Chondrocytes

Chapter 8

Translation and its precise regulation are critical for chondrocytes and articular cartilage homeostasis. Disturbances in this homeostasis are a cause of osteoarthritis (OA). OA is an active disease process that affects all the tissues of the articular joint. In particular, chondrocytes residing in the articular cartilage undergo cellular phenotypic changes fueled by pivotal alterations in their protein expression programs. This results in the remodelling and degeneration of articular cartilage and gradual failure of joint integrity. While gene transcriptional regulation behind these phenotypic and proteomic changes has been extensively studied in the field of osteoarthritis, mechanisms of protein translation regulation have been largely overlooked. The aim of the work presented in this thesis was to broaden the understanding of molecular mechanisms behind translation (de)regulation in chondrocytes and in OA in particular.

The translation capacity of the cell is dependent on ribosomes, ribonucleoprotein complexes that catalyze protein synthesis. The process of ribosome biogenesis is tightly regulated and relies on the assistance of small nucleolar RNAs (snoRNAs), which facilitate ribosomal RNA (rRNA) maturation. Previous studies showed that in the course of osteoarthritis, chondrocytes modify their translation rate. However, the signalling molecules and pathways involved in this regulation were poorly studied. In this thesis, we demonstrated that TGF-β, a cytokine critical for cartilage homeostasis but also OA development, induces the rate of translation in chondrocytes (**Chapter 2**). Interestingly, while the total translation capacity was induced, the treatment of chondrocytes with TGF-β specifically inhibited IRES (internal ribosome entry site)-mediated translation. TGF-β treatment also provoked site-specific changes in rRNA modification profiles and alterations in ribosomal protein composition. This was accompanied by changes in the chondrocytes' proteome and protein secretome. These results uncovered that not only translation in general is subjected to regulation in OA-relevant cytokine conditions, but also the mode of translation initiation.

The majority of snoRNAs guide rRNA post-transcriptional nucleotide modifications (PTM, 2'-*O*-methylation and pseudouridylation), while others facilitate endoribonucleolytic cleavage of pre-rRNA precursors. In **Chapter 3** we investigated snoRNA expression profiles in human articular cartilage and identified a panel of snoRNAs differentially expressed in cartilage ageing and OA. We confirmed these findings in *in vitro* cultured primary human articular chondrocytes and non-OA chondrocytes exposed to osteoarthritic synovial fluid or IL-1β. Knockdown and

overexpression experiments uncovered that expression levels of OA-associated snoRNAs, SNORA26 and SNORA96a, affect total rRNA levels as well as the expression of chondrogenic, hypertrophic and other OA-related genes in chondrocytes.

Cartilage hair hypoplasia (CHH; OMIM #250250), is a rare form of metaphyseal chondrodysplasia characterized by disproportionate short stature and aberrant growth plate chondrocyte development. It is caused by mutations in the gene encoding for snoRNA RMRP (RNA component of mitochondrial RNA processing complex). RMRP mediates cleavage of the 47S pre-rRNA precursor, contributing to 5.8S rRNA maturation. In **Chapter 4** we analyzed rare CHH patient-derived fibroblasts subjected to FDC (fibroblast-derived chondrocyte) transdifferentiation, to investigate the roles of RMRP in chondrogenic differentiation. Firstly, we showed that FDC is suitable *in vitro* model for studying the terminal phase of chondrogenic differentiation which is crucial for longitudinal bone growth in vertebrates. We also uncovered that FDC chondrocytes of CHH patients have difficulties in committing to terminal differentiation and we identified the upregulation of several key factors of BMP, FGF, and IGF-1 signalling axes. These results are important not only for the field of chondrogenic differentiation and CHH pathobiology but also OA. This is because the changes in chondrocytes' expression profiles which occur during chondrogenic differentiation are to some extent replicated in OA development. Altogether, the snoRNA work presented in this thesis highlights the multifaceted roles of snoRNAs in chondrogenic differentiation and OA by determining the chondrocyte phenotype and regulating cellular signalling.

The differential expression of snoRNAs in osteoarthritic cartilage identified in **Chapter 3** indicated that OA chondrocytes might have distinctly modified, heterogeneous ribosomes. In this thesis, we tested end-stage osteoarthritic synovial fluid (OA-SF) as an OA-mimicking microenvironment for its capacity to induce changes in rRNA PTM profiles of human primary chondrocytes (**Chapters 5** and **6).** The mapping of OA-sensitive rRNA PTM heterogeneity revealed several sites whose modification levels were regulated in the OA-mimicking microenvironment. This was investigated in greater depth by depleting snoRNAs which guide the modification of selected OA-sensitive PTMs. Depletion of *SNORD71*, the snoRNA guiding 2'-*O*-methylation of OA-sensitive U14 on 5.8S rRNA, significantly affected the mode and fidelity of translation (**Chapter 5**). It also altered the cellular proteome and, in particular, increased the translation efficiency of the fibrochondrocytic *COL1A1* mRNA which resulted in increased levels

of COL1A1 protein. These data demonstrate that chondrocytes employ rRNA epitranscriptomic mechanisms to control the translation of specific OA-related mRNAs. Another OA-sensitive rRNA PTM, pseudouridylation (ψ) of U4966 on 28S rRNA, was investigated by depleting its guide *SNORA33* (**Chapter 6**). The loss of this rRNA PTM induced changes in the cellular proteome and, relevant for OA, decreased expression levels of several inflammation-attenuating protein factors. The loss of 28S-ψ4966 affected the protein composition of the cellular ribosome pool and increased the capacity to initiate translation from specific IRES elements. IRES-mediated translation is generally linked to stress conditions and putative IRES elements have been recently identified in several OA-relevant genes. Our data represent the first empirical indication that IRES-mediated translation regulation plays a role in OA pathobiology.

Taken together, in this thesis, we established that translation regulation and ribosome heterogeneity play an important role in chondrocyte differentiation and OA pathobiology. As discussed in **Chapter 7**, our data offer novel insights into translation-regulatory mechanisms relevant to OA, with an emphasis on ribosomes. We provide the first evidence of chondrocytes adapting their ribosome pool in response to the OA microenvironment. These discoveries are in line with identified changes in snoRNA expression profiles in cartilage ageing, osteoarthritis and during chondrogenic differentiation. More importantly, we demonstrated that specific changes in rRNA PTMs have distinct consequences for the chondrocytes' proteome. These new insights into translation dynamics in OA chondrocytes provide the foundation for future translation and ribosome-based research in the OA field and open up new opportunities for the development of OA treatment strategies (**Chapter 8**).