Characterizing extramedullary megakaryocytes in the knee synovium of multiple joint diseases

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INTRODUCTION: Osteoarthritis (OA), rheumatoid arthritis (RA), anterior cruciate ligament tear (ACL), and degenerative meniscal tear (DMT) are joint diseases that share common symptoms such as pain, joint swelling, and stiffness. OA is the most prevalent form of joint diseases and is driven by low-grade synovial inflammation and cartilage degeneration. Megakaryocytes are hematopoietic cells, which produce platelets. The traditional view of megakaryocytes is that in the bone marrow, megakaryocytes are derived from hematopoietic stem cells, and once mature, undergo a terminal differentiation process to form platelets. However, studies have identified extramedullary megakaryocytes outside the bone marrow. Megakaryocytes and platelets play important roles in wound healing and are implicated in myeloproliferative disorders like myelofibrosis. Few studies focus on megakaryocytes as contributors of join diseases. In this study, we characterize the transcriptomic profiles of extramedullary synovial megakaryocytes in OA, RA, ACL, and DMT to determine the potential involvement of these cells in joint diseases pathogenesis.

METHODS: We isolated synovial cells from OA patients who had undergone total knee replacements. OA synovial cells were subjected to smartseq2 single-cell RNA sequencing (Smartseq2) and 10x single-cell RNA-sequencing (scRNA-seq). We also cultured synovial cells for 14 days with or without stem cell factor (SF), a ligand that binds to the KIT receptor on hematopoietic lineage progenitors, such as those of mast cells, progenitors of megakaryocyte, to drive differentiation of these cells. Total RNA was isolated from cultured synovial cells, and synovial tissue samples were taken from OA, RA, ACL, and DMT patients. Total RNA was used for bulk RNA-seq. All RNA-seq datasets were analyzed in R.

RESULTS: We identified the *KITLG*, a gene encoding SF, positive cells from the Smartseq2 sequenced synovial cells (**Figure Panel A.**). These seven *KITLG*+ cells are located within the sub-lining synovial cell population. By using the most characteristic megakaryocyte marker *ITGA2B* (CD41), we also identified four *ITGA2B*-positive synovial cells out of 192 Smartseq2-sequenced cells (2.08%) (**Figure Panel B.**). These *ITGA2B*+ cells expressed *CD34*, *CD151*, *ITGA3* (CD49c), *CD46*, *MCAM* (CD146), *CD226*, and *TIE1*. *ITGA2B*-positive cells also expressed lipid receptor *LDLR*, and inflammatory mediators such as *CCL5*, *VWA5B1*, *VWCE*, *CCL28*, *IL11*, and *CXCL16*. In addition, *ITGA2B*+ cells expressed Platelet Activating Factor Acetylhydrolase *PAFAH2*, and Prostaglandin I2 Synthase *PTGIS*, fibroblast growth factor FGF5. The scRNA-seq data from ten OA patients had a similar genomic profile that suggest the presence of megakaryocytes in the synovium of OA patients. We developed a megakaryocyte transcriptomic signature by generating a list of genes expressed by megakaryocytes, which include both mature megakaryocyte markers including *ITGA2B*, *ITGA3*, *CD151*, *CD46*, and *PAFAH2*, and immature megakaryocyte markers including *CD34*, *MCAM*, *CD226*, *TIE1*, *CCL5*, *VWA5B1*, *VWCE*, *CCL28*, *CXCL16*, *PTGIS*, *IL11*, *LDLR*, and *FGF5*. Bulk RNA-seq data showed transcriptomic signatures of megakaryocytes in synovial tissues from ACL, DMT, RA, and OA patients. Bulk RNA-seq of OA synovial cells treated with 10ng/mL SF for 14 days showed not only a higher percentage of mast cell gene signature markers such as *CPA3* and *TPSAB1*, but also a higher percentage of mature megakaryocyte markers and a lower percentage of immature megakaryocyte markers (**Figure Panel C.**), indicating differentiation, proliferation and maturation of the megakaryocyte progenitors in the culture condition.

DISCUSSION: Together, our data suggests the presence of abnormal extramedullary megakaryocytes in the synovium of joint diseases. We found a sub-lining synovial cell population that generate SF, a multi-lineage hematopoietic progenitors' proliferation and differential growth factor, in the synovium of patients with joint diseases such as OA. As an important multi-lineage hematopoietic growth factor, SF is not only involved in hematopoietic stem cell maintenance and mast cell development, but also drives the megakaryocyte differentiation and proliferation. Joint swelling is driven by inflammation. Proinflammatory factors are released in the synovial capsule and either recruit immune cells or induce the inflammation reactions. Previous studies showed that megakaryocytes and platelets not only produce abundant proinflammatory factors such as CXCL4, CCL5 and PAF, but also are directly involved in cellular inflammation processes as immune cells. Therefore, we hypothesize that megakaryocytes and platelets play an under-appreciated role in joint diseases pathology.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data shows that there are extramedullary megakaryocyte differentiation and proliferation, which may drive local inflammation in the synovium of patients with joint diseases. Anti-megakaryocyte therapy may be considered in treating these conditions.

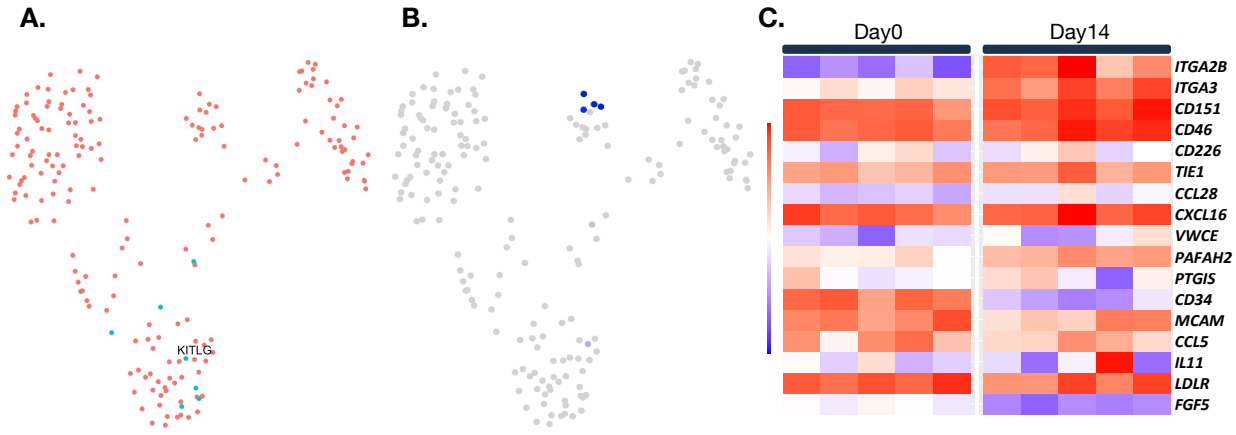

Figure: Stem cell factor generated by the *KITLG*+ synovial cells supported the differentiation and proliferation of *ITGA2B*+ megakaryocytes in the OA synovium. Panel A. Stem cell factor (SF)-producing cells (*KITLG* +) (teal dots) in Smartseq2 OA synovial cells. **Panel B.** Megakaryocytes (*ITGA2B* +) (dark blue dots) in Smartseq2 OA synovial cells. **Panel C.** The gene expression heatmap of megakaryocyte in the SF-cultured OA synovial cells indicated higher expression levels of mature megakaryocyte marker and lower expression levels of immature megakaryocyte markers after 14 days culture with SF.