Leveraging cutting-edge transcriptomics to inform precision therapeutic targeting for soft tissue joint disease

Jessica E. Ackerman[1], Stephanie G. Dakin[1]

[1] NDORMS, University of Oxford, Oxford, United Kingdom

scRNAseq technologies have rapidly advanced understanding of the cell types comprising healthy and diseased musculoskeletal tissues, however a significant knowledge gap remains in 'medicalizing' these cellular atlases to generate effective new therapies to treat joint disease. A call to arms is required to enable the musculoskeletal research community to cohesively advance the translation of 'omics' research into effective therapeutics.

Fibrotic soft tissue diseases are debilitating and costly to treat, with no consensus pharmacological intervention to restore native structure and function. It is resoundingly clear that better characterization of these tissues; synovium, tendon, ligament, and joint capsule, is necessary for the development of targeted therapeutics. In recent years, there has been an exponential increase in the use of transcriptomic-based technologies to study soft tissue joint disease, resulting in the generation of large datasets that provide unparalleled characterization of these tissues. These techniques include single-cell RNA-sequencing to define the transcriptomes of individual cells and identify novel cell populations, CITE-seq to confirm expression of surface proteins, spatial transcriptomics to define the molecular profile of a tissue while retaining the spatial localization of transcriptomic signatures, and bulk and single nuclei ATAC sequencing to understand the epigenetic scaffolding that underpins cell behavior. Despite the expansion of these techniques, the potential translational impact of the resulting large datasets is often unclear to the broader scientific and clinical community. In this Editorial, we highlight the current state of play for omics research on soft tissues of the joint and outline a call to arms to overcome the necessary challenges to translate new knowledge into effective therapies for patients with arthritis, arthrofibrosis and tendinopathy.

Current state of play

Recent advances in scRNAseq and spatial platforms facilitate the identification of distinct stromal cell subsets comprising healthy and diseased soft tissues of the joint, spatially mapping newly identified populations to their respective microanatomical niches. Research groups analyze their datasets independently using their own quality control and analysis pipelines. This absence of standardization presents several significant issues, most salient of which is the lack of consensus annotation. Without a common-use database to identify distinct cell states and populations, bespoke annotations vary widely and hinder cross-comparative studies between disease states and animal models. Initiatives such as the Human Cell Atlas and the MSK portal[1] facilitate comparison of data sets from healthy human musculoskeletal tissues between research groups. However, a third challenge presents, as a mechanism to compare healthy and diseased human musculoskeletal tissues with respective animal tissues is also required. This cross-species comparison is critical to bridge the knowledge gap between observational studies using human musculoskeletal tissues (giving a snapshot into the biology at a particular time point) and functional *in vivo* longitudinal studies using small and large animal musculoskeletal disease models providing mechanistic insights into pathobiology of joint tissues. There is currently no consensus as to which treatments are clinically superior for the management of common musculoskeletal diseases including osteoarthritis, arthrofibrosis and tendinopathy. Advancing understanding of the cellular basis of disease affecting musculoskeletal tissues in humans and animals could inform new therapeutic strategies to precisely target pathogenic cell types driving disease.

Call to arms: Requirements to advance the field

For cutting edge transcriptomics to inform the development of effective new therapies to treat soft tissue joint disease, it is necessary to address the following challenges:

1. *Consensus in annotating stromal cell populations of joint soft tissues:* It is essential to reach a consensus in annotating the cell types comprising synovium, tendon and ligament across healthy and diseased states. Standardizing annotations for $CD45^{neg}$ stromal cells and their respective sub-populations would greatly facilitate comparative studies between datasets from different research groups, between different musculoskeletal tissues and across species. This is exemplified in a study reporting the existence of fibroblast populations common to human skin, synovium, salivary gland and lung tissues whereby $CXCL10^{+}CCL19^{+}$ immune-interacting and $SPARC^{+}COL3A1^{+}$ vascular-interacting fibroblasts were expanded in diseased states from all tissue types[2].

2. *Cross species analysis of healthy & diseased musculoskeletal tissues:* A platform that enables the sharing, integration and comparison of human, small and large animal musculoskeletal single cell data sets of healthy and diseased musculoskeletal tissues is pivotal to connect research findings from human and animal model datasets. Importantly, animal models can provide mechanistic insights into the earlier stages of musculoskeletal disease, in contrast human tissues are frequently collected once chronic disease is well established. Combining transcriptomic data from human and animal provides powerful insights to determine if disease mechanisms are conserved across species and could reduce and refine the future use of animal models to study musculoskeletal disease.

3. *Medicalizing cellular atlases to advance therapeutic target discovery:* Transcriptomic studies have real potential to identify the distinct cell types and molecules that may be used as targets to effectively treat soft joint tissue disease. 'Medicalizing' cellular atlases of the joint (i.e., harnessing the knowledge gained from understanding the phenotypes of cells populating joint soft tissues to inform therapeutic target discovery) will address current challenges to find effective translational therapies for common musculoskeletal diseases, a prime example being the identification of distinct fibroblast subsets as novel therapeutic targets[3,4]. Wei et al[3]. outline a variety of novel therapeutic approaches that only become possible by delineating pathological fibroblasts present in inflammatory diseases, via disruption of their mechanism of activation or downstream effector molecules to halt disease progression. Engagement of industry and pharma partners will subsequently be essential to fully accomplish this objective.

Strategy to address these challenges

For this initiative to have the necessary impact on soft tissue joint disease research, there must be accordance amongst scientists, thus it is of utmost importance to maximize the benefits to the field. We propose the formation of a joint soft tissue omics consortium to unify this effort in moving these datasets towards clinical translation. Such a consortium will serve as a platform to initiate strategies to move towards a consensus in annotating scRNAseq atlases of joint soft tissues, develop improved strategies to perform cross species analysis of musculoskeletal soft tissues and catalyse the transfer of this knowledge into new therapeutics to effectively target diseases of joint soft tissues. This comment serves as a call to arms to researchers in the field to begin the work of engaging others towards this goal. To address consensus annotation, there is a requirement for funding of bioinformatic scientists to allocate time into coordinating data analysis of current datasets. Finally, generation of an online data depository for researchers to utilize, along with tools to standardize analysis pipelines and disseminate data will be critical for facilitating the most impactful and translational science. Fortunately, other fields have set a precedent for achieving these goals, The Brain Initiative Cell Census Network (BICCN) has recently set up a multimodal cell atlas of the mammalian

primary motor cortex, the result of a coordinated effort among neuroscience researchers[5]. The NIH allocates funding for large, coordinated projects of this kind, in the form of UM1 grants, and given the urgent need to align current interpretations of omics data in this field, we are optimistic that this framework will be successful for soft tissue joint tissues.

Integration of animal and human datasets is another significant challenge that will be key to clinical translation of omics data. While human samples are best suited for investigation of precision medicine, animal models can crucially recapitulate earlier stages of musculoskeletal disease and assess potential effects of treatment. Direct therapeutic translation is difficult given heterogeneity between humans and animal models; however, we believe that understanding conserved cellular populations and mechanisms between species will give researchers unprecedented insight into how *in vivo* interventions may behave in humans. Collectively, the incredible potential of this common database drives the goal of this initiative: to build a cross-species, multimodal analysis of joint soft tissues for clinical application. While the initial investment is substantial, it will undoubtedly reshape this field and streamline the use of next generation sequencing technologies for clinical translation.

Figure 1.

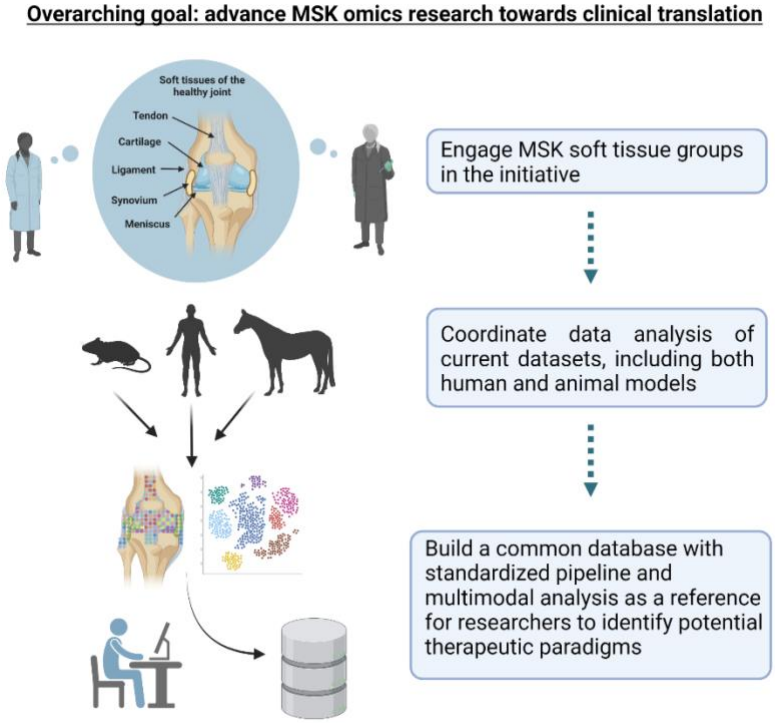

Figure 1. Stepwise approach necessary to move basic musculoskeletal soft tissue omics data from the bench towards clinical translation via creation of a common 'language' to identify new therapeutic strategies to treat musculoskeletal diseases.

References

1. Rivadeneira, F. & Westendorf, J.J. Bringing Genomic Discoveries to the Clinic: Integrating Omic Data Into the Musculoskeletal Field Through International Teamwork and Collaboration. *Journal of Bone and Mineral Research* **35**, 1623-1625 (2020).
2. Korsunsky, I.*, et al.* Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases. *Med* **3**, 481-518 e414 (2022).
3. Wei, K., Nguyen, H.N. & Brenner, M.B. Fibroblast pathology in inflammatory diseases. *J Clin Invest* **131**(2021).
4. Mizoguchi, F.*, et al.* Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat Commun* **9**, 789 (2018).
5. Callaway, E.M.*, et al.* A multimodal cell census and atlas of the mammalian primary motor cortex. *Nature* **598**, 86-102 (2021).

Competing interests

The authors declare no competing interests.

Related links

https://www.humancellatlas.org/
https://chanzuckerberg.com/science/programs-resources/single-cell-biology/seednetworks/the-tendon-seed-network/
https://msk.hugeamp.org/
https://biccn.org/