3D Synovial Organoid Culture Reveals Cellular Mechanisms of Tissue Formation and Inflammatory Remodeling

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Introduction: The synovial membrane is a distinctly organized structure with two layers: a densely packed lining layer that sits on top of a more loosely organized sublining layer. During the course of arthritis, the synovium becomes hyperplastic and demonstrates thickening of the lining layer and cellular condensation at the sublining layer. Using a three-dimensional synovial organ culture system, we explore cellular mechanisms of synovial tissue formation and inflammatory remodelling.

Methods: Fibroblast-like synoviocytes (FLS) derived from patients with rheumatoid arthritis (RA) were cultured in 3D Matrigel micromasses. To mimic synovial inflammation, micromasses were challenged with TNF. For histological analyses, micromasses were embedded in paraffin, sections were stained with haematoxylin and eosin; reticular fibres were dyed using the Gomori silver staining technique. Ki67 labelling was performed to identify proliferating cells. Two-photon laser scanning microscopy was used to measure lining layer thickness during the culture period and to visualize newly formed collagenous fibres (Second Harmonic Generation; SHG) 3D confocal micrographs were analysed using Imaris® Bitplane software. mRNA levels for various genes expressed in FLS were determined by qPCR.

Results: Synovial micromasses demonstrated thickening of the lining layer over time. When stimulated with TNF, hyperplasia of the lining layer and cellular aggregation at the sublining layer was observed. In order to identify the origin of cells contributing to the thickening of the lining layer, proliferation studies were conducted. Intriguingly, in the early phase of the culture period, the percentage of proliferating cells in the lining

layer was higher when compared to the sublining layer. This proliferative activity, however, was no longer present in the late phase, after the lining layer was established (mature phase). In the presence of TNF, an increased number of proliferating cells at the lining layer was maintained for an extended period of time, consistent with higher rates of cellular proliferation at the synovial lining in sections of RA synovial tissues when compared to OA synovial tissues. During the course of lining/sublining layer maturation, mRNA expression levels of genes of interest were measured. qPCR data indicate that MMP1, MMP3, and IL-6 are differentially expressed during the early phase (one week old) and the mature phase (four weeks old) of the culture period. By contrast, lubricin, cadherin-11, CCL20, and STAT1 gene expression did not show a significant difference.

Conclusions: The three-dimensional FLS micromass culture reveals spontaneous formation of a tissue structure that strikingly resembles the lining/sublining architecture of the in-vivo synovial tissue. This process involves FLS proliferation as well as expression of genes that allow for tissue remodeling. In inflammatory conditions similar cellular programs are re-activated resulting in synovial lining hyperplasia and a pannus-like condensed mass of cells.