterization and invasive properties of synovial fluid-derived adherent cells in rheumatoid arthritis. *Inflammation*. 2008;31(6):365-71.

[97] Orr C, Vieira-Sousa E, Boyle DL, Buch MH, Buckley CD, Cañete JD, et al. Synovial tissue research: a state-of-the-art review. *Nature reviews Rheumatology*. 2017;13(8):463-75.

[98] Gerlag DM, Tak PP. How to perform and analyse synovial biopsies. *Best practice & research Clinical rheumatology*. 2013;27(2):195-207.

[99] Kelly S, Humby F, Filer A, Ng N, Di Cicco M, Hands RE, et al. Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality synovial tissue from both large and small joints in early arthritis patients. *Annals of the rheumatic diseases*. 2015;74(3):611-7.

[100] Lazarou I, D'Agostino MA, Naredo E, Humby F, Filer A, Kelly SG. Ultrasound-guided synovial biopsy: a systematic review according to the OMERACT filter and recommendations for minimal reporting standards in clinical studies. *Rheumatology*. 2015;54(10):1867-75.

[101] Svensson MND, Zoccheddu M, Yang S, Nygaard G, Secchi C,

Doody KM, et al. Synoviocyte-targeted therapy synergizes with TNF inhibition in arthritis reversal. *Sci Adv*. 2020;6(26):eaba4353.

[102] Stougaard J, Lomholt S, Ommen P, Kelsen J, Kragstrup TW. The antifibrotic drug pirfenidone inhibits spondyloarthritis fibroblast-like synoviocytes and osteoblasts in vitro. *BMC Rheumatology*. 2018;2(1):33.

[103] Siebert S, Pratt AG, Stocken DD, Morton M, Cranston A, Cole M, et al. Targeting the rheumatoid arthritis synovial fibroblast via cyclin dependent kinase inhibition: An early phase trial. *Medicine (Baltimore)*. 2020;99(26):e20458.

[104] Brennen WN, DL JT, Jiang W, Krueger TE, Antony L, Denmeade SR, et al. Overcoming stromal barriers to immuno-oncological responses via fibroblast activation protein-targeted therapy. *Immunotherapy*. 2021;13(2):155-75.

[105] Xu T, Zhao Y, Ding H, Cai L, Zhou Z, Song Z, et al. [(68)Ga] Ga-DOTA-FAPI-04 PET/CT imaging in a case of prostate cancer with shoulder arthritis. *European journal of nuclear medicine and molecular imaging*. 2020.

[106] Montero-Melendez T, Nagano A, Chelala C, Filer A, Buckley CD, Perretti M. Therapeutic senescence via GPCR activation in synovial fibroblasts facilitates resolution of arthritis. *Nature communications*. 2020;11(1):745.

[107] Wei K, Korsunsky I, Marshall JL, Gao A, Watts GFM, Major T, et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature*. 2020;582(7811):259-64.

[108] Kragstrup TW, Sohn DH, Lepus CM, Onuma K, Wang Q, Robinson WH, et al. Fibroblast-like synovial cell production of extra domain A fibronectin associates with

inflammation in osteoarthritis. *BMC Rheumatol.* 2019;3:46.

[109] Kriegsmann J, Berndt A, Hansen T, Borsi L, Zardi L, Bräuer R, et al. Expression of fibronectin splice variants and oncofetal glycosylated fibronectin in the synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Rheumatology international.* 2004;24(1):25-33.

[110] Kumra H, Reinhardt DP. Fibronectin-targeted drug delivery in cancer. *Advanced drug delivery reviews.* 2016;97:101-10.

[111] Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nature reviews Rheumatology.* 2020;16(6):316-33.

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