

Activation of Fibroblast-like Synoviocytes Triggers Impaired Endothelial Cell Functioning in Inflammatory Arthritis

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INTRODUCTION: Fibroblast-like synoviocytes (FLSs) form a unique cell type that populates the joint synovium. FLSs are essential for the maintenance of joint homeostasis. They become the main drivers of persistent inflammation in arthritic joints. During an inflammatory state, the FLSs become permanently altered and function as imprinted aggressors by developing a long-term epigenetic memory (1). This aggressive phenotype of FLSs persists even after the inflammation has subsided and may abundantly co-stimulate various other cells lying in proximity to them. Since inflammation and angiogenesis are closely integrated (2), we hypothesized that the activated FLSs cause impaired functioning of adjoining cells within synovium such as endothelial cells. Therefore, the aim of the present study was to investigate the mechanisms underlying the endothelial cell dysfunction induced by activated FLSs.

METHODS: Animal procedures were approved by an institutional animal care and use committee. We assessed doxycycline-induced inflammatory arthritic changes in doxycycline-inducible human TNF α -transgenic (iTNF-tg) mice (3) which developed inflammatory arthritis exclusively after doxycycline administration by histological techniques. Synovia from DOX-ON mice (1mg/ml doxycycline & 5% sucrose for 3 weeks), DOX-OFF mice (1mg/ml doxycycline & 5% sucrose for initial 3 weeks & regular water for next 3 weeks), and control mice (5% sucrose for 3 weeks) were digested for bulk RNA-seq to study the transcriptomic changes. The differentially expressed genes (DEGs) were identified using the BioJupies web tool and were visualized using heatmaps, volcano plots, and principal component analysis. RNA-seq data analyses were carried out to study the gene expression for different conditions assayed by RT-PCR. In order to understand the growth rate, cell cycle, and cell viability, we performed cell proliferation assay using FLSs from three groups of mice. We assessed the interaction between FLSs and endothelial cells by studying the effects of factors released by FLSs on HUVECs with the help of cell proliferation assay, in-vitro angiogenesis assay, and gene expression levels by RT-PCR. We assessed the expression of critical genes involved in inflammatory pathways in TNF-stimulated FLSs by selecting some genes associated with the adaptive immune system and key transcriptomic signatures from our RNA-seq analysis. Finally, we performed an ATAC-seq experiment using FLSs from three groups of mice, in order to assess the availability of open chromatin and the epigenomic reprogramming in healthy, inflammatory, and resolution phases of arthritis. The data analysis of ATAC-seq is ongoing.

RESULTS: The histological analysis confirmed that inflammatory changes in doxycycline-iTNF-tg mice were reversible as the synovial hyperplasia, enthesitis, cartilage and bone alterations, formation of pannus tissue reduced after the treatment of mice with doxycycline was removed. We next performed RNA-Seq experiments from FLSs obtained from control, DOX-ON, and DOX-OFF mice and discovered that most transcriptomic changes were not reversible, which clearly indicated that FLSs possessed inflammatory memory (Fig 1A). The most significant downregulated pathways in the DOX-OFF and DOX-ON mice were related to cell cycle, cell cycle checkpoints, and mitosis whereas the upregulated pathways were related to glutathione conjugation, immune system, cytokine signaling, interferon signaling, biological oxidation, etc (Fig 1B). We selected seven genes after RNA-seq which showed a similar difference in expression levels (log2 scale) among controls, DOX-ON, and DOX-OFF after real-time qPCR. Cell proliferation assay revealed that there was a G0/G1-phase arrest, cell death induction, and reduced viability in DOX-OFF FLSs. We also observed that the supernatant of DOX-OFF FLSs induced early apoptosis in HUVECs with cell proliferation assay. Analysis of in-vitro angiogenesis assay revealed that HUVECs possessed a poor angiogenic potential when induced with DOX-OFF FLSs supernatant. Interestingly, we also observed that there was an upregulation in the expression levels of pro-inflammatory genes and a downregulation of pro-angiogenic genes in HUVEC cells treated by supernatant of DOX-OFF FLSs. There was a differential expression of genes related to the adaptive immune system under inflammatory conditions by stimulating FLSs with TNF, a well-known pro-inflammatory inducer that activates synovial fibroblasts, which causes the overproduction of cathepsins and MMPs.

DISCUSSION: Our study highlighted that the persistent aggressive phenotype of FLSs not only causes the increased invasiveness of synovium into the extracellular matrix and further exacerbates joint damage but also triggers impaired endothelial cell functioning. The abrogation of the inflammatory phase by the removal of doxycycline 3 weeks after beginning stimulation resulted in faster resolution of the histologic features, but a clear persistence of inflammatory transcriptomic expression. It is known that FLSs in inflammatory state are resistant to programmed cell death which is induced by defective apoptotic stimulus signals which further leads to abnormal synovial hyperplasia, pannus formation, and inflammatory cell infiltration, resulting joint destruction and functional loss (4). Here we show that despite possessing the transcriptional inflammatory memory with downregulation of cell cycle pathways, FLSs showed an increased apoptotic ability and reduced cell proliferation during the resolution phase of arthritis along with a decline in angiogenic potential and lower expression of pro-angiogenic genes. Furthermore, the results of chromatin accessibility may provide deeper insights into epigenomic reprogramming and its sequelae on endothelium functioning.

SIGNIFICANCE/CLINICAL RELEVANCE: The pathological behavior of FLSs residing in synovium directly contributes to the synovial and endothelial pathology in chronic inflammatory diseases such as rheumatoid arthritis (RA). The remission phase of RA is an important phase that determines the disease course and future treatment plan but remains understudied. Since there is no permanent cure for RA, our study provides the possible mechanisms underlying endothelial cell dysfunction induced by activated FLSs in this resolution phase which may offer a comprehensive picture for the development of therapies.

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