Discovery of Telocytes Integrated into Mast Cells Adjacent to Joint-Draining Lymphatic Vessels and their Potential Role in Synovial Lymphatic System

Andriy Kobryn[1,4], Yue Peng[1,2], H. Mark Kenney[1,3], Karen L de Mesy Bentley[1,2], Lianping Xing[1,2], Benjamin D. Korman[1,3], Christopher T. Ritchlin[1,3], Edward M. Schwarz[1,2,3,4]

[1]Center for Musculoskeletal Research, [2]Department of Pathology & Laboratory Medicine, [3]Department of Dermatology, [4]Department of Orthopaedics, University of Rochester Medical Center, Rochester, NY, USA.

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Introduction: Rheumatoid arthritis (RA) patients and TNF-transgenic mice exhibit lymphatic dysfunction linked to joint disease.[1] scRNAseq on cells isolated from popliteal lymphatic vessels (PLV) identified *Efhd1* as a selective marker for PLVs.[2] Efhd1-CreER^{T2} x Ai9 mice showed tamoxifen-induced labeling of peri-PLV telocytes, which are reduced in RA synovium.[3] Our transmission electron and confocal microscopy showed peri-PLV telocytes directly contacting mast cells. Based on this, we hypothesized that peri-PLV telocytes participle in the synovial lymphatic system (SLS) by sensing osmotic pressure and transmitting signals to mast cells that release vasoconstrictive factors to induce contractions in resting PLV.

Methods: PLVs from tamoxifen-treated *Efhd1*-CreER^{T2} x Ai9 mice were cultured ex vivo to establish co-cultures of tdT^{+} telocytes and tdT^{-} fibroblasts. Osmotic stress responses were assessed using sucrose, with Fluo-4 and MitoSOX-Green dyes measuring Ca^{++} flux and mitochondrial ROS respectively. We also performed Matrigel invasion assays +/- TGF-β1, and bulk RNAseq of FACS purified tdT^{+} telocytes cultured on low, intermediate, and high stiffness-defined extracellular matrix (ECM) substrates. Statistical analyses were performed using one-way ANOVA.

Results: Telocytes exhibited significantly greater Ca^{++} flux in response to sucrose-induced hyperosmotic stress than fibroblasts **(Fig. 1a, p<0.01).** Increased mitochondrial ROS production was only observed in telocytes **(Fig. 1b, p<0.0001).** Fibroblasts displayed greater invasive capacity in Matrigel vs. telocytes **(Fig. 2a).** Bulk RNAseq of tdT^{+} telocytes demonstrated their ability to differentiate into myofibroblasts on stiffness ECM **(Fig. 2b).** Consistently, scRNAseq trajectory analysis of PLV cells predicted telocyte-to-fibroblast differentiation (data not shown), supporting their potential contribution to pathogenic fibroblast populations.

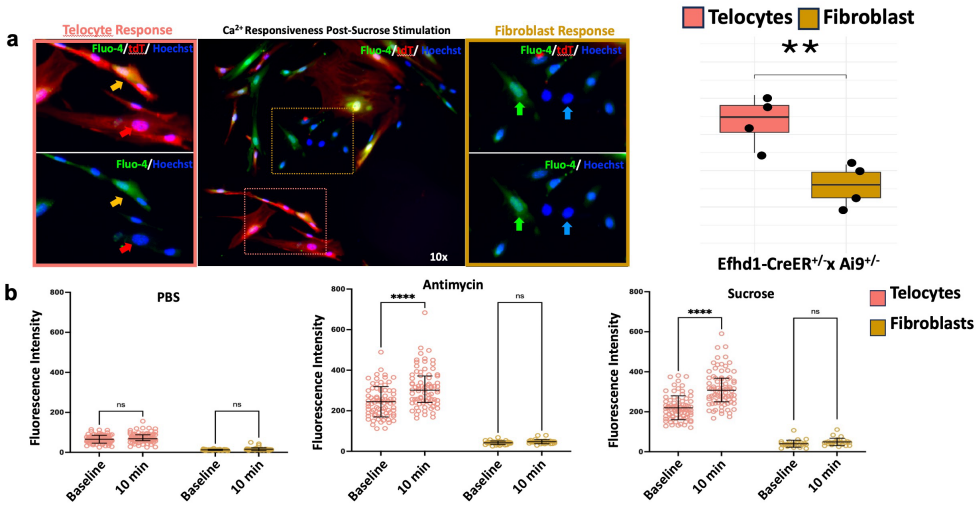

Fig. 1 **a** Representative 10x fluorescent images of a coculture post-sucrose stimulation are shown to illustrate Ca^{++} responsiveness seen as green fluorescence (center). The ROI (boxes) are magnified to illustrate four cell types: non-responsive telocytes (red), responsive telocytes (yellow), non-responsive fibroblasts (blue), and responsive fibroblasts (green) with (top) and without (bottom) the superimposed tdT channel. The response to 100 μM (424.5 mOsm) sucrose was quantified as the % Fluo-4 positive telocytes or fibroblasts (** p<0.01). **b** Quantification of MitoSOX-Green fluorescent intensity from the first and last frames demonstrates that telocytes generate significantly more ROS than fibroblasts (**** p<0.0001).

Conclusions/Discussion: Our findings support a model in which Efhd1^{+} telocytes regulate joint homeostasis by sensing osmotic stress and relaying signals to mast cells, thereby promoting lymphatic clearance. Mechanistically, telocytes exhibit high cytosolic Ca^{++} flux and increased mitochondrial ROS (Mitoflash), coupled with low invasive capacity, consistent with Efhd1 mediated regulation of mitochondrial Ca^{++} uptake and inhibition of YAP/TAZ metastasis pathway.[4] In contrast, under conditions of chronic inflammation and stiff ECM, telocytes lose Efhd1 expression, acquire myofibroblast features, and activate YAP/TAZ pathway that enhance invasiveness.

Significance/Clinical Relevance: Our study identifies telocytes as a critical stromal population that senses osmotic and mechanical cues to regulate SLS functions, which highlights their potential as therapeutic targets to restore joint drainage and limit damage from arthritis.

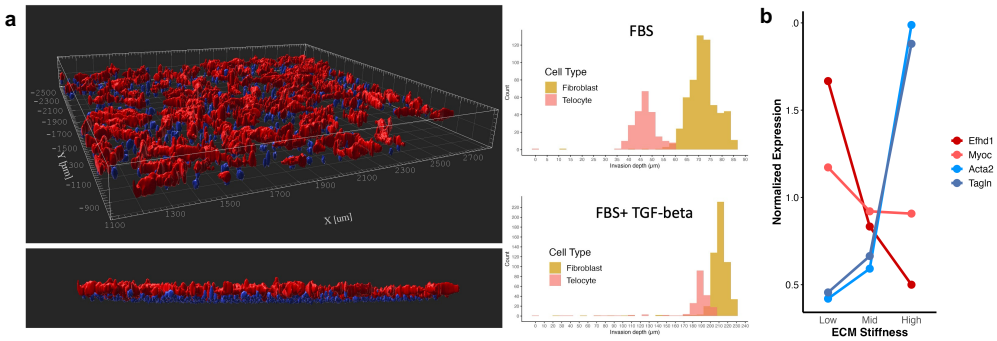

Fig. 2 **a** Matrigel invasion assays were performed using transwell inserts containing cells in serum-free media that invaded towards 10% FBS containing media -/+ 5ng/ml TGF-beta in the bottom chamber for 24hr. The culture was Hoechst-stained prior to fluorescent confocal microscopy, and representative 3D reconstructed images are shown with top and side views illustrating the red telocytes on top of the blue nuclei within the fibroblasts. The migration depth of each nuclei in the Matrigel was calculated and presented in the histograms, which are representative of three independent experiments. **b** Primary cultures of the FACS purified tdT^{+} cells were cultured on collagen-coated CytoSoft® Imaging 24-well plates with varying stiffness levels: 0.2 kPa (soft), 8 kPa (intermediate), and 64 kPa (stiff). After 24hrs of culture, the cells were harvested for bulk RNA sequencing and gene expression analyses. Normalized expression of telocyte (*Efhd1* and *Myoc*) and myofibroblast (*Acta2* and *Tagln*) marker genes are graphed as a function of the ECM stiffness.

References: [1] Bouta E.M. et al. Nat Rev Rheumatol (2018); [2] Kenney H.M. et al. Arthritis Res Ther (2022); [3] Rosa I. et al. J Cell Mol Med (2021); [4] Meng K. et al. Cancer Sci (2023).

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