Characterization of the osteoarthritic joint microenvironment

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Summary

One of the hallmarks of osteoarthritis (OA) is an altered joint microenvironment, which results in compositional changes of the synovial fluid. The impact of this changing microenvironment on the deterioration of joint tissues and how it corresponds to OA development has been poorly studied. This thesis describes a novel approach to monitor synovial fluid-induced signaling changes during OA development. In addition, phenotypic changes of articular chondrocytes in response to OA synovial fluid were characterized.

A cell-based reporter gene screening platform was developed to monitor synovial fluid-induced pathway activities. An unstable Nano luciferase (NLucP) driven by either gene promoters (i.e. IL6, IL8, ADAMTS5 and WISP1) or transcription factor response elements (i.e. NFκB-RE, SRF-RE, SRE, AP1-RE, CRE, ISRE, NFAT5-RE, ARE, SIE, SBE, TCFLEF-RE, GRE and NBE) were selected as OA-relevant reporter systems. OA- or disease-associated genes can be rapidly screened using gene promoter reporters, as evidenced by our IL6 and WISP1 reporters that predicted the inhibition of mesenchymal stromal cell chondrogenesis by OA conditioned media with 87% accuracy (Chapter 3). In addition, we demonstrated that transcription factor response element reporters are valuable tools for monitoring the activity of cellular signal transduction pathways in response to small molecules, signaling proteins and, more importantly, OA synovial fluid (Chapter 2/4/5/6).

Characterization of OA synovial fluid was performed on various levels, including signaling protein content, chondrocyte intracellular signaling patterns, gene expression responses, collagen production and cell proliferation (Chapter 4/5). Synovial fluids derived from end stage-OA patients were generally enriched in signaling proteins, such as interleukins, growth factors and chemokines. In direct comparison to non-OA, these OA synovial fluid-enriched signaling proteins provoked higher MAPK, AKT, RhoGTPase, NFκB and cell cycle signaling in primary articular chondrocytes (Chapter 5). The OA-specific microenvironment activated AKT and cell cycle signaling-induced cellular processes, such as protein synthesis and cell proliferation. Long-term OA synovial fluid exposure of chondrocytes sustained their proliferation, which was not halted by contact inhibition. Epidermal growth factor
receptor (EGFR) signaling was found to be critical for OA synovial fluid-induced chondrocyte proliferation (Chapter 4). Analysis of transcription factor activity downstream of MAPK (i.e. SRE, AP1-RE), RhoGTPase (i.e. SRF-RE) and NFκB (i.e. NFκB-RE) pathways demonstrated an OA synovial fluid-dependent increase in activity. Secretomes of OA joint tissues (i.e. cartilage, synovium, meniscus and infrapatellar fat pad) contributed to the inflammatory profile of the OA microenvironment, as they provoked clear NFκB-RE (e.g. cytokines/DAMPs), CRE (e.g. prostaglandins), SIE (e.g. IL6) and ISRE (e.g. IFNα/β) signaling (Chapter 5). These distinct OA synovial fluid pathway signatures led to accelerated chondrocyte dedifferentiation, mainly via ERK signaling and promoted chondrocyte fibrosis through JNK, PI3K and classical PKC activation. Long-term (13 days) culture of chondrocytes with OA synovial fluid gradually induced many fibrosis-associated genes, including SERPINF1, CXCL12, SRPX, GPNMB, TMEM119 and CEMIP, which are upregulated in vivo in fibrochondrocytes of OA patients. Moreover, OA synovial fluid facilitated collagen type I processing and deposition, whereas collagen type II production was inhibited (Chapter 4). Interleukin, chemokine and DAMP-enriched OA synovial fluid provoked a pronounced inflammatory response, which was highly dependent on classical PKC signaling. Chondrocytes in response to OA synovial fluid transcriptionally activated several chemokine genes. Furthermore, the p38 and PI3K signaling routes mainly mediated OA synovial fluid-induced expression of ECM-degrading enzymes (Chapter 5). Overall, the multi-level characterization of the OA synovial fluid microenvironment highlights key molecular processes that underlie OA development.

After we distinguished and characterized non-OA and OA synovial fluid on multiple levels, we explored heterogeneity among OA patients based on their patient-specific synovial fluids. Development of diagnostics to molecularly endotype OA patients is essential for future stratification medicine. Until now, stratification based on synovial fluid biomarkers remains challenging, due to compositional complexity, lack of specificity and patient heterogeneity. As opposed to previous synovial fluid biomarker research, we investigated the integrated chondrocytic signal transduction in response to synovial fluid as one complex mixture, rather than by single biological factors (Chapter 6). Our cluster analysis of 160 synovial fluid signaling patterns
identified two main subgroups. The first group was characterized by high inflammatory (i.e. NFκB-RE), RhoGTPase (i.e. SRF-RE) and MAPK (i.e. SRE, AP1-RE, CRE) pathway activity. In contrast, the second group revealed an overall higher STAT3 (i.e. SIE) signaling. These findings highlight the existence of two distinct OA endotypes, which hints towards different mechanisms or processes that occur during end-stage knee OA.

Taken together, the findings described in this thesis contribute to a better understanding of the joint microenvironment-induced molecular disease mechanisms that underlie OA. Moreover, endotyping tools using synovial fluid as OA indicator hold great promise to facilitate future OA precision medicine.