INTRODUCTION

In this review, we highlight new approaches and applications of single-cell profiling techniques and how these data are leading to unique insights into the phenotypic and functional heterogeneity of fibroblasts in the joint. Finally, we will discuss how defining this previously underappreciated fibroblast heterogeneity in rheumatoid arthritis (RA) is leading to the identification of pathological fibroblast cell states that could be therapeutically targeted in inflammatory joint disease.

1.1 Rheumatoid arthritis

RA is a prototypic immune-mediated inflammatory disease characterized by persistent synovial joint inflammation that, if untreated, leads to progressive joint damage. While the

Abstract

Rheumatoid arthritis is an immune-mediated inflammatory disease in which fibroblasts contribute to both joint damage and inflammation. Fibroblasts are a major cell constituent of the lining of the joint cavity called the synovial membrane. Under resting conditions, fibroblasts have an important role in maintaining joint homeostasis, producing extracellular matrix and joint lubricants. In contrast, during joint inflammation, fibroblasts contribute to disease pathology by producing pathogenic levels of inflammatory mediators that drive the recruitment and retention of inflammatory cells within the joint. Recent advances in single-cell profiling techniques have transformed our ability to examine fibroblast biology, leading to the identification of specific fibroblast subsets, defining a previously underappreciated heterogeneity of disease-associated fibroblast populations. These studies are challenging the previously held dogma that fibroblasts are homogeneous and are providing unique insights into their role in inflammatory joint pathology. In this review, we discuss the recent advances in our understanding of how fibroblast heterogeneity contributes to joint pathology in rheumatoid arthritis. Finally, we address how these insights could lead to the development of novel therapies that directly target selective populations of fibroblasts in the future.
introduction of biological disease-modifying anti-rheumatic drugs (bDMARDs) targeting either leukocytes or their derived products has led to a step change in the management of RA. 30%-40% of patients do not respond to such therapies, regardless of the mechanism of action of the drug used.9-13 These observations suggest the existence of additional pathways of disease persistence that remain to be identified and therapeutically targeted.14 While autoimmunity and systemic immune dysregulation are fundamental underlying disease processes in RA, the primary site of pathology during the effector phase of the disease occurs in the lining of synovial joints (called the synovial membrane).7 As a result, the pathogenic role of cells resident in synovial tissue, most notably fibroblasts and macrophages, has gained considerable attention as potential therapeutic targets in inflammatory joint disease.15-18

1.2 | Synovial tissue architecture

The synovium is a thin mesenchymal tissue that encapsulates the joint cavity and provides a barrier and lubricates the joint during locomotion.19,20 The membrane comprises a complex cellular ecosystem of tissue-resident macrophages, fibroblasts, nerves, and endothelial cells organized into a distinct microanatomy. The synovium is compartmentalized into histologically distinct zones: the lining layer and sub-lining layer with each compartment serving as a specialized tissue niche, adapted to perform specific tissue functions and unique roles in tissue homeostasis.19

In a healthy joint, the lining of the synovium is only 1-3 cell layers thick and is composed of tissue-resident macrophages and fibroblasts.21 This zone of the synovium controls cellular and molecular trafficking between the synovial membrane and the joint cavity, maintaining the integrity of the joint and regulating the composition of the synovial fluid, which ensures sufficient lubrication for joint locomotion and exchange of nutrients between the synovial tissue and the synovial fluid. In contrast, the sub-lining is comprised of fibroblasts and tissue-resident macrophages distributed throughout a loose connective tissue that contains blood and lymphatic vessels to ensure effective transport of nutrients and cells to and from the systemic circulation to the joint.

The synovial membrane undergoes extensive remodeling in response to inflammation and can expand to as much as 10-20 cell layers thick.22,23 Infiltrating immune cells and proliferation of fibroblasts result in synovial hyperplasia, characterized by a heterogeneous population of fibroblasts and macrophages that promote tissue inflammation and damage.23,24 The formation of pannus tissue, a well-described architectural feature of the chronically inflamed joint,25 is comprised of hypertrophic synovium, composed of macrophages and fibroblasts that produce destructive enzymes that degrade articular cartilage and bone.26 The cellular components and mechanisms underlying the disease-specific remodeling of the synovium have yet to be fully elucidated.27

1.3 | Definitions of tissue fibroblasts in health and disease

Fibroblasts are ubiquitous mesenchymal cells that make up the stromal compartment of organ tissues.1 Historically identified by their morphology, ability to adhere to plastic and absence of epithelial, vascular, and leukocyte lineage markers, their study has been compounded by a lack of fibroblast-specific cell markers.29,30 Under steady-state conditions, fibroblasts are more generally defined by their expression of collagen 1-alpha, platelet-derived growth factor receptor-alpha (PDGFRα), and THY1 (CD90).31-33 although these markers are not fibroblast-specific and expression does not reflect the underlying heterogeneity of this cellular population. Current evidence indicates that fibroblast populations consist of diverse cellular subsets based on their developmental origin,34,35 anatomical location,36,37 and tissue function.38 Emerging single-cell profiling data of the transcriptional landscape of fibroblasts across different tissues are providing a framework for a consensus definition of fibroblast phenotypes and description of their heterogeneity.4,29,40 This is the first crucial step in developing a universal definition of fibroblast phenotypes, but must now be extended to incorporate definitions based on cellular function that associate with the expression of specific gene programs.

1.4 | Fibroblasts in synovial joints

Synovial fibroblasts are tissue-resident cells only found in the synovium of diarthrodial joints.41 Specifically adapted to this microenvironment, these cells have an essential function in maintaining joint homeostasis and have key effector roles in inflammatory joint disease. Their true phenotypic and functional heterogeneity has only recently been fully appreciated. The molecular mechanisms leading to the functional specialization of synovial fibroblasts are an area of intense research and are leading to potentially new therapeutic avenues, including selectively targeting pathogenic subsets of fibroblasts in inflammatory joint disease.26 It is hoped that targeting these cells directly in the joint microenvironment could break the therapeutic ceiling in RA and re-establish joint homeostasis.

2 | SYNOVIAL FIBROBLASTS IN THE HEALTHY JOINT

Under resting conditions, the synovial microanatomy is characterized by a well-defined thin synovial lining layer containing a high density of fibroblasts and tissue-resident macrophages.42,43 The sub-lining layer by contrast is less well defined, comprising a loose connective tissue, sparsely populated by fibroblasts within a collagenous extracellular matrix, interspersed with adipose cells and blood vessels. The healthy joint cavity is a fluid-containing sterile space that functions to lubricate the joint cavity and lacks immune cell trafficking.19 Lining layer fibroblasts directly contribute to the composition of the synovial fluid by producing lubricin
established that synovial fibroblasts are key effectors in joint inflammation and damage. They have diverse functions that impact on many disease pathways and as a result are an attractive therapeutic target. However, they remain difficult to study in human disease largely due to a lack of specific cell surface markers which would allow for their identification from other cell types. The application of single-cell profiling technologies such as single-cell RNA sequencing and high dimensional imaging studies has revealed the true cellular diversity of synovial fibroblasts and defined their tissue-specific niches in the joint. While progress has been made recently in our understanding of the phenotypic diversity of these cells, linking this phenotypic heterogeneity to cellular function and ultimately role in disease pathology is an essential part of future research.

3.1 | Inflammatory remodeling of the synovium

During a chronic inflammatory insult, the synovial microenvironment is remodeled with the expansion and formation of a pathological, highly organized sub-lining tissue with diverse cellularity, including a significant expansion of tissue-resident synovial fibroblasts and the formation of an invasive hyperplastic pannus tissue (see Figure 1). The synovial membrane expands to 10-20 cell layers thick, with remodeling involving selective expansion of distinct cellular populations both immune and non-immune and organization of these cells into specialized compartments within the synovial tissue. These changes in the synovial microenvironment also lead to changes in the phenotype of both tissue-resident synovial cells and infiltrating inflammatory cells. These individual features of synovial remodeling all involve synovial fibroblasts which have a critical role in mediating inflammatory joint pathology, as emphasized by early studies of cadherin 11 (CDH11), an adhesion protein expressed by synovial fibroblasts during inflammation. CDH11 determines adhesion between fibroblasts and is critical for the formation of synovial tissue hyperplasia, also increases fibroblast migration and invasion, and synergizes in the activation of fibroblasts to produce matrix metalloproteinases (MMPs), cytokines, and chemokines. The genetic deletion of Cdhl11 in mice results in the impaired formation of a hyperplastic synovium leading to attenuation of synovial inflammation in mice. These studies emphasize the critical role of the synovial remodeling in driving inflammatory joint pathology and its importance as a potential site for modulating the disease process.

3.1.1 | Cellular expansion

The mechanism of pathological cellular expansion in the synovium is multifactorial, including (a) active proliferation in response to growth factors and cytokines produced by immune cells; (b) a low rate of apoptosis due to increased pro-survival factors and resistance to stress induced apoptosis (compared to other cell types); and (c) an increased accumulation of senescent cells. The differential effects of these processes on different subsets of synovial fibroblasts

(proteoglycan 4) and hyaluronic acid that lubricate the joint during locomotion. These cells also regulate ion transport and deposit extracellular matrix made up of type III, IV, V, and VI collagen and laminin allowing nutrient exchange between the synovial fluid and the synovial membrane.

The lining layer fibroblasts have traditionally been thought of as a loose association of cells that lack tight junctions but are supported by a porous basement-like membrane. A recent study in mice and humans has challenged our understanding of the cellular architecture of the synovium, demonstrating the presence of tissue-resident macrophages directly adjacent to the lining layer of barrier macrophages with lining layer fibroblasts has yet to be explored, but fibroblasts in this context may play a supportive role, helping to sustain an effective macrophage barrier. This reciprocal relationship is observed in large cavities, where fibroblasts act to prevent cavity lining macrophages from entering the tissue. The potential for reciprocal fibroblast-macrophage relationship under steady-state and pathological conditions in the synovium requires further study.

The function of fibroblasts located in sub-lining tissue under resting conditions has yet to be fully defined; however, studies of global populations of fibroblasts isolated from the healthy joint display an immunosuppressive function similar to that observed in mesenchymal stem cells. While significant progress has been made in understanding fibroblast heterogeneity under pathological conditions, little is known about the true heterogeneity of synovial fibroblasts under healthy conditions. In particular, it is not known whether the subsets of fibroblasts identified in the disease context also exist under resting healthy conditions or are simply a function of disease-driven differentiation or changes in cell activation state. The study of the resting joint has been compounded by a lack of access to healthy synovium, although this is being addressed through large cell atlas consortia studies, such as the human cell atlas.
FIGURE 1  Synovial structure in health and disease. The healthy synovium consists of a lining layer containing lubricin and hyaluronic acid secreting fibroblasts (THY1⁺ CD55⁺ PRG4⁺) adjacent to an outer lining of tissue-resident barrier macrophages (CX3CR1⁺ TREM2⁺) that maintain immune privilege in the joint.43,47 This macrophage population is replenished from CX3CR1⁻ MHCII⁺ macrophages embedded within the sub-lining layer. Under resting conditions, the sub-lining layer and the synovial membrane are not well defined. During chronic joint inflammation, the macrophage barrier is lost and there is pathological expansion and remodeling of the synovial sub-lining layer leading to extensive fibroblast heterogeneity.3,47 Some fibroblasts are detached from the synovium and found free-floating in the synovial fluid.202
and how this contributes to quantitative changes of different populations of fibroblasts during pathology are yet to be elucidated. In addition, the contribution of migrating cell populations from other tissue sources is still under active debate (see “Migratory potential of synovial fibroblasts” and Figure 2).

3.1.2 Cellular organization

The synovial microanatomy also undergoes an organizational response to inflammation, resulting in well-defined synovial tissue niches that compartmentalize pathogenic synovial cells. Lining and sub-lining cell zones are present, but joint inflammation is associated with the loss of the CX3CR1-expressing barrier macrophages and the development of a more defined sub-lining tissue characterized by replacement of adipose tissue with dense cellular infiltrates of inflammatory cells and expansion of tissue-resident macrophages and fibroblasts. Fibroblasts define this sub-lining cellular landscape, providing an address code to localize immune cells in this tissue compartment by contributing to chemokine gradients. The positional identity of fibroblasts in the synovial tissue is mediated by microenvironmental instructive signals that polarize fibroblast phenotype, depending on tissue location, by regulating the expression of specific gene expression programs. Using single-cell RNA sequencing followed by trajectory analysis and spatial localization of fibroblast subsets within human RA synovial tissue, it has been shown that human synovial fibroblasts exhibit positional identity along a spatial axis that extends from the perivascular space in the sub-lining tissue to the synovial lining layer. This is mediated by endothelium-derived NOTCH3 signaling, which is a key positional determinant of perivascular fibroblast identity. Other positional cues and polarizing signals that facilitate fibroblast specialization and positional identity have yet to be defined, but these could represent potentially important therapeutic avenues. In summary, the synovial architecture also has an organizational response to inflammation co-ordinated by synovial fibroblasts whose phenotype defines compartmentalized cellular niches within the synovial tissue that contain pathogenic cells.

3.1.3 Tissue pathotypes

Synovial histopathology in RA is heterogeneous and has been defined as pauci-immune (scant infiltration of immune cells and prevalence of tissue resident fibroblasts, diffuse-myeloid (predominant macrophages within the sublining tissue and lacking B/plasma cell aggregates) and lympho-myeloid (characterized by well-organized B or plasma cell aggregates and rich in macrophages). Synovial tissue transcriptomic profiles from untreated RA patients suggest that synovial tissue pathotype signatures are associated with specific disease trajectories, treatment response, and disease outcomes. Patients with a fibroid (pauci-immune) pathotype have an enriched fibroblast gene signature and tend to have the poorest response to treatment. These findings support a role for fibroblasts as effector cells in treatment-refractory disease pathways. Understanding how fibroblast phenotype and heterogeneity contribute to treatment response will therefore be vital in our understanding of how and when to target fibroblasts in RA.

Ectopic lymphoid structures

It has also been shown that aggregates of lymphocytes can form structures in the synovial tissue that resemble secondary lymphoid organs. They display varying degrees of organization, but are characterized by a T cell-rich zone enclosing a central B cell-rich zone served by a network of high endothelial venules. These ectopic lymphoid follicle-like structures, resembling germinal centers, are characterized by a follicular dendritic cell network (specialized stromal cell), essential for affinity maturation of B cells. These structures have been observed with variable prevalence in RA and are associated with severe disease, T cell priming and are surrounded by plasma cells producing autoantibodies locally in the tissue.

The pathophysiological role of these structures in promoting chronic joint inflammation is still uncertain, but their presence characterizes a distinct pathotype of synovitis that can be detected by
Pannus tissue

It represents another distinct cellular niche in the synovium, and pannus-resident fibroblasts may represent a distinct population of fibroblasts with specialized functions. 27 Galectin 3, a secreted β-galactoside-binding protein, is present almost exclusively in the pannus of the inflamed RA synovium. 81,82 Its expression is upregulated at sites of cartilage invasion after the attachment of synovial fibroblasts to cartilage oligomeric matrix protein. 83 Galectin 3 is then able to directly activate pannus-resident fibroblasts, stimulating the secretion of pro-inflammatory cytokines, chemokines, and MMP3, leading to the recruitment of inflammatory cells to the pathogenic tissue site. 84 At the pannus-cartilage interface, fibroblast-mediated production of MMPs, such as MMP1, MMP3, and MMP13, damages the collagen-rich structures of cartilage and enables fibroblasts to invade. 85,86

3.1.4 | Cellular phenotypes

The proliferative and organizational response to inflammation, observed at the tissue level in the synovium, is also associated with changes in cellular phenotypes leading to a diverse repertoire of tissue-resident cells. 87 There is also a global shift toward pro-inflammatory cell states with active suppression of pro-resolving cell states, resulting in a diverse cellular ecosystem that supports tissue inflammation and inhibits resolution. 22,23,88 Synovial fibroblasts can acquire a destructive phenotype, forming part of the invasive pannus tissue and along with macrophages, and release tissue destructive enzymes responsible for degradation of cartilage and bone. RA synovial fibroblasts attach to and invade cartilage directly, an effect that is independent of an intact adaptive immune system. 85,89 The pannus microenvironment becomes hypoxic, and fibroblasts within this tissue niche up-regulate tissue-degrading enzymes and apoptosis-inhibiting factors. 70-94 These destructive fibroblasts have been found to have an epigenetically imprinted phenotype that is maintained even when these cells are removed from the joint and cultured for prolonged periods of time, before being re-introduced into the cartilage implant. 85,95,96 This re-programming of fibroblast phenotype might explain why joint destruction and damage in RA do not always directly correlate with the severity of inflammation.

In summary, joint inflammation is associated with significant remodeling of the synovial microenvironment observed at a spatiotemporal and phenotypic cellular level. These processes direct site-specific aspects of pathology and may underlie the clinical heterogeneity of RA. Synovial fibroblasts are critical cells in this process and orchestrate the synovial microanatomy into distinct anatomical compartments and drive the spatial organization of synovial cells to develop functional cellular networks. As detailed cellular atlases emerge of the developing, healthy, and inflamed joints, we will gain further critical insights into the cellular ecosystem of the joint and its role in disease.

3.2 | Synovial fibroblast phenotypic and functional diversity

Tissue fibroblasts have historically been considered as functionally homogeneous cells, involved primarily in extracellular matrix production. However, it is now widely accepted that these cells perform a number of specialized functions. 97,98 In the joint, the lining and sub-lining of the synovial membrane are anatomically separated and contain morphologically distinct populations of fibroblasts. 53,99 Numerous human studies have demonstrated potential heterogeneity in the expression of cell surface marker proteins, 100 proteoglycans, 101 and various chemokines 28 between different compartments of the synovial membrane, consistent with the existence of different synovial fibroblasts within each of these tissue compartments. Early studies also implied a stratification of function between the lining and sub-lining layer fibroblasts, with a more pro-inflammatory function attributed to the sub-lining layer fibroblasts. 100,102 For example, a single-cell RNA-sequencing analysis of the human RA synovial tissue initially identified two main fibroblast phenotypes: a CD55+ population in the lining and a THY1+ population in the sub-lining. 102 The CD55+ fibroblasts were enriched for HAS1 (encoding a hyaluronan synthase), and the THY1+ fibroblasts were enriched for genes related to MMP expression and organization of the extracellular matrix. The phenotypic diversity of synovial fibroblasts has, however, only recently been addressed comprehensively using single-cell profiling technologies from enzymatically digested synovial tissue in mice and humans. 38,62 These studies have clearly defined transcriptomically distinct populations of fibroblasts under inflammatory conditions. Initially, Mizoguchi et al 28 explored this diversity of human RA synovial fibroblasts by using flow cytometry to sort purify putative fibroblast subsets based on the expression of stromal markers PDNP, CDH11, CD34, and THY1. These proteins were chosen as they were expressed at low levels under resting conditions and significantly upregulated during joint inflammation. 42,55,103-107 Following analysis by microarray and low-input, bulk-cell RNA sequencing followed by principal component analysis, three putative synovial fibroblast subsets were identified with unique transcriptomic profiles: PDNP+ CD34+ THY1+ lining fibroblasts, PDNP+ CD34+ THY1+ sub-lining fibroblasts, and PDNP+ CD34+ THY1+ sub-lining fibroblasts. 38 Of these, the THY1+ fibroblast subsets were expanded...
in the inflamed synovium in RA and correlated with the severity of joint inflammation. In contrast, in the synovia of patients with osteoarthritis (OA), a non-inflammatory joint disease, the PDPN⁺ CD34⁺ THY1⁺ fibroblasts were expanded. Although these populations differed in their location within the joint, almost all subsets were positive for protein CDH11, which was previously shown to be associated with pathological behavior of fibroblasts in vitro and in experimental arthritis mouse models.⁴²

The observation that sub-lining fibroblasts are expanded in inflamed synovia in RA has been confirmed in several studies including Zhang et al.⁴³ The authors used mass cytometry and unbiased single-cell RNA-sequence clustering to identify four synovial fibroblast populations with distinct transcriptomic profiles in a collaborative study, as part of the Accelerating Medicines Partnership-Rheumatoid Arthritis/Systemic Lupus Erythematosus (AMP RA/SLE) Consortium. These subsets included a CD34⁺-, THY1⁺-, and HLA-DRAhigh-expressing subset, found in both the sub-lining layer and a PRG4⁺ (gene encoding lubricin) lining layer population of fibroblasts. THY1⁺ HLA-DRAhigh sub-lining fibroblasts were >15-fold expanded in RA synovium and are considered pro-inflammatory, expressing high amounts of IL-6, CXCL12, and CCL2. In addition to the above, Zhang et al.³ identified a novel sub-lining fibroblast subset with high expression of DKK3 encoding Dickkopf3, a protein upregulated in OA that prevents cartilage degradation in vitro.¹⁰⁸ DKK3 overexpression in fibroblasts suppresses cell proliferation and promotes apoptosis via TGFβ1/SMAD signaling¹⁰⁹ as well as impairing angiogenesis and inhibiting tumor growth through induction of endoplasmic reticulum stress.¹¹⁰ Interestingly, Zhang et al.³ demonstrated that DKK3⁺ sub-lining fibroblasts also expressed high levels of OPG, CADM1, and MFAP2 which are involved in bone formation,¹¹¹ enhanced intestinal barrier function,¹¹² and elastic fiber structural formation.¹¹³ Thus, DKK3⁺ fibroblasts may play a role in immuno-regulation and/or restoring joint homeostasis. Finally, Zhang et al.³ demonstrated lining layer subsets consisting of (species-specific) CD55⁺ CD34⁻ THY1⁺ fibroblasts and express high levels of genes MMP1, MMP3, PRG4, HAS1, and CD55.³,³⁸,⁴⁷,⁶² See Table 1 for a summary of fibroblasts subsets, along with evidence of the proposed function.

Our work has defined the synovial fibroblast heterogeneity in both human and mouse arthritis.⁴⁷ We found that stromal markers were highly expressed in the inflamed synovia (tissue biopsy studies) in those patients who developed RA, compared to those in which joint inflammation resolved.⁹⁸ Of these markers, high expression levels of fibroblast activation protein-α (FAPα), a cell membrane dipeptidyl dipeptidase expressed by synovial fibroblasts,¹¹⁴ were the strongest predictor of chronic joint inflammation.⁹⁸ This led us to develop the hypothesis that FAPα may be a biomarker of a pathogenic subset of synovial fibroblasts. Deletion of FAPα⁺ cells attenuated joint inflammation and bone erosion in serum transfer-induced arthritis in mice, suggesting that FAPα-expressing fibroblasts play an important pathological role in arthritis. Flow cytometry confirmed FAPα⁺ fibroblasts were split into two discrete subsets: THY1⁻ and THY1⁺ FAPα⁺-expressing cells. Bulk population RNA-seq of sorted purified populations confirmed that FAPα⁺ THY1⁺ cells displayed a transcriptional signature of lining fibroblasts, whereas FAPα⁻ THY1⁺ cells expressed a sub-lining phenotype. The most significant differences in transcriptional profiles were observed between the lining and sub-lining fibroblast populations, suggesting that anatomical location in the tissue was a major determinant of phenotype and gene expression programs.

Using single-cell transcriptomics of enzymatically digested mouse synovia, we confirmed the presence of several transcriptionally distinct populations of fibroblasts with one THY1⁺ lining layer population (also expressing Cd55, Prg4, and Clic5) and three THY1⁻ sub-lining layer subsets. A cross-comparison between human and mouse single-cell datasets revealed two THY1⁺ subsets that are shared between human and mouse, including a CD34⁺ C3⁺ (this subset is localized within the perivascular zone around blood vessels and involved in immuno-inflammatory processes and stromal remodeling) and a COL8A1⁺, COL1A1⁺ MDK⁺ (relating to bone, cartilage, and ECM remodeling). In addition, a single lining layer fibroblast phenotype was identified by a cassette of gene expression markers CLIC5⁺, TSPAN15⁺, and PRG4⁺. Re-analysis of the Zhang et al.³ human synovial tissue dataset revealed FAPα expression within the pathogenic subset of fibroblasts (THY1⁺ HLA DRhigh) identified in this dataset and predominately within the sub-lining cell fibroblast populations.⁴⁷

The transcriptional gene expression programs that define these fibroblast subsets correlate with different effector cell functions. As mentioned above, Zhang et al. showed in human synovial tissue that THY1⁺ HLA DRhigh fibroblasts express the highest level of IL-6, CXCL12, CCL2, and interferon-stimulated genes, suggesting a pro-inflammatory phenotype.³ In mouse studies, the Fapα⁺ THY1⁺ subset is located in the sub-lining and has an immune effector profile characterized by high expression of a number of cytokines and chemokines, including IL-6, IL-33, and IL-34.⁷⁷ The Fapα⁺ THY1⁻ subset is located in the lining and has a damage effector profile that includes high expression of inducers of osteoclast activity (CCL9 and TNFS11) and MMPs involved in cartilage degradation (MMP3, MMP9, and MMP13), indicating that the cells mediate bone and cartilage damage.⁷⁷ Consistent with these findings, subsets of synovial sub-lining fibroblasts have been found to have a secretory profile consistent with an inflammatory state in both mouse and human fibroblasts, secreting chemokines and cytokines (eg, IL-6, CXCL12, and CCL2) in response to TNFα stimulation in vitro, that have the potential to modulate the inflammatory response in the joint.⁸⁸ The proportion of CD34⁺ THY1⁺ cells in the joint correlated positively with the proportion of leukocytes and the extent of synovitis in the human RA synovium.⁸⁸ In contrast, lining fibroblasts are the pre-dominant source of MMP1 and MMP3 and are able to stimulate osteoclastogenesis through the production of RANKL, and these findings are consistent with the lining fibroblasts having a destructive phenotype with the ability to invade and degrade articular cartilage and bone in mice.⁴¹ We have previously shown that PDPN⁺ human synovial fibroblasts attached to, invaded, and degraded articular cartilage following implantation to immunodeficient mice.¹⁰⁰

The definitive evidence that these transcriptionally distinct populations of fibroblasts have non-overlapping effector cell functions
came from our adoptive transfer studies of selected fibroblasts in vivo using mouse models of arthritis. We were able to demonstrate that the intra-articular injection of murine FAPα+ THY1+ immune effector sub-lining fibroblast subsets into the inflamed mouse synovium exacerbated joint inflammation, whereas the injection of FAPα+ THY1− subsets did not affect the severity of inflammation but resulted in more joint damage. These data collectively demonstrate that the immune effector functions of synovial fibroblasts are conferred by sub-lining subsets, whereas damage is mediated predominately by the lining layer fibroblasts. The co-independence of these two cell populations remains uncertain as lining fibroblasts only had a destructive phenotype when stimulated ex vivo with pro-inflammatory cytokines, perhaps suggesting that damage also requires an intact pathological sub-lining layer in vivo. We further demonstrated an expansion of immune effector fibroblasts expressing FAPα and THY1 in the synovial tissue of individuals with RA, compared to patients with OA, consistent with previous studies demonstrating an expansion of sub-lining fibroblast subsets in RA.

The differential expansion of these two fibroblast populations in the synovium could explain the inflammatory pathotype of RA, in contrast to a more destructive pathotype observed in OA.

The molecular mechanism driving the expansion of sub-lining layer fibroblasts in RA has recently been elucidated. Wei et al recognized that expansive fibroblast populations in the sub-lining were

### TABLE 1 Major fibroblast subsets and probed function in the inflamed synovium

| Subsets | Tissue location | Proposed function | Mouse homologous Subset | Disease pathology | Common markers |
|---------|----------------|-------------------|-------------------------|------------------|----------------|
| DKK3+Croft et al47 Zhang et al3 | Sub-lining layer | Express genes related to the extracellular matrix, Bone formation, cell junction formation, and elastic fiber structural integrity | Not yet defined | Not yet defined—may have a role in tissue remodeling. | DKK3, CADM1, Mfap2, OPG, Col1a2, |
| THY1HLA-DRh3 Zhang et al3 | Sub-lining layer (perivascular and interstitial) | Immunoregulatory, pro-inflammatory effect, NOTCH3-mediated differentiation. | Yes | Expanded by >15-fold in RA in synovial tissue containing high levels of leukocyte infiltration compared with synovial tissue in OA. IL-6 expression. Express genes related to MHC class II presentation and the IFNγ-mediated signaling pathway. | Col11a1, Mdk, Col8a1, Postn, MMP13, Col1a1, |
| CD55+ PRG4+ Croft et al47 Zhang et al3 Stephenson et al102 | Lining layer | Lining layer function under resting conditions—secretes lubricin and hyaluronic acid | Yes | Increased osteoclast activity and structural joint damage. Less abundant in RA in synovial tissue containing high levels of leukocyte infiltration than in synovial tissue in OA | CLIC5, Tspan15, PRG4, Hbegf, Htra1, Sema3a |
| THY1 CD34+ Croft et al47 Fričič, J. et al163 Mizoguchi et al38 | Sub-lining layer (perivascular) | Immunoregulatory, tissue priming via C3 activation, pro-inflammatory effect, perivascular location, NOTCH3-mediated differentiation. | Yes | Expression of genes involved in complement activation, responsible for tissue priming response that underlies progression of arthritis. Express genes related to the extracellular matrix. Increased invasive and migratory properties in vitro. Increased ability to recruit peripheral blood monocytes in vitro | Apod, C3, CD34, Mfap5, Clip, CXCL14 |

Note: Recent studies38,102 have identified different fibroblast subsets in the joint defined by their transcriptomic signatures, anatomical location, and potential function and role in disease pathology.
located around the endothelium in the perivascular space, suggesting that vascular endothelial-derived signals could be important in modulating fibroblast phenotype. The investigators determined that the pathogenic sub-lining fibroblast phenotype and the expansion of this population are driven by endothelial-derived NOTCH3 signaling. The critical role of NOTCH3 signaling in arthritis was confirmed by the genetic deletion of the Notch3 gene and antibody blockade of NOTCH3 signaling during experimental arthritis in mice, with both approaches leading to attenuated synovial inflammation and damage. These studies demonstrate that the inhibition of NOTCH3-mediated induction of pathogenic fibroblast differentiation could be an effective therapeutic approach in RA.

It is interesting that targeting a sub-lining layer fibroblast is also effective at inhibiting joint damage, given the observation that immune regulatory and joint destructive functions may be carried on two different effector fibroblast subsets. These findings suggest that an intact pathogenic synovial sub-lining may be required to mediate the destructive abilities of lining fibroblasts or that redundancy in function exists between fibroblast subsets in the synovium. Indeed, we only observed a destructive phenotype in lining layer fibroblasts in vitro following stimulation with TNFα. The genetic deletion of Cdh11 gene which encodes the CDH11 protein that mediates cell-to-cell contact between synovial fibroblasts in mice disrupts the synovial membrane architecture, inhibiting synovial hyperplasia, and protects the joint against both damage and inflammation. Collectively, these data suggest that the architectural reorganization or function of cells in this compartment in response to inflammation can be protective.

In conclusion, recent studies have described a diverse repertoire of fibroblast phenotypes with effector functions mapped to individual subsets, summarized in Table 1. However, several challenges and uncertainties still remain. Firstly, a detailed functional analysis of individual fibroblast subsets is still needed. Secondly, in order to understand the contribution of fibroblast heterogeneity to inflammatory joint pathology, it is important to understand the spatiotemporal changes of these fibroblast populations as disease progresses and in response to treatment. Thirdly, it is vital that we understand how changes in fibroblast phenotype and differentiation are regulated and whether pathogenic fibroblast subsets defined by single-cell analysis represent reversible changes in cell status. Collectively, this knowledge will allow us to determine the role of synovial fibroblasts in driving specific disease pathologies and determine how and when to utilize anti-fibroblast therapy in the future.

3.3 Synovial fibroblasts as immune effector cells

Two important functional changes occur during the progression of RA. Firstly, synovial fibroblasts lose their immunosuppressive capability and secondly become actively immune-stimulatory. The persistent pathological phenotype of fibroblasts in established RA demonstrates that synovial fibroblasts are not merely "passive responders" to the inflammatory milieu, but can independently influence the inflammatory status of the joint.

3.3.1 Fibroblast/endothelial cross talk determines immuno-phenotype

The interaction between leukocytes and synovial fibroblasts during an acute inflammatory response ultimately leads to resolution of the inflammatory focus. However, such interactions at sites of chronic inflammation lead to sustained leukocyte survival and retention within the joint, leading to the persistence of the inflammatory lesion. For example, upon coculture with endothelial cells (EC), fibroblasts isolated from patients with resolving synovitis and non-inflamed joints suppressed lymphocyte adhesion in response to TNFα stimulation. This immune-protective effect was lost in fibroblasts isolated from patients with very early RA (≤3 months since disease onset), allowing increased lymphocyte recruitment. The stimulatory phenotype was characterized by high levels of IL-6 and TGF-β1 production. Hence, fibroblasts cultured from the synovial tissues of patients with divergent disease outcomes (resolving vs persistence) are also functionally distinct. Moreover, fibroblast-EC interactions evolve with RA progression. In contrast, fibroblasts from very early RA have not yet acquired the ability to autonomously activate EC in the absence of exogenous cytokines.

IL-6 and TGF-β1 are each able to induce divergent pro-inflammatory or anti-inflammatory effects depending on the inflammatory context or cell type. Emerging evidence reveals complex cross talk between IL-6 and TGF-β1 signaling pathways, in which each cytokine can positively or negatively regulate the expression or activity of the other depending on the inflammatory context. IL-6 and TGF-β1 were identified as the bioactive agents required for the inhibitory effects on recruitment of cocultured resolving fibroblasts. Furthermore, neutralization of IL-6 and TGF-β1 inhibited the recruitment effect of cocultured very early RA fibroblasts. This suggests that in very early RA, IL-6 and TGF-β1 have not simply lost efficacy, but trigger stimulatory rather than inhibitory downstream events.

Synovial fibroblasts are a major source of IL-6 in RA. Recent evidence suggests synovial fibroblasts isolated from patients with RA sustain IL-6 production (as well as heightened production of other chemotactic mediators such as IL-8) by creating a positive feedback loop involving autocrine LIF, LIF receptor, and STAT4 signaling. IL-6 released during coculture with EC signals through CD126 expressed by EC, but not fibroblasts. Given that fibroblasts cannot respond to IL-6 generated during coculture, distinct fibroblast-EC interactions must regulate EC responses to IL-6 and produce the discrete patterns of lymphocyte recruitment. High SOCS3 expression (ie, negative regulation of STAT activation), as seen in the EC from cocultures with fibroblasts isolated from patients with resolving synovitis, triggers an immuno-protective IL-6 response. Conversely, failure to induce SOCS3 was associated with loss of immunosuppressive responses in the EC from cocultures very early.
3.3.2 | Fibroblasts as innate immune cells

In addition to loss of immune-protective function, synovial fibroblasts also play an important role in RA pathology through cross talk with other cell types. Historical evidence has shown synovial fibroblasts can act as innate immune cells through the expression of pattern recognition receptors (PRRs) such as TLR2, TLR3, TLR4, TLR7, and TLR9. TLR4 recognizes anti-citrullinated protein antibodies (ACPAs) and has been demonstrated to play an important role in RA pathology. Recently, it has been shown that soluble CD14 can also induce the expression of inflammatory cytokines such as IL-6, TNFα, IL-8, adhesion molecules, MMPs, and RANKL through TLR4. TLR4 has been shown to activate TLR3 and induce the production of pro-inflammatory mediators through NFκB signaling. Silencing of regulator of G-protein signaling (RGS1) inactivates TLR3 and suppresses inflammation and angiogenesis in collagen-induced arthritis in mice. In addition, when cocultured with T cells, synovial fibroblast TLRs induce cell-cell contact and cytokine-mediated TH1 and TH17 expansion resulting in IFNγ and IL-17 production. Thus, like innate cells, synovial fibroblasts are capable of recognizing pathogen-associated molecular patterns and endogenous ligands which contribute to the pathogenesis of RA.

3.3.3 | Fibroblast/macrophage coupling serves as an immune-regulatory checkpoint in the synovium

Under steady-state conditions, the other major cellular component of the synovial membrane is macrophages. Macrophages derive from two main cellular lineages: bone-marrow-derived monocytes and monocyte-independent macrophages derived from cells that disperse into the tissues during embryonic development. The tissue-resident macrophages from the latter lineage have distinctive gene expression profiles that depend on the particular tissue in which they reside. Under resting conditions in mice, 40% of synovial tissue macrophages are CX3CR1+ macrophages that constitute a barrier layer adjacent to lining layer fibroblasts. These macrophages have an immunoregulatory phenotype expressing Trem2 and genes encoding TAM receptors such as Axl and Meger that mediate the clearance of apoptotic cells. These barrier CX3CR1+ macrophages are derived from CX3CR1+ MHCIIC cells situated deep within the sub-lining layer. These MHCIIC+ synovial macrophages also give rise to CX3CR1+ RELMα+ sub-lining macrophages which are found to be expanded during experimental arthritis in mice and in synovial tissue in RA. Depletion of CX3CR1+ macrophages during experimental arthritis in mice leads to synovial barrier breakdown and cellular infiltration of neutrophils and monocyte-derived cells into the synovium. Although CX3CR1+ lining macrophages do not expand during

3.4 | Fibroblast/macrophage coupling serves as an immune-regulatory checkpoint in the synovium

Under steady-state conditions, the other major cellular component of the synovial membrane is macrophages. Macrophages derive from two main cellular lineages: bone-marrow-derived monocytes and monocyte-independent macrophages derived from cells that disperse into the tissues during embryonic development. The tissue-resident macrophages from the latter lineage have distinctive gene expression profiles that depend on the particular tissue in which they reside. Under resting conditions in mice, 40% of synovial tissue macrophages are CX3CR1+ macrophages that constitute a barrier layer adjacent to lining layer fibroblasts. These macrophages have an immunoregulatory phenotype expressing Trem2 and genes encoding TAM receptors such as Axl and Meger that mediate the clearance of apoptotic cells. These barrier CX3CR1+ macrophages are derived from CX3CR1+ MHCIIC cells situated deep within the sub-lining layer. These MHCIIC+ synovial macrophages also give rise to CX3CR1+ RELMα+ sub-lining macrophages which are found to be expanded during experimental arthritis in mice and in synovial tissue in RA. Depletion of CX3CR1+ macrophages during experimental arthritis in mice leads to synovial barrier breakdown and cellular infiltration of neutrophils and monocyte-derived cells into the synovium. Although CX3CR1+ lining macrophages do not expand during

3.3.4 | Fibroblast subsets promote T cell recruitment

It is well documented that synovial fibroblasts can present antigens to CD4+ T cells during RA via MHCIIC. In addition, the production of chemokines such as CXCL10 and CXCL19 by RA synovial fibroblasts is key promoters of lymphocyte recruitment. A recent cross-disease analysis which included human fibroblasts isolated from the RA synovium has demonstrated SPARC+ COL3A1+ synovial fibroblasts interact with blood vessels via NOTCH signaling to alter the EC compartment, priming it to promote cellular infiltration which is further supported through the differentiation of CXCL10+ CXCL19+ synovial fibroblasts. CXCL10+ CXCL19+ fibroblasts interact directly with T cells and are likely analogous to Zhang et al defined HLA-DRα+ pathogenic subset that displays a strong response to IFNγ (a pro-inflammatory molecule abundantly secreted by CD8+ T cells). The expression of RA synovial fibroblast CX3CL1 and CX3CR1 by CD8+ and CD4+ T cells has also been shown to positively correlate with RA pathology. In support of this, treatment targeting CX3CL1 production has shown efficacy in early phase clinical trials in RA.

While the global effect of depleting sub-lining layer synovial fibroblast subsets has been determined to have an anti-inflammatory effect in mice, the individual contribution of distinct sub-lining fibroblast subsets in regulating differential aspects of inflammation has yet to be determined. In RA, THY1+ HLA-DRα+ fibroblasts express an interferon-stimulated phenotype suggesting that IFNγ-producing lymphocytes that infiltrate the tissue may stimulate an interferon-activated gene signature in this fibroblast population and drive their differentiation into a pro-inflammatory phenotype. It is likely that other critical interactions between synovial fibroblasts and other infiltrating inflammatory cells result in the modulation of the tissue fibroblast phenotype. It will be vital to define the effect of these cellular interactions on the regulation of individual fibroblast subset phenotypes in the future, since these interactions are likely to evolve as disease progresses, and in response to treatment with specific immunomodulatory therapies.
inflammation, they do, however, maintain an immunoregulatory phenotype and actively clear apoptotic cells.

These data suggest that CX3CR1+ macrophages are the first immune-regulatory checkpoint that attempts to suppress or limit synovial inflammation and the failure of this checkpoint leads to established joint inflammation. The functional purpose of the anatomical coupling of lining macrophages with lining layer fibroblasts (which are functionally, spatially and anatomically distinct from sub-lining fibroblasts) is currently unknown. We hypothesize that the loss of this spatial coupling during inflammation may lead to the emergence of a pathogenic lining layer fibroblast phenotype or a compensatory repair like phenotype.

In the sub-lining tissue, MHCII+ interstitial macrophages replenish the end-stage CX3CR1+ macrophages, although the exact role of end-stage interstitial macrophages is currently unclear. However, as MHCII+ macrophages also give rise to pro-inflammatory sub-lining RELMα-expressing macrophages, it is important to understand the mechanism in which CX3CR1+ and RELMα+ macrophage differentiation is determined. As lining and sub-lining macrophages are long-lived, it is also possible there is a pool of MHCII+ macrophages which is rapidly depleted in response to an inflammatory stimulus. Further studies are required to explore the interactions between lining layer macrophages and fibroblasts and the molecular mechanisms that lead to disruption of the synovial macrophage barrier and expansion of the invasive fibroblast population seen in RA.

The second fibroblast-macrophage regulatory checkpoint is observed between fibroblasts and sub-lining macrophages. Sub-lining macrophages comprise heterogeneous populations of monocye-derived and tissue-resident macrophages. A recent comprehensive analysis of their heterogeneity has revealed the transcriptional and functional diversity of synovial tissue macrophages.87 The investigators isolated macrophages from patients with early/active RA, treatment-refractory/active RA, and RA in sustained clinical remission. The analysis revealed four distinct subsets that comprised nine discrete phenotypic states. Two of these subpopulations (MERTK+ TREM2high CD48+ SPP1+ macrophages and MERTK+ LYVE1+ macrophages) were enriched in the healthy synovium and in patients with RA whose disease was in remission compared with those with active disease. A reduction in these cellular subsets in the tissue was associated with an increased risk of future disease flare. In vitro evidence showed MERTK+ macrophages produced high amounts of pro-resolving lipids and when cocultured with synovial fibroblasts induced a repair response in fibroblasts. In contrast MERTK, MerTK macrophages are expanded during active disease compared to remission. This population comprises subsets of macrophages expressing alarmins (CD48+ S100A12+), bone remodeling molecules (CD48+ SPP1+), an interferon signature (HLA+ ISG15+ cluster), and an antigen-presenting cell signature (HLA+ CLEC10A+). MERTK macrophages produce TNFα and IL-6 and induce pathological activation of fibroblasts.

As a result, MERTK+ macrophages may act as an off switch by negatively regulating pro-inflammatory fibroblast phenotypes. We hypothesize the failure of this checkpoint could lead to the emergence of persistently activated, pathogenic fibroblast subsets. In addition, another study has revealed the existence of macrophages in the RA synovium that are positive for the growth factor HB-EGF.136 These macrophages promoted fibroblast invasiveness in an epidermal growth factor receptor-dependent manner. Thus, indicating that intercellular networks between these pathogenic populations of synovial tissue cells can contribute to inflammatory joint pathology.

In summary, these studies demonstrate that fibroblast phenotypes can be regulated by synovial tissue macrophages. The coupling of these tissue-resident cell types appears to be critical in determining if joint inflammation either persists or resolves. If these checkpoints fail to limit or restrict inflammation, then disease persists. Certain macrophage subsets may therefore act as critical “off” switches, resulting in the suppression of pro-inflammatory fibroblast phenotypes. It is not yet known, however, how inflammation-induced epigenetic changes and metabolic re-programming of fibroblasts observed in chronic disease might impact on their ability to respond to these regulatory changes in their phenotype induced by macrophages. In addition, quantitative changes in these two cell populations may underpin disease progression and treatment response. Understanding the nature of the functional networks established by these tissue-resident synovial cells at different stages of disease and in response to treatment will be critical in developing novel therapeutic approaches that aim to restore joint homeostasis.

3.5 | Synovial fibroblasts—passive responders or transformed aggressors

Current evidence suggests that the phenotype (based on positional identity) of synovial fibroblasts is acquired from microenvironmental instructive signals from within the synovial tissue, rather than a pre-determined phenotype. One instructive signal of fibroblast phenotype and positional identity in the tissue has been identified as endothelial-derived NOTCH3 signaling which drives a sub-lining pathogenic fibroblast phenotype, responsible for driving arthritis pathology in the joint.62 The removal of these fibroblasts from the joint resulted in the rapid loss of their tissue phenotype and the acquisition of a transitional fibroblast phenotype. These findings are consistent with observations that culture of synovial fibroblasts can lead to alterations in their phenotype over time.137,138 These observations suggest a high degree of cellular plasticity in fibroblast phenotype that is dependent on external signals that regulate specific programs of gene expression within these tissue-resident cells.

However, a large body of evidence supports the concept that disease-associated fibroblast phenotypes evolve with the chronicity of disease, resulting in a stably activated and aggressive phenotype in established disease.51 The strongest evidence that an autonomous destructive fibroblast phenotype is established in chronic disease is the finding that invasive behavior is retained by human RA synovial fibroblasts in ex vivo invasion assays and in human RA fibroblast-cartilage co-implantation assays in mice.26,51,139 Thus, the
invasive phenotype of fibroblasts in RA is dependent on both autonomous and local microenvironmental signals and is highly likely to be epigenetically imprinted in chronic disease. Epigenetic mechanisms can induce stable changes in gene expression without altering the genome, and have been extensively reviewed elsewhere. These mechanisms are essential during embryonic development and define tissue- and cell-specific gene expression. In postnatal tissues, these mechanisms operate to allow cells to adopt to microenvironmental changes.

However, changes in the epigenome can lead to slowly, imprinted, and pathologically driven changes in gene expression. These pathogenic programs of gene expression could lead to the emergence of disease-associated effector cell phenotypes that ultimately fail to respond to negative regulation by immunosuppressive signals, resulting in the persistence of joint inflammation.

There are three main types of epigenetic modifications reported in RA fibroblasts: methylation, histone modifications, and microRNAs (miRNAs). Such modifications are disease-specific displaying differences between RA and OA synovial fibroblasts. This suggests epigenetic modifications can determine disease pathotype. Hypo- and hypermethylation (unmethylated or methylated CpG site that is normally or not normally methylated, respectively) are associated with an aggressive fibroblast phenotype, inducing expression of pro-inflammatory genes which favor RA synovial fibroblast proliferation and survival as well as destructive endogenously activated pathways which activate MMPs. Changes in fibroblast methylene can occur in early RA and may be useful as potential prognostic biomarker of disease progression.Histone degradation, acetylation, and methylation have also been implicated in RA fibroblast phenotype where prolonged exposure to TNFα in vitro can increase chromatin accessibility in RA fibroblasts by decreasing total histone4 (H4) and hyperacetylating the remaining H4, priming the cell for enhanced pro-inflammatory cytokine and chemokine production. Hyperacetylation of synovial fibroblast H3 also causes the chromatin region of the IL-6 promoter to loosen resulting exacerbated expression. The inflammatory milieu also deregulates the expression of fibroblast miRNA. For example, inhibiting histone acetylation in mouse synovial fibroblasts enhances miR-124 production and resolves synovial hyperplasia and inflammation in experimental arthritis by suppressing JAK/STAT signaling. Furthermore, a recent study demonstrated plasma levels of miR-23a-5p are significantly lower in RA blood. Overexpression of miR-23a-5p suppressed IL-6 and IL-1b production by inhibiting TLR4/NFκB signaling.

In addition to the above, the role of long noncoding RNAs (lncRNAs) has recently been addressed and has shown they are key players in regulating epigenetic and chromatin remodeling and transcriptional and post-transcriptional modifications. Over the last five years, there have been an increasing number of publications demonstrating the important role in which lncRNAs play in RA pathology (reviewed in Lao et al.). In RA, lncRNAs have both suppressive and pathological effects in synovial fibroblasts, respectively, sequestering or activating NFκB signaling and ultimately affecting fibroblast proliferation, migration, invasiveness, and apoptosis.

For example, a recent publication shows IncRNA NEAT1 is upregulated in RA fibroblasts and by targeting and repressing miR-204-5p exacerbates IL-1β and IL-6 production in a TNFα-dependent manner. In contrast, plasma concentrations of IncRNA DILC negatively correlate with IL-6 production in RA fibroblasts and therefore disease severity. Overexpression of DILC in RA fibroblasts induces apoptosis and inhibits IL-6 production.

In summary, synovial fibroblasts are plastic and local signals from within the joint microenvironment determine the induction of specific gene expression programs that determine their phenotype. During chronic inflammation, it is highly likely that pathogenic gene expression programs become imprinted as a result of persistent epigenetic modifications, in response to the inflammatory microenvironment. As a result, therapeutic interventions aimed at restoring physiological fibroblast cell states will need to either prevent the acquisition of these epigenetic modifications or reverse the process in established disease. Characterizing the epigenetic landscape of synovial fibroblasts and the disease-driven alterations in the gene expression programs that underlie specific changes in their phenotype may open new therapeutic avenues for modulating pathogenic fibroblast phenotype in disease.
to maintain viability and function in the hypoxic and inflammatory environment of the joint.\textsuperscript{165,166} An essential component of this adaptation is a glycolytic shift in synovial fibroblast metabolism that correlates with the acquisition of a pathogenic phenotype. Hexokinase-2, an enzyme that phosphorylates glucose to produce glucose-6-phosphate resulting in an increase rate of glycolysis in rapidly proliferating cells, has been shown to mediate synovial tissue hypertrophy underpinning the severity of joint inflammation and damage during arthritis.\textsuperscript{167} Targeting these metabolic pathways could be a viable therapeutic approach in RA.\textsuperscript{168}

The mechanism driving the metabolic re-programming of synovial fibroblasts in response to repeated inflammatory insults have been shown to be the result of an amplified production of C3 and its receptor activation in response to inflammation.\textsuperscript{163} This results in inflammasome activation and protection from cell senescence via mammalian target of rapamycin (mTOR) and hypoxia-inducible factor (HIF) 1a signaling. This mechanism of inflammatory re-programming operating in a selective population of pathogenic fibroblasts underpins tissue priming and the persistence of joint inflammation. It is highly likely that similar fibroblast subsets exist in other tissues and can be primed, promoting site-specific recurrence of inflammation. Importantly, these findings further support the rationale for therapeutically targeting the complement system in RA.\textsuperscript{169,170}

### 3.7 Migratory potential of synovial fibroblasts

Studies in mice have previously suggested that synovial fibroblasts also have migratory potential and can leave the joint to migrate to distant joints in vivo, potentially spreading disease from one joint to another.\textsuperscript{95,96,171} Several studies in mice have supported this hypothesis; firstly, migration of mesenchymal precursors from the blood into the joint prior to the onset of inflammatory arthritis has been observed in experimental arthritis models\textsuperscript{172} and secondly the observation that fibroblasts are indispensable for triggering arthritis in joints and can be directly arthrogenic.\textsuperscript{173,174} Furthermore, it has been shown that human RA synovial fibroblasts implanted with a cartilage/sponge matrix into an immunodeficient mouse can travel from the site of engraftment (cartilage/cell implant) to a distant cartilage implant.\textsuperscript{96} Cartilage damage stimulates fibroblasts to leave the vasculature and enter the joint, before they attach and invade articular cartilage.\textsuperscript{175} For example, in TNFx transgenic mice, loss of cartilage proteoglycan is a prerequisite for the destruction of cartilage by synovial fibroblasts.\textsuperscript{176} Furthermore, collagenase or IL-1 injection (IL-1 results in the loss of cartilage proteoglycan) into mouse synovial joints attracts intravenously injected human RA synovial fibroblasts to the joint and promotes attachment and degradation of cartilage.\textsuperscript{96,175} The exact phenotype of fibroblasts that have this migratory potential is not fully known. Our own work has shown that migrating cells that invade a distal cartilage implant in vivo, expressed PDPN\textsuperscript{+} and not, initially sub-lining layer fibroblast markers.\textsuperscript{100} However, as the implant matures and vasculature became established, the organization of fibroblasts at the site represented the synovial tissue architecture, in which cells were organized into lining and sub-lining layer zones with the differential expression of cell surface markers. Although these findings have not been confirmed in the context of newly characterized synovial fibroblast subsets,\textsuperscript{3,4} the data are consistent with the observations of fibroblast phenotype plasticity in response to microenvironmental cues.\textsuperscript{62}

Despite the importance of these findings, fibroblast migration in human disease has only recently been reported. The discovery of pre-inflammatory mesenchymal cells (PRIME cells) that are detectable in the blood of patients prior to an arthritis flare has re-opened the debate on “migrating” synovial fibroblasts. Orange et al.\textsuperscript{177} identified PRIME cells as potential cellular biomarkers that predict disease flare in patients with RA and demonstrated that these cells have a mesenchymal cell phenotype and are recruited by naïve B cells leading up to the flare and then decrease upon symptom onset (see Figure 2). The authors suggest that a comparison with single-cell fibroblast data generated by Zhang et al.\textsuperscript{177} revealed the gene expression signature of PRIME cells to be most closely related to sub-lining layer fibroblasts in the joint. Deep phenotyping and functional analysis of these cells has yet to be performed, and as a result, their direct pathogenic role in disease is yet to be determined. Importantly, it is assumed by the authors that these cells have the ability to enter the joint and directly contribute to inflammatory joint pathology, triggering an arthritis flare. However, the exact mechanism by which these cells contribute to arthritis flare currently remains unproven and is speculative.\textsuperscript{178}

### 3.8 Joint-specific features of synovial fibroblasts

It has long been recognized that pathognomonic disease patterns are associated with different types of inflammatory arthritis. The cellular and molecular mechanisms underlying this tissue tropism are poorly understood. There is now emerging evidence of significant differences in the transcriptional and epigenetic profiles of synovial fibroblasts isolated from different joint locations.\textsuperscript{37} This anatomically driven transcriptional diversity follows a pattern imprinted in embryonic development and translates to joint-specific phenotypes of synovial fibroblasts.\textsuperscript{179}

The spatial and temporal expression of key developmental genes, such as the homeobox (HOX) family of transcription factors, tightly controls the correct establishment of the body axis during embryonic development.\textsuperscript{180-182} Studies analyzing synovial fibroblasts isolated from specific human joints have detected differences in transcriptional expression of HOX genes and WNT signaling pathways\textsuperscript{179} (involved in both cellular differentiation and development).\textsuperscript{183} Hierarchical cluster analysis of transcriptional data from synovial fibroblasts isolated from the knees, hands, and shoulders found that the cells segregate according to anatomical joint location and that HOX gene signature detected in these fibroblasts shared similar features to the embryonic positional HOX gene expression pattern during limb development. Investigators have further
identified that DNA methylation and histone marks tightly regulate joint-specific HOX gene expression in synovial fibroblasts.\textsuperscript{184,185} It is not yet known whether the epigenetic imprinting of synovial fibroblasts occurs during embryonic development at sites distant to the joint or during postnatal development when supporting the unique biomechanical features of a specific joint.

This epigenetically driven anatomical diversity of synovial fibroblasts also translates into joint-specific fibroblast phenotypes and functional specialization that is dependent on joint location.\textsuperscript{37} For example, fibroblasts isolated from the hand joints have differential responses to cytokine stimulation and produce higher levels of matrix-degrading enzymes. They are more proliferative and have distinct chemotactic properties when compared to synovial fibroblasts from other joints.\textsuperscript{179} As a result, these differences in cellular phenotype may underlie the specific patterns of joint involvement observed in different types of arthritis by creating unique tissue microenvironments that confer a susceptibility of specific joints to certain types of arthritis.\textsuperscript{3,26} The existence of developmentally determined, positionally imprinted risk signatures of synovial fibroblasts may provide important mechanistic insights into the tissue tropism observed in immune-mediated inflammatory diseases and the disease pathways underlying the persistence of inflammation at distinct anatomical sites.\textsuperscript{37}

Implications for therapy have also been described due to differences in synovial fibroblasts isolated from different affected joints (eg, hip versus knee joint) in patients with RA.\textsuperscript{179,185,186} Differences in expression of cytokine signaling pathways and differential sensitivity to cytokine neutralization or signaling blockade may contribute to variable treatment responses at different joint locations. It will now be important to integrate joint-specific differences in fibroblast function with the recent description of specific fibroblast subset heterogeneity defined by single-cell profiling technologies. This may allow for the identification of shared and unique fibroblast subsets that confer specific functions and pathogenic roles across different joints within the same disease.

4 | THERAPEUTIC TARGETING OF FIBROBLASTS

There are several potential therapeutic approaches to targeting fibroblast-mediated pathology in inflammatory joint disease, including both direct and indirect approaches.\textsuperscript{16,26} Indirect approaches include the neutralization of fibroblast-specific activating factors such as cytokines or growth factors (including those that drive autonomic, primary cell amplification loops that sustain specific fibroblast phenotypes or activation status) and the blockade of fibroblast derived factors, such as chemokines and cytokines that mediate inflammatory joint pathology.\textsuperscript{26,122,126} However, such indirect approaches are already a component of some currently available treatments such as anti-cytokine therapies or signal transduction inhibitors.\textsuperscript{187} These therapies have not been sufficient to break the current ceiling of therapeutic response in RA, and as a result, new therapeutic approaches are needed.\textsuperscript{188} There are several potential approaches to directly target fibroblasts described below.

4.1 | Direct targeting of pathogenic subsets of fibroblasts

Linking the transcriptionally defined cellular phenotype of fibroblasts more clearly to cellular function and pathogenic role in disease makes targeting distinct subsets of fibroblasts increasingly possible.\textsuperscript{26} Targeting pathological immune cells by selective depletion has proved highly efficacious in RA and other immune-mediated inflammatory diseases, such as the use of rituximab (targeting CD20 on B-cells)\textsuperscript{189} for example. A prerequisite for such a therapeutic approach to be effective in targeting fibroblasts is the ability to selectively deplete a population of pathogenic fibroblasts accurately defined by a cell surface marker, expressed only by the pathogenic population of cells to be targeted. In the case of fibroblasts, we have used a diphtheria toxin system to selectively deplete a pathogenic population FAPα-expressing fibroblasts leading to attenuated synovial inflammation and joint damage in experimental arthritis in mice.\textsuperscript{47} These findings provide a therapeutic rationale for the selective targeting of FAPα-expressing fibroblasts in human inflammatory joint disease. However, for such an approach to be effective in humans, we must be able to identify fibroblast- and tissue-specific markers to localize these cytotoxic therapies to the joint and reduce the risk of "off" target effects in other tissues. Targeting FAPα-expressing fibroblasts with chimeric antigen receptor (CAR)–specific T cells has proven to be efficacious in reducing cardiac fibrosis, as well as restoring function and improving recovery following cardiac injury in mice.\textsuperscript{190} Furthermore, a phase I clinical trial has proven that local delivery of FAPα CAR T cells is safe in patients with pleural mesothelioma.\textsuperscript{191} These findings are promising, prompting development of such therapeutic approaches in RA and other autoimmune diseases.\textsuperscript{192}

Strategies for targeting fibroblasts based on specific cell surface receptors such as CDH11 have previously been evaluated. However, a phase II clinical trial was discontinued due to inefficacy.\textsuperscript{134} Many of the markers expressed by immune effector and synovial fibroblast subsets, such as THY1, CD34, and PDPN, are also expressed by other cellular lineages.\textsuperscript{107,193,194} Comprehensive analysis of the cell surface expression profile of pathogenic fibroblast subsets mapped to their transcriptional phenotype, coupled with the development of bivalent or trivalent antibody targeted cell depletion strategies and novel cellular drug delivery systems (eg, liposomes), may allow for more accurate fibroblast targeting in the future.

4.2 | Targeting the differentiation of synovial fibroblasts

Another potential therapeutic avenue involves targeting the underlying molecular mechanisms driving the differentiation of
fibroblasts toward specific effector cell phenotypes. This has been demonstrated by the inhibition of NOTCH3 signaling in experimental arthritis in mice. Wei et al. reported significant attenuation of joint inflammation and damage in mice, following the genetic deletion of the Notch3 gene or therapeutic blockade of NOTCH3 signaling. The cellular mechanism underlying this effect was the inhibition of fibroblast differentiation toward a pathogenic sub-lining phenotype, preventing the expansion of perivascular fibroblasts. NOTCH3 signaling blockade also significantly diminished the fraction of SPARC⁺ COL3A1⁺ but not CXCL10⁺ CXCL19⁺ fibroblasts. As SPARC⁺ COL3A1⁺ fibroblasts potentially remodel the synovial vasculature to promote cellular infiltration and CXCL10⁺ CXCL19⁺ directly communicate with T cells to maintain activation and recruitment, NOTCH3 inhibition could be more effective in early disease.

4.3 Modulating the pathogenic behavior of fibroblasts

Cellular metabolism is a major regulator of fibroblast behavior. Targeting dysregulated metabolism in synovial fibroblasts using metabolism modifiers may be another therapeutic option. Examples include targeting hexokinase-2—the glycolytic enzyme that is a critical driver of synovial hyperplasia in response to joint inflammation. This enzyme is modulated by currently available treatments such as tofacitinib that reduces the expression of this enzyme in synovial tissue. More targeted interventions could include the therapeutic blockade of the complement system preventing fibroblast-mediated synovial tissue priming. While complement targeted therapies in RA have not yet been shown to be effective, possible therapeutic interventions could include the direct targeting of C3 or reversal of metabolic re-programming in CD34⁺ fibroblasts in the joint using inhibitors of glycolysis or small molecular inhibitors of the inflammasome.

Therapeutic modulation of the epigenome has the potential to re-establish a normal cell state in previously imprinted fibroblast phenotypes. For example, a recent study on fibroblasts isolated from patients with RA demonstrated that 280 TNFα-inducible genes escape repression owing to persistent H3K27 acetylation and increased chromatin accessibility of their regulatory elements. Further analysis revealed that these regulatory elements were enriched for binding motifs for NFκB, interferon regulatory factors, and AP-1 transcription factors. As a result, targeting histone acetylation reader proteins such as BET proteins could modulate the persistently activated fibroblast phenotype. For example, BET inhibitor I-BET151 has been shown to suppress the production of cytokines and MMPs by synovial fibroblasts in vitro. A comprehensive analysis of the gene regulatory pathways that modify fibroblast subset phenotype may reveal other specific therapeutic targets.

5 CONCLUDING REMARKS

Synovial fibroblasts are specially adapted for their role in joint homeostasis, and this functional specialization is retained within distinct anatomical compartments of the synovial membrane. These specialized synovial tissue niches undergo cellular remodeling in response to inflammation and contain specific subsets of fibroblasts. The spatial and temporal heterogeneity of synovial fibroblasts underpins their pathogenic role in inflammatory joint disease. Fibroblast heterogeneity is driven by anatomical location in the tissue, microenvironmental instructive signals, and interaction with tissue-resident leukocytes. These regulatory signals and cellular interactions result in both subset differentiation and changes in cell activation status. This phenotypic heterogeneity gives rise to functional cellular diversity reflected in the existence of synovial fibroblast subsets with different effector cell functions.

The link between phenotypic heterogeneity and cellular function needs to be further explored in order to provide a clear link between transcriptional diversity and functional role in health and disease. Comprehensive analysis of synovial fibroblast subsets at different stages of disease in response to therapeutic intervention will be vital in exploring the role of specific fibroblast subsets in determining the response to treatment and disease progression and in dictating the most effective timing for the use of fibroblast targeted therapies. As yet, the impact of chronic disease on fibroblast heterogeneity is unknown. The identification of key positional cues within the synovial tissue that regulate fibroblast identity/phenotype suggests a large degree of residual plasticity in phenotype. However, this plasticity may be modulated in chronic disease, as suggested by numerous previous studies that demonstrate the development of an epigenetically imprinted fibroblast phenotypes with chronicity of joint inflammation. This raises the possibility that the success of fibroblast targeted therapies is dependent on the stage of disease. For example, early changes in fibroblast phenotype may underpin a treatment-refractory disease trajectory and therefore targeting these cells in early disease may be more effective at altering the course of arthritis.

Future research strategies should focus on linking single-cell transcriptomic analysis of cellular heterogeneity obtained from different pathogenic tissues to fibroblast function and build a consensus on the definition of fibroblast identity and heterogeneity, based on function rather than gene expression profiles. As we now define the cellular landscape of the joint, at different stages of disease and in different sub-groups of patients, it is vital to link these findings to function and this requires comprehensive in vitro and in vivo functional studies linked to the cell atlas of the tissue. Furthermore, the exploration of shared and distinct fibroblast phenotypes across different tissues and inflammatory diseases will be vital for developing cross-indication therapeutic targeting of specific pathogenic fibroblasts in the future, perhaps as an adjuvant to existing biologic therapies such as anti-TNFα treatment, an approach that will hopefully break the ceiling of therapeutic response in RA.
ACKNOWLEDGEMENTS
The authors would like to thank the Rheumatology Research Group at the University of Birmingham for useful discussions. Dr Adam P. Croft is funded by the Kennedy Trust for Rheumatology Research (Senior Clinical Fellowship KENN 19 20 06), Foundation for Research in Rheumatology (FOREUM) (Project ID:173), and the Academy of Medical Sciences.

CONFLICT OF INTEREST
The author(s) declare no conflicts of interest.

AUTHOR CONTRIBUTION
All authors contributed equally to the manuscript.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID
Adam P. Croft https://orcid.org/0000-0002-9487-0511

REFERENCES
1. Yofe I, Dahan R, Amit I. Single-cell genomic approaches for developing the next generation of immunotherapies. Nat Med. 2020;26:171-177.
2. Jaitin DA, Kenigsberg E, Keren-Shaul H, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. Science. 2014;343:776-779.
3. Zhang F, Wei K, Slowikowski K, et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol. 2019;20:928-941.
4. Kiliaraki V, Prados A, Aramaka M, Kollias G. The mesenchymal context in inflammation, immunity and cancer. Nat Immunol. 2020;21:974-982.
5. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388:2023-2038.
6. Smolen JS, Aletaha D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. Nat Rev Rheumatol. 2015;11:276-289.
7. Catrina AI, Svensson CI, Malmström V, Schett G, Klæreskog L. Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. Nat Rev Rheumatol. 2017;13(2):79-86.
8. Winthrop KL, Weinblatt ME, Bathon J, et al. Unmet need in rheumatology: reports from the Targeted Therapies meeting 2019. Ann Rheum Dis. 2019;78:99-93.
9. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007;7:429-442.
10. van der Heijde D, Klæreskog L, Rodriguez-Valverde V, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum. 2006;54(4):1063-1074. https://doi.org/10.1002/art.21655
11. Genovese MC, Becker J-C, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med. 2005;353:1114-1123.
12. Cohen SB, Emery P, Greenwald MW, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum. 2006;54:2793-2806.
13. Emery P, Keystone E, Tony HP, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. Ann Rheum Dis. 2008;67:1516-1523.
14. Schett G, Tanaka Y, Isaacs JD. Why remission is not enough: underlying disease mechanisms in RA that prevent cure. Nat Rev Rheumatol. 2021;17:135-144.
15. Patel R, Filer A, Barone F, Buckley CD. Stroma: fertile soil for inflammation. Best Pract Res Clin Rheumatol. 2014;28:565-576.
16. Filer A. The fibroblast as a therapeutic target in rheumatoid arthritis. Curr Opin Pharmacol. 2013;13:413-419.
17. Kurowska-Stolarska M, Aliverini S. Synovial tissue macrophages: friend or foe? RMD Open. 2017;3:e000527.
18. Yang X, Chang Y, Wei W. Emerging role of targeting macrophages in rheumatoid arthritis: focus on polarization, metabolism and apoptosis. Cell Profil. 2020;53:e12854.
19. Smith MD. The normal synovium. Open Rheumatol J. 2011;5:100-106.
20. Rhee DK, Marcelino J, Baker M, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J Clin Invest. 2005;115:622-631.
21. Kiener HP, Watts GFM, Cui Y, et al. Synovial fibroblasts self-direct multicellular lining architecture and synthetic function in three-dimensional organ culture. Arthritis Rheum. 2010;62:742-752.
22. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205-2219.
23. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature. 2003;423:356-361.
24. Derer A, Böhmer N, Görtzsch B, et al. Rsk2 controls synovial fibroblast hyperplasia and the course of arthritis. Ann Rheum Dis. 2016;75:413-421.
25. O’Sullivan FX, Fassbender HG, Gay S, Koopman WJ. Etiopathogenesis of the rheumatoid arthritis-like disease in MRL/lpr mice. I. The histomorphologic basis of joint destruction. Arthritis Rheum. 1985;28:529-536.
26. Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. Nat Rev Rheumatol. 2020;16:316-333.
27. Buckley CD, Ospelt C, Gay S, Midwood KS. Location, location, location: how the tissue microenvironment affects inflammation in RA. Nat Rev Rheumatol. 2021;17:195-212.
28. LeBlou VS, Neilson EG. Origin and functional heterogeneity of fibroblasts. FASEB J. 2020;34:3519-3536.
29. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6:392-401.
30. Filer A, Pitzalis C, Buckley CD. Targeting the stromal microenvironment in chronic inflammation. Curr Opin Pharmacol. 2006;6:393-400.
31. Lynch MD, Watt FM. Fibroblast heterogeneity: implications for tissue-specific antifibrotic therapies. Sci Transl Med. 2018;10.
32. Roelofs AJ, Zupan J, Riemen AHK, et al. Joint morphogenetic cells in the adult mammalian synovium. Nat Commun. 2017;8:15040.
33. Driskell RR, Lichtenberger BM, Hoste E, et al. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature. 2013;504:277-281.
36. Rinn JL, Bondre C, Gladstone HB, Brown PO, Chang HY. Anatomic demarcation by positional variation in fibroblast gene expression programs. PLoS Genet. 2006;2:e119.
37. Ospelt C, Frank-Bertoncelj M. Why location matters - site-specific factors in rheumatic diseases. Nat Rev Rheumatol. 2017;13:433-442.
38. Mizo-guchi F, Slowikowski K, Wei K, et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. Nat Commun. 2018;9:789.
39. Buechler MB, Pradhan RN, Calviello AK, Muller S, Bourgon R and Turley SJ. Cross-tissue single-cell transcriptomics reveals organizing principles of fibroblasts in health and disease. 2021;1-58. bioRxiv. https://doi.org/10.1101/2021.05.42.469121
40. Korsunsky I, Wei K, Pohin M, et al. Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases. bioRxiv. 2021.11.426253-51 (2021). https://doi.org/10.1101/2021.11.426253
41. Huber LC, Distler O, Tarner I, Gay RE, Gay S, Pap T. Synovial fibroblasts: key players in rheumatoid arthritis. Rheumatology (Oxford). 2006;45:669-675.
42. Lee DM, Kiener HP, Agarwal SK, et al. Cadherin-11 in synovial lining formation and pathology in arthritis. Science. 2007;315:1006-1010.
43. Culemann S, Grünéboom A, Nicolás-Ávila JÁ, et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. Nature. 2019;572:670-675.
44. Buechler MB, Kim K-W, Onufer EJ, et al. A stromal niche defined by expression of the transcription factor WT1 mediates programing and homeostasis of cavity-resident macrophages. Immunity. 2019;51:119-130.e5.
45. Filer A, Ward LSC, Kemble S, et al. Identification of a transcriptionally distinct fibroblast function in very early rheumatoid arthritis. Ann Rheum Dis. 2017;76:2105-2112.
46. McGettrick HM, Ward LSC, Rainger GE, Nash GB. Mesenchymal stromal cells as active regulators of lymphocyte recruitment to blood vascular endothelial cells. Methods Mol Biol. 2017;1591:121-142.
47. Croft AP, Campos J, Jansen K, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature. 2019;570:246-251.
48. ABOUT HUMAN CELL ATLAS. https://www.humancellatlas.org 1-1 (2021).
49. Baldwin MJ, Criibbs AP, Gullik F, Snelling SJB. Mapping the musculoskeletal system one cell at a time. Nat Rev Rheumatol. 2021;17:247-248.
50. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev. 2010;233:233-255.
51. Bottini N, Firestein GS. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. Nat Rev Rheumatol. 2013;9:24-33.
52. Chang SK, Noss EH, Chen M, et al. Cadherin-11 regulates fibroblast inflammation. Proc Natl Acad Sci USA. 2011;108:8402-8407.
53. Kiener HP, Lee DM, Agarwal SK, Brenner MB. Cadherin-11 induces rheumatoid arthritis fibroblast-like synoviocytes to form lining layers in vitro. Am J Pathol. 2006;168:1486-1499.
54. Kiener HP, Niederreiter B, Lee DM, Jimenez-Boj E, Smolen JS, Brenner MB. Cadherin 11 promotes invasive behavior of fibroblast-like synoviocytes. Arthritis Rheum. 2009;60:1305-1310.
55. Valencia X, Higgins JMG, Kiener HP, et al. Cadherin-11 provides specific cellular adhesion between fibroblast-like synoviocytes. J Exp Med. 2004;200:1673-1679.
56. Lafaytis R, Remmers EF, Roberts AB, Yocom DE, Sporn MB, Wilder RL. Anchorage-independent growth of synoviocytes from articular and normal joints. Stimulation by exogenous platelet-derived growth factor and inhibition by transforming growth factor-beta and retinoids. J Clin Invest. 1989;83:1267-1276.
57. Korb A, Ravenstädth H, Pap T. Cell death in rheumatoid arthritis. Apoptosis. 2009;14:447-454.
58. 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 Rheumatol. 2014;66:40-48.
59. Shin Y-J, Han S-H, Kim D-S, et al. Autophagy induction and CHOP under-expression promotes survival of fibroblasts from rheumatoid arthritis patients under endoplasmic reticulum stress. Arthritis Res Ther. 2010;12:R19.
60. Firestein GS, McInnes IB. Immunopathogenesis of rheumatoid arthritis. Immunity. 2017;46:183-196.
61. Burman A, Haworth O, Hardie DL, et al. A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. J Immunol. 2005;174:1693-1700.
62. Wei K, Korsunsky I, Marshall JL, et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. Nature. 2020;582:259-264.
63. Dennis G, Holweg CTJ, Kummerfeld SK, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. Arthritis Res Ther. 2014;16:R90.
64. Pitzalis C, Kelly S, Humby F. New learnings on the pathophysicsology of RA from synovial biopsies. Curr Opin Rheumatol. 2013;25:334-344.
65. Lewis MJ, Barnes MR, Bligh K, et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. Cell Rep. 2019;28:2455-2470.e5.
66. Humby F, Lewis M, Ramamoorthy N, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis. 2019;78:761-772.
67. Lliso-Ribera G, Humby F, Lewis M, et al. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). Ann Rheum Dis. 2019;78:1642-1652.
68. Bombardieri M, Lewis M, Pitzalis C. Ectopic lymphoid neogenesis in rheumatic autoimmune diseases. Nat Rev Rheumatol. 2017;13:141-154.
69. Humby F, Bombardieri M, Manzo A, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. PLoS Medicine. 2009;6:e1.
70. Scheel T, Gursche A, Zacher J, Häuptl T, Berek C. V-region gene analysis of locally defined synovial B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in rheumatoid arthritis. Arthritis Rheum. 2011;63:63-72.
71. Manzo A, Bombardieri M, Humby F, Pitzalis C. Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmune and tissue damage/remodeling. Immunol Rev. 2010;233:267-285.
72. Takemura S, Braun A, Crowson C, et al. Lymphoid neogenesis in rheumatoid synovitis. J Immunol. 2001;167:1072-1080.
73. Rosengren S, Wei N, Kalunian KC, Zvaifler NJ, Kavanaugh A, Boyle DL. Elevated autoantibody content in rheumatoid arthritis synovium with lymphoid aggregates and the effect of rituximab. Arthritis Res Ther. 2008;10:R105-R109.
74. Harada S, Yamamura M, Okamoto H, et al. Production of interleukin-7 and interleukin-15 by fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Rheum. 1999;42:1508-1516.
75. Takayanagi H, Iizuka H, Juji T, et al. Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum. 2000;43:259-269.
76. Pickens SR, Chamberlain ND, Volin MV, Pope RM, Mandelin IIAM, Shahhara S. Characterization of CCL19 and CCL21 in rheumatoid arthritis. Arthritis Rheum. 2011;63:914-922.
77. Bombardieri M, Kam N-W, Brentano F, et al. BAFF/APRIL-dependent TLR3-stimulated pathway enhances the capacity of rheumatoid synovial fibroblasts to induce AID expression and Ig class-switching in B cells. *Ann Rheum Dis*. 2011;70:1857-1865.

78. Burger JA, Zvaifler NJ, Tsukada N, Firestein GS, Kippis TJ. Fibroblast-like synoviocytes support B-cell pseudoeniperiploiosis via a stromal cell-derived factor-1 and CD106 (VCAM-1)-dependent mechanism. *J Clin Invest*. 2001;107:305-315.

79. Ohata J, Zvaifler NJ, Nishio M, et al. Fibroblast-like synoviocytes of mesenchymal origin express functional B-cell activating factor of the TNF family in response to proinflammatory cytokines. *J Immunol*. 2005;174:864-870.

80. Buckley CD, Barone F, Nayar S, Bénézech C, Caamaño J. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. *Annu Rev Immunol*. 2015;33:715-745.

81. Mendez-Huergo SP, Hockl PF, Stupirski JC, et al. Clinical relevance of galectin-1 and galectin-3 in rheumatoid arthritis patients: differential regulation and correlation with disease activity. *Front Immunol*. 2018;9:3057.

82. Ohshima S, Kuchen S, Seemayer CA, et al. Galectin 3 and its binding protein in rheumatoid arthritis. *Arthritis Rheum*. 2003;48:2788-2795.

83. Neidhart M, Zaucke F, von Knoch R, et al. Galectin-1 and galectin-3 in rheumatoid synovium: Inhibitor of apoptosis protein (IAP) family in synovial tissue. *Arthritis Rheum*. 2003;48:2796-2809.

84. Nickerson JM, Copeland D, Duffey TL, et al. Galectin-1 and galectin-3 in rheumatoid arthritis synovial fibroblasts after adhesion to cartilage oligomeric matrix protein. *Oncogene*. 2005;24:1419-1424.

85. Filer A, Naylor AJ, Marshall JL, et al. Rheumatoid synovial fibroblasts differentiate into distinct subsets in the presence of cytokines and cartilage. *Arthritis Res Ther*. 2016;18:270.

86. Kurowska-Stolarska M, Distler JHW, Jüngel A, et al. Inhibitor of apoptosis protein (IAP) family in synovial tissue. *Arthritis Res Ther*. 2001;3:R40.

87. Palmer DG, Selvendran Y, Allen C, Revell PA, Hogg N. Features of synovial membrane identified with monoclonal antibodies. *Clin Exp Immunol*. 1985;59:529-538.

88. Stephenson W, Donlin LT, Butler A, et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. *Nat Commun*. 2018;9:791.

89. Malhotra D, Fletcher AL, Astarita J, et al. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat Immunol*. 2012;13:499-510.

90. Ekwall A-KH, Eisler T, Anderberg C, et al. The tumour-associated glycoprotein podoplanin is expressed in fibroblast-like synoviocytes of the hyperplastic synovial lining layer in rheumatoid arthritis. *Arthritis Res Ther*. 2011;13:R40.

91. Warren MD, Gutierrez-Clell AH, Hafrick MJ, et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. *Nat Commun*. 2018;9:791.

92. Miyake K, Nishida K, Kadota Y, et al. Cytokine-induced expression of vasoohibin-1 by rheumatoid synovial fibroblasts. *Acta Med Okayama*. 2009;63:349-358.

93. Middleton J, Americh L, Gayon R, et al. A comparative study of endothelial cell markers expressed in chronically inflamed human tissues: MECA-79, Duffy antigen receptor for chemokines, von Willebrand factor, CD31, CD34, CD105 and CD146. *J Pathol*. 2005;206:260-268.

94. Snelling SJ, Davidson RK, Swinger TE, et al. Dickkopf-3 is up-regulated in osteoarthritis and has a chondroprotective role. *Osteoarthr Cartil*. 2016;24:883-891.

95. Li Y, Liu H, Liang Y, Peng P, Ma X, Zhang X. DKK3 regulates cell proliferation, apoptosis and collagen synthesis in keloid fibroblasts via TGF-β/Smad signaling pathway. *Biomed Pharmacother*. 2017;91:174-180.

96. Nakamura M, Tsukada N, Matsuura S, et al. Osteoprotegerin regulates bone formation through a coupling mechanism with bone resorption. *Endocrinology*. 2003;144:5441-5449.

97. Sun S, Liu W, Li Y. CADM1 enhances intestinal barrier function in a rat model of mild inflammatory bowel disease by inhibiting the STAT3 signaling pathway. *J Biol Chem*. 2020;295:1103-1114.

98. Ramirez-Montagut T, Blachere NE, Sviderskaya EV, et al. FAPalpha, a surface peptidase expressed during wound healing, is a tumor suppressor. *Oncogene*. 2004;23:5435-5446.

99. Neumann E, Lefèvre S, Zimmermann B, et al. Migratory potential of rheumatoid arthritis synovial fibroblasts: additional perspectives. *Cell Cycle*. 2010;9:2286-2291.
116. Buckley CD. Why does chronic inflammation persist: An unexpected role for fibroblasts. *Immunol Lett.* 2011;138:12-14.

117. Jones S, Horwood N, Cope A, Dazzi F. The anti-proliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells. *J Immunol.* 2007;179:2824-2831.

118. Haniffa MA, Wang X-N, Holtick U, et al. Adult human fibroblasts are potent immunoregulatory cells and functionally equivalent to mesenchymal stem cells. *J Immunol.* 2007;179:1595-1604.

119. McGlacken HM, Butler LM, Buckley CD, Rainger GE, Nash GB. Tissue stroma as a regulator of leucocyte recruitment in inflammation. *J Leukoc Biol.* 2012;91:385-400.

120. Zhang XL, Topley N, Ito T, Phillips A. Interleukin-6 regulation of T cell responses in rheumatoid arthritis. *Arthritis Rheum.* 2005;58:1001-1009.

121. Dominitzki S, Fantini MC, Neufert C, et al. Cutting edge: transcriptional regulation of toll-like receptor expression in early and long-standing arthritis. *Arthritis Rheum.* 2006;55:4384-3869.

122. Carromán M, Juarzán Y, Pérez-García S, et al. RNA sensors in human osteoarthritis and rheumatoid arthritis synovial fibroblasts: immune regulation by vasoactive intestinal peptide. *Arthritis Rheum.* 2011;63:1626-1636.

123. Ichise Y, Saegusa J, Tanaka-Natsui S, et al. Soluble CD14 induces pro-inflammatory cytokines in rheumatoid arthritis fibroblast-like synovial cells via toll-like receptor 4. *Cells.* 2020;9:1689.

124. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther.* 2020;5:209-223.

125. Xu H, Tang J, Zeng G, et al. RGS1 silencing inhibits the inflammatory response and angiogenesis in rheumatoid arthritis rats through the inactivation of Toll-like receptor signaling pathway. *J Cell Physiol.* 2019;234:20432-20442.

126. Kato H, Endres J, Fox DA. The roles of IFN-γ versus IL-17 in pathogenic effects of human Th17 cells on synovial fibroblasts. *Mod Rheumatol.* 2013;23:1140-1150.

127. Yoshitomi H. Regulation of immune responses and chronic inflammation by fibroblast-like synoviocytes. *Front Immunol.* 2019;10:1395.

128. Tran CN, Davis MJ, Tesmer LA, et al. Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum.* 2007;56:1497-1506.

129. Carmona-Rivera C, Carlucci PM, Moore E, et al. Synovial fibroblast-neutrophil interactions promote pathogenic adaptive immunity in rheumatoid arthritis. *Sci Immunol.* 2017;2:eaaag3358.

130. Ueno A, Yamamura M, Iwahashi M, et al. The production of CXCR3-agonistic chemokines by synovial fibroblasts from patients with rheumatoid arthritis. *Rheumatol Int.* 2005;25:361-367.

131. Tanaka Y, Takeuchi T, Umehara H, et al. Safety, pharmacokinetics, and efficacy of E6011, an anti-fibractalkine monoclonal antibody, in a first-in-patient phase 1/2 study on rheumatoid arthritis. *Mod Rheumatol.* 2018;28:58-65.

132. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol.* 2013;14:986-995.

133. Kuo D, Ding J, Cohn IS, et al. HBEGF+ macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci Transl Med.* 2019;11.

134. Tanaka Y, Takeuchi T, Umehara H, et al. Safety, pharmacokinetics, and efficacy of E6011, an anti-fibractalkine monoclonal antibody, in a first-in-patient phase 1/2 study on rheumatoid arthritis. *Mod Rheumatol.* 2018;28:58-65.

135. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol.* 2013;14:986-995.

136. Kuo D, Ding J, Cohn IS, et al. HBEGF+ macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci Transl Med.* 2019;11.

137. Neumann E, Riepl B, Knedla A, et al. Cell culture and passaging alters gene expression pattern and proliferation rate in rheumatoid arthritis synovial fibroblasts. *Arthritis Res Ther.* 2010;12:R83.

138. Zimmermann T, Kunisch E, Pfeiffer R, et al. Isolation and characterization of rheumatoid arthritis synovial fibroblasts from primary culture–primary culture cells markedly differ from fourth-passage cells. *Arthritis Res.* 2001;3:72-76.

139. Firestein GS. Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressors? *Arthritis Rheum.* 1996;39:1781-1790.

140. Ham S, Bae J-B, Lee S, et al. Epigenetic analysis in rheumatoid arthritis synoviocytes. *Exp Mol Med.* 2019;51:1-13.

141. Nemtsova MV, Zaletaev DV, Bure IV, et al. Epigenetic changes in the pathogenesis of rheumatoid arthritis. *Front Genet.* 2019;10:570.

142. Karami J, Aslani S, Tahmasebi MN, et al. Epigenetics in rheumatoid arthritis: fibroblast-like synoviocytes as an emerging paradigm in the pathogenesis of the disease. *Immunol Cell Biol.* 2020;98:171-186.

143. Gibney ER, Nolan CM. Epigenetics and gene expression. *Heredit.* 2010;105:4-13.

144. Karouzakis E, Gay RE, Gay S, Neidhart M. Epigenetic deregulation in rheumatoid arthritis. *Adv Exp Med Biol.* 2011;711:137-149.

145. Ciechomska M, Roszkowski L, Maslinski W. DNA methylation as a future therapeutic and diagnostic target in rheumatoid arthritis. *Cells.* 2019;8:953.

146. Horiuichi M, Morinobu A, Chin T, Sakai Y, Kurosaka M, Kumagai S. Expression and function of histone deacetylases in rheumatoid arthritis synovial fibroblasts. *J Rheumatol.* 2009;36:1580-1589.

147. Nakano K, Whitaker JW, Boyle DL, Wang W, Firestein GS. DNA methylation signature in rheumatoid arthritis. *Ann Rheum Dis.* 2013;72:110-117.

148. Whitaker JW, Shoemaker R, Boyle DL, et al. An imprinted rheumatoid arthritis methylome signature reflects pathogenic phenotype. *Genome Med.* 2013;5:1-12.

149. Gaur N, Karouzakis E, Glück S, et al. MicroRNAs interfere with DNA methylation in rheumatoid arthritis synovial fibroblasts. *RMD Open.* 2016;2:e000299.

150. Karouzakis E, Raza K, Kolling C, et al. Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. *Sci Rep.* 2018;8:7370-7376.

151. Sohn C, Lee A, Qiao Y, Loupasakis K, Ivashkiv LB, Kalliolias GD. Prolonged tumor necrosis factor α primes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. *Arthritis Rheumatol.* 2015;67:86-95.

152. Wada TT, Araki Y, Sato K, et al. Aberrant histone acetylation contributes to elevated interleukin-6 production in rheumatoid arthritis synovial fibroblasts. *BMC Biophys Res Commun.* 2014;444:682-686.

153. Stanczyk J, Pedrioli DML, Brettano F, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* 2008;58:1001-1009.

154. Meng C, Qi H, Wang X, et al. Expression and methylation levels of suppressor of cytokine signaling 3 in rheumatoid arthritis synovial fibroblasts. *Exp Mol Pathol.* 2020;113:104361.

155. Bao X, Ma L, He C. MicroRNA-23a-5p regulates cell proliferation, migration and inflammation of TNF-α-stimulated human fibroblast-like MHH7A synoviocytes. *J Immunol.* 2010;184:609-618.
arthritis mice via miR-6089/AKT1/NF-κB axis. *Cell Death Dis.* 2020;11:833.

158. Xiao J, Wang R, Zhou W, Cai X, Ye Z. LncRNA NEAT1 regulates the proliferation and production of the inflammatory cytokines in rheumatoid arthritis fibroblast-like synoviocytes by targeting mir-204-5p. *Hum Cell.* 2021;34:372-382.

159. Wang G, Tang L, Zhang X, Li Y. LncRNA DILC participates in rheumatoid arthritis by inducing apoptosis of fibroblast-like synoviocytes and down-regulating IL-6. *Biosci Rep.* 2019;39.

160. Huber R, Bikker R, Welz B, Christmann M, Brand K. TNF Tolerance in monocytes and macrophages: characteristics and molecular mechanisms. *J Immunol Res.* 2017;2017:9570129.

161. Crowley T, O’Neil JD, Adams H, et al. Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. *Arthritis Res Ther.* 2017;19:35.

162. Klein K, Frank-Bertoncej M, Karouzakis E, et al. The epigenetic architecture at gene promoters determines cell type-specific LPS tolerance. *J Autoimmun.* 2017;83:122-133.

163. Friščić J, Böttcher M, Reinwald C, et al. The complement system orchestrates synovial fibroblast activation and synovial tissue repair in rheumatoid arthritis. *Cell Death Dis.* 2018;9:618.

164. Bustamante MF, Oliveira PG, Garcia-Carbonell R, et al. Hexokinase 2 as a novel selective metabolic target for rheumatoid arthritis. *Nat Rev Rheumatol.* 2020;16:1027-1027.

165. Falconer J, Murphy AN, Young SP, et al. Review: Synovial cell metabolism and chronic inflammation in rheumatoid arthritis. *Arthritis Rheumatol.* 2018;70:1959-2038.

166. Neumann E, Lefèvre S, Zimmermann B, Gay S, Müller-Ladner U. Synoviolar fibroblasts regulate the switch from acute to chronic inflammation of synovial fibroblasts. *Arthritis Rheumatol.* 2020;11:603237.

167. Hillen J, Geyer C, Heitzmann M, et al. Structural cartilage damage attracts circulating rheumatoid arthritis synovial fibroblasts into unaffected joints. *Arthritis Res Ther.* 2017;19:1-11.

168. Armaca M, Ospelt C, Pasparakis M, Kollias G. The p55TNFR-1/IIK2-Ripk3 axis orchestrates arthritis by regulating death and inflammatory pathways in synovial fibroblasts. *Nat Commun.* 2018;9:618.

169. Armaca M, Apostolakis 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. *J Exp Med.* 2008;205:331-337.

170. Hillen J, Geyer C, Heitzmann M, et al. Structural cartilage damage attracts circulating rheumatoid arthritis synovial fibroblasts into unaffected joints. *Arthritis Res Ther.* 2017:19:1-11.

171. Korb-Pap A, Stratis A, Mühlenberg K, et al. Early structural changes in cartilage and bone are required for the attachment and invasion of inflamed synovial tissue during destructive inflammatory arthritis. *Ann Rheum Dis.* 2012;71:1004-1011.

172. Orange DE, Yao V, Sawicka K, et al. RNA identification of PRIME cells predicting rheumatoid arthritis flares. *N Engl J Med.* 2020;383:218-228.

173. Sifkakis PP, Vlachogiannis NI, Sakkou M. PRIME cells predicting rheumatoid arthritis flares. *N Engl J Med.* 2020;383:1594-1595.

174. Frank-Bertoncej M, Trenkmann M, Klein K, et al. Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat Commun.* 2017;8:14852.

175. Harfe BD, Scherz PJ, Nissim S, Tian H, McMahon AP, Tabin CJ. Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell.* 2004;118:517-528.

176. Koshiha-Takeuchi K, Takeuchi JK, Arruda EP, et al. Cooperative and antagonistic interactions between Sall4 and Tbx5 pattern the mouse limb and heart. *Nat Genet.* 2006;38:175-183.

177. Montavon T, Le Garrec J-F, Kerszberg M, Duboule D. Modeling Hox gene regulation in digits: reverse collinearity and the molecular origin of thumbness. *Genes Dev.* 2008;22:346-359.

178. Pazin DE, Gamer LW, Cox KA, Rosen V. Molecular profiling of synovial joints: use of microarray analysis to identify factors that direct the development of the knee and elbow. *Dev Dyn.* 2012;241:1816-1826.
199. Tough DF, Tak PP, Tarakhovsky A, Prinjha RK. Epigenetic drug discovery: breaking through the immune barrier. *Nat Rev Drug Discov.* 2016;15:835-853.

200. Loh C, Park S-H, Lee A, Yuan R, Ivashkiv LB, Kalliolias GD. TNF-induced inflammatory genes escape repression in fibroblast-like synoviocytes: transcriptomic and epigenomic analysis. *Ann Rheum Dis.* 2019;78:1205-1214.

201. Klein K, Kabala PA, Grabiec AM, et al. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann Rheum Dis.* 2016;75:422-429. https://doi.org/10.1136/annrheumdis-2014-205809

202. Stebulis JA, Rossetti RG, Atez FJ, Zurier RB. Fibroblast-like synovial cells derived from synovial fluid. *J Rheumatol.* 2005;32:301-306.

**How to cite this article:** Marsh L-J, Kemble S, Reis Nisa P, Singh R, Croft AP. Fibroblast pathology in inflammatory joint disease. *Immunol Rev.* 2021;00:1-21. https://doi.org/10.1111/imr.12986