The epigenetic landscape of osteoarthritis: Investigating histone modifications in primary human articular chondrocytes

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Osteoarthritis (OA) affects 500.000 patients worldwide, yet its molecular mechanisms remain poorly understood. Transcriptional and protein-level changes are likely linked by epigenetic marks, especially histone modifications. We aim to map the epigenetic profile of human articular chondrocytes (hACs) from OA patients and non-OA controls at single cell resolution using CyTOF. However, no existing protocol supports the analysis of freshly isolated hACs, capturing dynamic post-translational modifications like histone marks. We developed a procedure tailored to the low abundance and adhesive properties of hACs.

For CyTOF, hACs were stained using metal isotope-labelled antibodies, DNA was detected by Iridium and chondrocyte identity and quality were assessed via CD44 and SOX9. Preliminary optimisations were performed by flow cytometry.

Freezing of freshly isolated hACs resulted in poor cell recovery, promoting a switch to storage of pre-fixed cells. Protein-free buffers increased adhesion and were replaced by protein-containing options. Multiple permeabilization regimes were tested; Treatment with 1% SDS and 0.1% Triton X-100 for 10 min effectively disrupted the pericellular matrix, permeabilised membranes, and achieved chromatin accessibility. Combining permeabilization and blocking minimized cell loss. BSA-coated and low-retention tips increased cell loss, while FBS-coated tubes improved retention.

Our optimised protocol provides sufficient hAC recovery and allows detection of chondrocyte markers and histones modifications on single-cell level. Next, we will analyse patient samples to discover epigenetic modifications promoting OA and locate the disease relevant chondrocytes using imaging mass cytometry.