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Precision medicine in rheumatoid arthritis

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Abstract

Rheumatoid arthritis is an autoimmune disease that causes significant morbidity. Application of cellular profiling techniques such as single-cell transcriptomics and spatial transcriptomics has uncovered novel pathogenic cell types in RA joint tissues and revealed marked heterogeneity in the cellular composition among RA patients. Together, these insights provide exciting opportunities to translate discoveries into precision medicine in RA. The present review aims to highlight novel insights into RA pathology and discuss key steps needed to translate these discoveries into actionable changes in clinical practice. We review efforts to identify surrogate biomarkers that could be used to predict RA synovial tissue phenotypes and the corresponding responses to therapy. Finally, we discuss the opportunity to develop novel patient-derived organoid systems as a platform for therapeutic target validation.

Keywords

rheumatoid arthritis; precision medicine; single-cell RNAseq; spatial transcriptomics; organoids

I. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that affects 1% of the population[1][2]. RA causes significant morbidity and carries a substantial societal burden[1]. The treatment of rheumatoid arthritis has advanced significantly over the past decades with the advent of numerous targeted therapies [1]. However, despite these advances, several challenges remain. The first challenge is that we cannot yet predict which therapeutic agent will lead to the optimal response with the least toxicity for each patient.

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The second challenge is that many RA patients never achieve sustained remission[1], despite rapid advances in RA therapeutics. Third, there is marked disease heterogeneity within RA with regards to synovial tissue phenotypes [3–5] and extraarticular manifestations [6,7]. Together, these unmet needs pose a substantial challenge for precision medicine approaches in RA.

In this review, we start by discussing key technological breakthroughs that have enabled a disease “deconstruction” approach to defining RA pathology at a single-cell level. Next, we summarize recent findings on pathogenic cell states expanded in RA synovia, molecular pathways underlying their expansion, and new insights into RA disease heterogeneity and synovial tissue phenotypes. We describe key steps needed to translate these discoveries into clinical insights. We highlight approaches being taken to identify surrogate biomarkers for RA synovial tissue phenotypes and efforts to reconstruct RA through novel spatial transcriptomics methods. Finally, we discuss the development of novel synovial tissue organoid systems as an experimental platform for precision medicine and therapeutic target validation.

II. Deconstructing rheumatoid arthritis through cellular profiling

The application of transcriptomic and cellular profiling technologies to RA joint tissue previously has focused on preselected cell types, surveyed whole tissues rather than disaggregated cells, or only used a single technology platform. Recently, rapid advances in transcriptomic profiling, cytometry, and spatial transcriptomics have enabled the unprecedented examination of cellular and molecular heterogeneity in RA blood and synovium. We begin the review by discussing key advances in cellular profiling techniques that together, have transformed our ability to examine rheumatoid arthritis at a single-cell resolution.

While fluorescence-based cytometric analysis of cell surface markers has long been the gold standard for immune cell profiling [8], mass cytometry overcomes some of the limitations of conventional fluorescence-based cytometry by utilizing antibodies conjugated to metals[9]. In studies that utilize mass cytometry for cellular profiling, cells are simultaneously labeled with a large panel of antibodies (>40 markers) that encompass key markers that convey information regarding cell lineage, cell type, cytokines, intracellular signaling, and epigenetic status. Moreover, cellular profiling by mass cytometry is scalable, making it feasible to apply mass cytometry to a large number of patient samples.

Transcriptomic profiling through RNA-sequencing (RNA-seq)[10] has enabled a highly detailed examination of gene expression. In particular, the advent of single-cell RNA-seq (scRNAseq)[11] has fundamentally changed the way scientists approach profiling of cell populations by enabling unbiased transcriptomic profiling without a priori knowledge of cell types as defined by known intracellular or surface markers. This unbiased approach is particularly appealing when applied to target tissues of inflammatory disease.. In 2018, Stephenson et al was one of the first groups to apply this technique to the rheumatoid arthritis synovium[12]. Using droplet-based scRNAseq, the authors profiled 20,387 synovial

cells to identify 13 putative immune and stromal cell types, including T and NK lymphocyte subsets, B cell populations, and synovial fibroblast subsets[12].

III. Defining inflammatory cell states in RA at the single-cell resolution

The ability to leverage cutting-edge technologies to deconstruct chronic inflammatory diseases became possible as cellular profiling techniques has continued to mature. The overarching goal of the Accelerating Medicines Partnership (AMP) RA/SLE Consortium was to deconstruct inflammatory diseases by applying high-dimensional cellular profiling to organs and tissues affected by chronic inflammation. A major challenge for the network was to implement a standardized protocol for synovia tissue acquisition, processing, data generation, and data analysis across clinical sites. The AMP RA/SLE consortium addressed this issue by first testing different approaches to acquiring and processing synovial biopsy samples across multiple clinical sites[13]. A key advance that enabled synchronization of tissue processing and minimization of technical batch effects was the implementation of a robust fresh tissue synovial biopsy cryopreservation protocol[13]. The AMP RA/SLE consortium demonstrated that cryopreserved synovial tissue maintained high cell viability with intact transcriptomic profiles at a single-cell level[13]. Synovial cells obtained through a cryopreservation pipeline generated largely viable cells and were readily accessible for high-dimensional analyses, including mass cytometry and RNA-sequencing[13]. Next, the ability to recover robust cell yield from synovial tissue biopsies was critical to capture the synovial cellular heterogeneity from individual RA patients. The traditional tissue disaggregation methods utilize collagenase to dissociate cells from synovium which can result in cleavage of surface proteins and altered transcriptomic profiles due to prolonged tissue digestion time. Using a highly purified proteolytic enzyme combined with mechanical trituration, the AMP RA/SLE consortium developed an optimized synovial tissue dissociation protocol that delivers robust cell yield and enables high-quality transcriptomic analyses on a cell-by-cell level[13].

The AMP RA/SLE consortium applied mass cytometry and scRNAeq to over 40 joint tissues from patients with RA or osteoarthritis (OA). Combining mass cytometry and scRNAseq, the consortium identified 18 unique cell states in RA synovia[14]. Here, we discuss some of the novel cell states in RA synovia uncovered through the cellular profiling approach to deconstructing RA (Figure 1).

A. Pathogenic lymphocyte subsets expanded in RA synovium

The role of B cells in RA pathogenesis has long been appreciated[15] and the efficacy of B-cell depletion strategies strongly supports a role for B cells in driving disease pathogenesis in RA[16][17][18]. In the peripheral blood of RA patients, B cells exhibit abnormal phenotypes, characterized by an expansion of activated memory B cells populations[19][20]. By applying scRNAseq In RA synovia, the AMP RA/SLE consortium identified 4 distinct B cell states: plasmablasts, naive B cells, memory B cells, and autoimmune-associated B cells (ABCs) [14]. ABCs were first reported in aging mice and their presence has been documented in patients with various autoimmune diseases including SLE[21]. In RA, ABCs

are highly expanded in patients with active disease and express markers of recently activated B cells [14].

T cell - B cell aggregates can be observed in synovial tissue of patients with seropositive RA[22]. While it has been long appreciated that T cells are important mediators of chronic inflammation in RA[23,24], it was not known until recently which T cell populations drive B cell aggregation and proliferation in RA synovium. In 2017, Rao et al reported a CD4⁺ PD-1^{hi} CXCR5⁻ T cell population that is highly expanded in synovial tissue, synovial fluid, and peripheral blood of seropositive RA patients[25]. In RA synovia, CD4⁺ PD-1^{hi} CXCR5⁻ T cells are located adjacent to B cells within lymphoid aggregates[25]. CD4⁺ PD-1^{hi} CXCR5⁻ T cells express high levels of IL-21 and CXCL13 and induce memory B cell differentiation into plasma cells *in vitro*[25]. Unlike T follicular helper cells, these cells lack CXCR5 and instead express CCR2 which enables homing of these cells to inflamed peripheral tissue[25]. Due to their ability to shape the B cell responses in peripheral tissues, these cells have been described as T peripheral helper (Tph) cells. Subsequent studies have shown that T cells that resemble Tph are expanded in lupus kidneys[25–27] and celiac disease[28].

In addition to the identification of ABCs and Tph cells, cellular profiling of lymphocytes by scRNAseq also revealed CD8⁺ T cells as key producers of inflammatory cytokines interferon-gamma and TNF [14]. Interestingly, transcriptomic analysis of CD8⁺ T cells suggested distinct CD8⁺ T cell subsets characterized by differential expression of *GZMK* and *GZMB*[14]. Further work is warranted to gain insights into the function and origin of *GZMK*⁺ CD8⁺ T cells in RA.

B. Distinct synovial macrophages drive joint inflammation and disease remission

The healthy synovial lining consists of lining fibroblasts and resident macrophages. A recent study in mice demonstrated that synovial lining macrophages are derived from embryonic precursors which populate the synovium during embryogenesis[29]. Synovial lining macrophages renew locally, forming a protective epithelial-like barrier that is disrupted in experimental arthritis[29]. Recent scRNAseq analyses of monocytes and macrophages have shown tremendous heterogeneity among the myeloid compartment in RA synovia [29][30][14,31]. Among the myeloid compartment, two inflammatory monocyte populations were significantly expanded in RA patients with highly inflamed synovium: one myeloid population characterized by high expression of *IL1B* and *HBEGF*, and another characterized by an interferon activation signature[14,31]. The *IL1B*⁺ *HBEGF*⁺ inflammatory macrophages promoted fibroblast invasiveness *in vitro*[31], suggesting a potential role for *IL-1B*⁺ *HBEGF*⁺ inflammatory macrophages in driving fibroblast-mediated joint destruction. In a separate study, Alivernini et al examined gene expression profiles of synovial tissue macrophages across patients with early/active RA, treatment-refractory/active RA, and RA in sustained remission[30]. This study highlighted a population of synovial tissue macrophages uniquely expanded during disease remission. These macrophages, characterized by the expression of *MERTK* and *TREM2*, produce lipid mediators that suppress inflammation and induce a reparative response in synovial fibroblasts[30]. Interestingly, a reduced frequency of these macrophages in remission was

associated with an increased risk of disease flare[30], suggesting a role for local tissue macrophages in sustaining remission. Together, these studies highlight functionally distinct macrophages and monocytes in the synovium.

C. Sublining fibroblast expansion in RA

Fibroblasts are mesenchymal cells that maintain normal organ tissue function[32,33]. Synovial fibroblasts are mesenchymal cells in the joint that form the lining membrane, secrete proteoglycans to promote normal joint functions[34][35]. In RA, synovial fibroblasts take on pathological roles that contribute to joint damage by recruiting leukocytes, promoting neoangiogenesis, and degrading cartilage[34][33,35]. Advances in cellular profiling technologies have provided an unprecedented look at fibroblast heterogeneity in chronic inflammatory diseases[35], including inflammatory bowel disease[36–39], RA [40][14,41,42], pulmonary fibrosis[43][44], and cancer[45,46]. To identify pathological fibroblast subsets in RA synovia, Mizoguchi et al[41] applied flow cytometry followed transcriptomic analysis and identified 3 putative synovial fibroblasts subtypes: CD90- CD34- lining fibroblasts, CD90+ CD34+ sublining fibroblasts, and CD90+ CD34- sublining fibroblasts. Of these three fibroblast subsets, CD90+ sublining fibroblasts were highly expanded in RA and correlated with the severity of synovial tissue inflammation[41]. The concept of synovial fibroblast heterogeneity and the expansion of sublining fibroblasts in RA synovia were confirmed in separate studies from the AMP RA/SLE consortium. In the first study, integration of mass cytometry and scRNAseq identified 4 synovial fibroblast subsets: CD90- lining fibroblasts, CD90+CD34+ sublining fibroblast population, CD90+ HLA-DRA+ fibroblasts, and DKK3+ fibroblasts[14]. Consistent with an inflammatory phenotype, CD90+ HLA-DR+ fibroblasts express high levels of IL-6, CCL2, and CXCL12[14]. Reflecting distinct regional functional compartmentalization between the lining and sublining fibroblasts, sublining fibroblasts secrete inflammatory cytokines whereas lining fibroblasts produce matrix-remodeling MMP1 and MMP3[41]. In an animal model of inflammatory arthritis, it was recently shown that CD90+ sublining fibroblasts undergo expansion in joints of arthritis mice[42]. Interestingly, adoptive transfer of CD90+ sublining fibroblasts into arthritic mice worsens joint inflammation, whereas adoptive transfer of CD90- lining fibroblasts worsened cartilage and bone erosion[42]. These results suggest that lining and sublining fibroblasts take on distinct pathological functions in inflammatory arthritis.

What drives the functional polarization of synovial fibroblasts? The enrichment of interferon-stimulated genes (ISGs) in CD90+HLA-DR fibroblasts suggests that the inflammatory phenotype is in part driven by interferon signaling[14][47]. Surprisingly, trajectory analysis of synovial fibroblasts suggests that lining and sublining are transcriptionally connected along an anatomical continuum, from synovial lining to the sublining vasculature[40]. We recently identified vascular endothelium-derived Notch signaling as a key driver of CD90+ sublining fibroblast differentiation[40]. Inhibition of Notch receptor signaling in a synovial organoid model abrogated CD90+ fibroblast differentiation, suggesting a critical role for endothelium-derived signals in driving fibroblast positional identity[40]. Together, these observations suggest disease-specific alterations in the fibroblast compartment.

D. Angiogenesis and angiocrine factors in RA.

Endothelial cells that line blood vessels have traditionally been perceived as passive, structural units that provide blood flow to target tissues. However, recent studies suggest that specialized endothelial cells found in niche tissue microenvironments actively orchestrate tissue remodeling through paracrine secretion of morphogenic signals termed angiocrine factors [48]. Across different organs and tissues, specialized capillary endothelial cells secrete angiocrine factors that act as morphogens to determine the shape, architecture, size, and patterning of developing and regenerating tissues[48]. In inflammatory diseases such as RA, the formation of new blood vessels, or angiogenesis, is critical for supplying oxygen and nutrients to immune and stromal cells in inflamed tissue[49]. In inflamed tissues, hypoxia induces the production of proangiogenic factors, including vascular endothelial growth factor (VEGF), placenta growth factor (PGF), fibroblast growth factor (FGF) which in turn induce angiogenesis, neovascularization, and remodeling of the surrounding tissue[49].

The concept of an angiocrine endothelium-mediated synovial tissue remodeling in RA was recently described by our group[40]. Unbiased scRNAseq analysis of synovial endothelial cells from RA patients suggests that synovial endothelial cells exist along the arterial to venous continuum[40]. Arterial endothelial cells are characterized by the expression of *PODXL* and *NOTCH4*, whereas venous endothelial cells can be identified by the expression of *CLU* and *ACKR1*[40]. Interestingly, Notch ligands delta-like ligand 4 (*DLL4*) and Jagged-1 (*JAG1*) were highly expressed by arterial endothelial cells. Endothelial-derived Notch signaling directs the maturation of undifferentiated fibroblasts into mural cells and sublining fibroblasts, leading to sublining expansion in RA. Beyond Notch signaling, synovial endothelial cells express many growth factors, cytokines, and chemokines that together help shape the inflammatory response in RA[40,49]. The identity of additional angiocrine factors in RA and their role in synovial tissue remodeling remains to be defined.

IV. Key steps towards reconstructing rheumatoid arthritis

As discussed above, recent studies utilizing cellular profiling techniques have provided new and exciting insights into key cellular changes relevant to RA pathogenesis. If we consider the overall approach of defining key cell states and molecular pathways in RA as an effort to “deconstruct” RA, what would be the approaches to “reconstruct” RA? As the “disease reconstruction” approach in RA continues to bring forward new insights into RA pathogenesis, it is important to consider the opportunities as well as challenges that are needed to overcome to translate these insights into practical, operational components of clinical therapeutic decision making. The first opportunity is to apply the cellular and molecular data to improve our understanding of RA disease heterogeneity and to guide RA treatment. By designing studies that combine cellular profiling data with deep clinical phenotyping, it may be possible to define distinct RA phenotypes, identify biomarkers that correlate with distinct RA phenotypes, and predict treatment response, leading to a more personalized treatment strategy (Figure 2). The second opportunity is to leverage the rich cellular profiling data to define novel therapies targeting specific cell populations or pathways that are pathologically enriched in RA. Below we discuss ongoing efforts to define

RA phenotypes and identify biomarkers that predict treatment response. We also highlight novel experimental approaches that could expedite drug discovery and facilitate target validation in RA. Together, these approaches are critically needed to translate discoveries into precision medicine in RA (Figure 2).

A. Biomarkers as key tools towards personalized medicine in RA.

A common theme for personalized medicine is the capability to accurately predict responses to therapies that take into account each patient's unique characteristics, disease severity, and the likelihood of response to specific targeted therapies [50]. To do this, a critical tool needed to guide personalized therapy in RA is biomarkers that capture each patient's unique biology, disease phenotype, and response to treatment. Current strategies towards biomarker discovery in RA rely on the utilization of proteomic, genomic, or metabolomic approaches[51]. Despite intense efforts over the past decades to identify markers that predict response to therapy in RA, currently, there are no biomarkers that accurately predict an individual's response to targeted therapy [50][51–53]. Nevertheless, recent advances in -omic technologies and clinical study design provide exciting opportunities in the space of RA biomarker discovery. Here, we discuss recent studies that utilized -omic approaches to identify novel molecular and cellular biomarkers that correlate with disease phenotypes or predict treatment response.

At the histopathological level, RA synovium exhibit marked heterogeneity at an individual level with regards to the degree of inflammation[54], lining hyperplasia[54], inflammatory cellular composition[55,56], presence of lymphoid aggregates[55,57]. In a recent study of RA phenotype heterogeneity[5], Lewis et al applied RNAseq to synovial tissue and peripheral blood to a large cohort of early, treatment-naive RA patients. Unbiased transcriptomic analysis identified 3 distinct RA phenotypes: fibroblast-enriched macrophage-enriched, and lymphoid-myeloid-enriched phenotypes[5]. Compared to the histologic assessment of RA phenotypes, RNAseq-based stratification of RA phenotypes demonstrated an improvement in predicting therapeutic response[58]. Consistently, several recent studies have similarly shown that synovial tissue phenotype correlates with response to biologics [59][3,5]. To translate the concept of RA synovial tissue phenotype predicting treatment response into routine clinical practice, a surrogate peripheral marker that accurately predicts RA synovial tissue phenotype would be needed since synovial tissue biopsy is not routinely performed as part of the RA management in many countries, including the United States. Transcriptomic profiling of peripheral blood from patients is an attractive approach since peripheral blood is collected routinely clinically for diagnostic and monitoring for drug toxicities. This approach also leverages the relative ease of RNA isolation and preservation from whole blood and rapid advances in deep gene expression profiling techniques [53]. Interestingly, whereas the synovial tissue RNAseq revealed marked transcriptional differences across the 3 RA phenotypes, blood RNAseq exhibited mild differences across distinct RA phenotypes, with type I interferon response genes enriched in the lymphoid-myeloid phenotype[5]. This observation reflects that, compared to the peripheral blood, there is marked variability and heterogeneity in the synovial tissue of patients with RA with regards to the tissue cellular composition. Likely, the local

signals from the synovial tissue microenvironment[31,40] are crucial in shaping the tissue inflammatory response across individuals with RA.

In a separate study, Orange et al [60] applied a novel approach utilizing transcriptomic analysis of peripheral blood to identify RNA signatures of flare in patients with established RA. Leveraging weekly home collection of blood in RA patients followed by transcriptomic profiling, Orange et al. established a longitudinal analysis of gene expression changes in the peripheral blood over 364-time points. Using this approach, they identified a gene expression signature consistent with B-cell activation, followed by mesenchymal cell gene signature, in the blood of RA patients before RA disease flare[60]. Interestingly, the mesenchymal gene signature observed from the peripheral blood of these patients is similar to the transcriptomic profiles of synovial fibroblasts in inflamed joints, raising the possibility of circulating mesenchymal cells in peripheral blood of RA patients before disease flares. While additional studies are needed to verify the tissue origin of the mesenchymal cell signature in these patients, this study nevertheless illustrated a novel concept of longitudinal genomics analysis as a strategy to identify surrogate biomarkers in RA patients.

Proteomic technologies enable large-scale profiling of proteins in patient samples[61]. Applying proteomic technologies to identify biomarkers in RA to improve diagnosis and management is an area of intense investigation [62]. The recent discovery of the “pre-RA” stage[63] [64]has led to the identification of autoantibodies against cyclic citrullinated peptides (anti-CCP antibodies) that may predict RA onset in individuals with a high risk for developing RA[65][66] [52] [51]. Identification of precise biomarkers in the pre-RA phase will be crucial for success in clinical trials aimed at preventing RA.

Metabolomics, the comprehensive analysis of metabolites in a biological specimen, is emerging as a powerful tool in precision medicine[67]. Applied to inflammatory arthritis, investigators utilizing metabolomic profiling have identified distinct metabolomic and lipidomic signatures for seronegative RA and psoriatic arthritis[68], RA versus reactive arthritis[69], altered glucose[70–72], and choline[73] metabolism in RA synovial fibroblasts. Utilizing a lipid profiling approach, Gomez et al reported that the concentrations of pro-resolving mediators in the peripheral blood of RA patients predict the responsiveness of DMARD treatment[74]. Furthermore, the levels of baseline lipid mediators are correlated with distinct RA synovial tissue phenotypes[74], indicating lipid profiling of the peripheral blood may serve as a surrogate biomarker for distinct RA phenotypes.

Together, these approaches illustrate that the combination of the cutting-edge omic approach with the deep phenotypic characterization of patients can lead to the novel discovery of peripheral blood markers that may help stratify RA patients, predict response to therapy, and predict disease flares. As single-cell multi-omic techniques continue to improve by incorporating new measurement parameters, including surface proteomics[75], metabolomics[76], and epigenetics[77], our ability to define specific biomarkers in RA will undoubtedly continue to improve. Furthermore, cellular profiling techniques that provide precise identification of cellular sources of relevant biomarkers may provide additional insight into RA disease pathogenesis and disease heterogeneity.

B. Spatial reconstruction of rheumatoid arthritis

Synovitis in RA involves complex cellular interactions between infiltrating immune cells and tissue-resident stromal cells and macrophages that lead to pathology and joint damage. Deconstructing RA synovitis using single-cell technologies has revealed previously underappreciated cellular heterogeneity in RA synovia[14,25,31,40–42]. However, the structural and organizational principles of synovitis in RA remain incompletely understood due to the lack of spatial context and understanding of the architectural design of the microenvironments within inflamed RA synovia. An important next step towards improving our understanding of the tissue context in chronic inflammation is to reconstruct synovitis by determining the spatial localization of key immune and stromal cells within RA synovia.

The ability to examine gene expression directly in the context of tissue anatomy is central to understanding disease pathology. Traditional imaging techniques, including immunohistochemistry and in situ hybridization are typically capable of detecting a few genes or protein markers of interest. Recently, the development of advanced multiplexed single-molecule fluorescence in situ hybridization (smFISH)[78] and spatial transcriptomic assays[79] offer exciting possibilities of measuring gene expression directly on tissue sections. Techniques such as spatial transcriptomics[80], MERFISH[81], Slide-seq[82], stereo-seq[83], enable the measurement of hundreds to thousands of genes simultaneously across a tissue section while retaining tissue architecture. Studies applying spatial transcriptomics to RA have begun to emerge over the past two years. In 2019, Carlberg et al. applied spatial transcriptomics to RA and psoriatic arthritis (PsA) synovia and identified an expanded adaptive immune response profile in RA, compared to PsA[84]. As spatial transcriptomic technologies continue to improve concerning spatial resolution and sensitivity, more in-depth studies of RA synovial tissue architecture will no doubt emerge.

C. Patient-derived synovial organoids as an experimental platform for target validation.

As the disease deconstruction approach reveals new disease-associated cell states and pathogenic pathways, there is now an exciting opportunity to identify novel therapeutic targets in RA. A key challenge in target validation is defining experimental systems that faithfully recapitulate synovial tissue pathology in RA. While several animal models of inflammatory arthritis exist and have been crucial in improving our understanding of arthritis pathology[85], there is currently no animal model of inflammatory arthritis that completely recapitulates all aspects of RA pathogenesis or joint pathology. Therefore, experimental systems that utilize patient-derived, organotypic models that recapitulate RA synovitis is highly desirable. In 2010, Kiener et al reported the first *in vitro* model of synovial lining membrane formation[86]. This “micromass” system leverages the ability of synovial fibroblast to self-assemble into compacted lining membrane when cultured in an extracellular matrix[86]. Since the initial report of the method, the micromass system has been widely used by RA investigators to examine the role of adhesion molecule Cadherin-11 during lining formation and arthritis pathology[87], fibroblast invasion[88], and inflammatory cytokine activation[89].

Recently, the observation that sublining fibroblasts are localized around synovial vascular endothelium led us to hypothesize that endothelial cells are crucial in directing sublining

fibroblast differentiation[40]. To reconstruct the synovial sublining, we developed a fibroblast plus endothelial cell organoid system where endothelial cells self-assemble into vascular tubules within the sublining compartment [40]. The formation of vascular tubules in synovial organoids then provides positional cues for fibroblasts, leading to fibroblast differentiation towards a sublining fibroblast phenotype in a NOTCH-dependent manner[40]. This de novo approach relies on the principle of mixing synovial parenchymal cells in a 3-dimensional matrix and then allowing them to self-assemble into their native architecture through homo- and hetero-typic cell-cell contacts. Additional work is needed to determine if this synovial organoid system can be used to determine differentiation cues for other synovial cell types, such as synovial tissue macrophages. Nevertheless, the prospect of using a reconstitution approach to build synovium in a dish is appealing because of its simplicity and the ease of tailoring the organoid system to model specific cell-cell interactions in the synovium.

One limitation of the reconstitution approach to reconstruct the synovium is that these systems often rely on cultured primary cells, often from different donors. The ability to retain patient-specific biology *ex vivo* is critical for leveraging organoid models for personalized medicine. In oncology, the recent development of patient-derived organoids (PDOs) has accelerated the ability to examine variation in patient response to targeted therapies [90–92]. Tumor PDOs utilize an “en-bloc” approach where tissue can be maintained *ex vivo* as organoids to assess response to therapeutic modulation[93]. While there has been tremendous technical development of PDOs for cancer therapeutics, little is known if synovial tissue, patient-derived organoids could be leveraged to guide RA therapeutics. The concept for developing synovial tissue PDOs is quite appealing: If a synovial tissue PDO can be developed to preserve the original cellular composition and the native synovial tissue microenvironment unique to each RA patient, synovial tissue PDOs may provide an opportunity to validate therapeutic targets and to determine the therapeutic response to existing therapies. Furthermore, if synovial tissue PDOs can be developed from synovial biopsies, it would provide a powerful platform for which novel therapeutics could be tested directly in RA patient tissues.

IV. Summary

Rheumatoid arthritis is a chronic autoimmune disease that causes significant morbidity. Emerging cellular profiling data from synovial tissue biopsies have revealed novel, pathogenic cell states in RA synovia and that distinct synovial cellular phenotypes exist in RA. There is an urgent unmet need to better understand the clinical implication of RA synovial tissue phenotypes with regards to disease prognosis and therapeutic response to targeted therapies. Defining surrogate biomarkers that could be used to predict RA synovial tissue phenotypes will be critical towards translating these findings into actionable insights in clinical practice. Precision medicine in RA also necessitates the development of novel experimental models that retain each patient’s characteristics. Patient-derived organoid system is a promising approach that may provide a platform to validate therapeutic targets and to determine the therapeutic response to existing therapies.

Practice points

- Rheumatoid arthritis (RA) is a heterogeneous disease defined by distinct synovial tissue phenotypes
- Emerging evidence for distinct RA phenotypes creates an urgent unmet need to determine how each RA phenotype affects the likelihood of patients' response to targeted therapies.

Research Agenda

- Cellular profiling through single-cell RNAseq enabled RA “disease deconstruction” which has led to the identification of several pathogenic cell states expanded in RA.
- Spatial transcriptomic techniques are promising approaches to “reconstruct” RA pathology by defining the anatomical localization of pathogenic cell states in RA synovia
- Biomarkers that correlate with RA phenotypes and predict response to targeted therapies are needed for precision medicine in RA
- The development of patient-derived synovial tissue organoids will provide a novel platform for drug discovery and target validation in RA.

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Cell state	Cell markers	Location in synovium	Suggested role in RA
 PRG4+ lining fibroblasts	<i>PRG4, CD55</i>	lining ^{12, 14, 40, 41, 42}	secrete MMPs ⁴¹ ; degrade cartilage ⁴²
 CD90+ CD34+ perivascular fibroblasts	<i>THY1, CD34, C3</i>	sublining, near blood vessels ^{40, 41}	secrete inflammatory cytokines ⁴¹
 CD90+ HLA-DR+ perivascular fibroblasts	<i>THY1, HLA-DRA</i>	sublining, near blood vessels ^{40, 41}	secrete inflammatory cytokines IL-6 ⁴¹ ; promote neovascularization ⁴⁰
 NOTCH4+ arterioles	<i>PODXL, NOTCH4</i>	sublining ⁴⁰	Induce perivascular fibroblast differentiation ⁴⁰
 T peripheral helper (TpH)	<i>CXCL13, PDCD1</i>	sublining, near T-B aggregates ²⁵	Recruit B cells: induce plasma cell differentiation ²⁵
 GZMK+ CD8 T cells	<i>GZMK, GZMB</i>	not yet determined	produce IFN-G ¹²
 autoimmune-associated B cells	<i>ITGAX, TBX21</i>	sublining ¹²	produce autoantibodies
 Plasmablasts	<i>MZB1, XBP1, CD27</i>	sublining	produce antibodies
 IFN-activated monocytes	<i>SPP1, LY6E, IFI6</i>	not yet determined	secrete chemokines CXCL
 pro-inflammatory monocytes	<i>IL1B, HBEGF, PLAUR</i>	sublining ^{12, 31}	secrete inflammatory cytokines ³¹ , promote fibroblast invasion ³¹
 synovial tissue macrophage	<i>MERTK, TREM2</i>	lining ^{29, 30}	induce a repair phenotype in fibroblasts ³⁰ ; promote joint homeostasis ²⁹

Figure 1. Pathogenic cell states in RA.

Summary of pathogenic cell states in RA synovia revealed by single-cell profiling. The specific marker genes for each cell state, their known sub-anatomical location within the synovium, and putative pathological functions are highlighted.

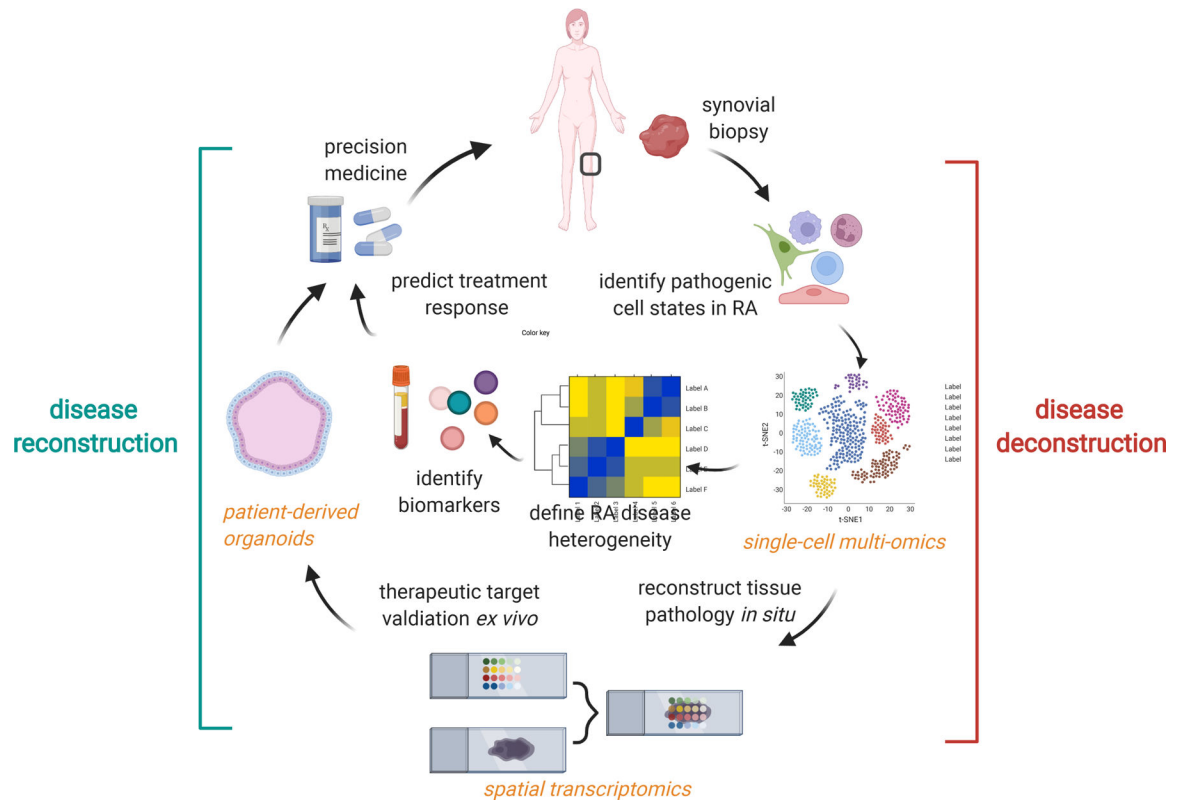


Figure 2. Precision in RA: from disease deconstruction to disease reconstruction.

In disease deconstruction (right), synovial biopsy followed by single-cell multi-omics profiling is the first step towards understanding cellular heterogeneity in RA. By integrating synovial cellular profiles with clinical phenotypes, we can begin to define RA phenotypes through molecular taxonomy. Several novel approaches are needed for RA reconstruction (left). First, spatially-resolved transcriptomics could provide new insights into pathological cellular interactions in RA synovia and identify new therapeutic targets. The development of experimental models that retain patient-specific phenotypes (i.e. patient-derived synovial organoids) could provide a platform to test treatment-response and validate new drug targets. Finally, surrogate biomarkers that correlate with distinct RA phenotypes and predict treatment response will be needed to guide clinical decision-making.