Chondrocytes

Impact paragraph

Chapter 8

Osteoarthritis (OA) is a prevalent chronic degenerative joint disease. It affects more than 500 million people globally and it is a leading cause of chronic pain and disability in developed nations [1,2]. Given its prevalence and debilitating consequences, OA imposes a considerable socioeconomic burden on healthcare systems around the world [3]. Yet, no OA-modifying drugs are currently available. The complex multifactorial and heterogeneous character of OA is one of the main reasons why the efforts to develop a universal one-treatment-fits-all drug therapy failed in the past. A deeper understanding of underlying molecular processes and their relative contribution to particular OA phenotypes and endotypes will be important for the development of targeted, precision medicine-based treatments in the future.

One of the hallmarks of OA is the degeneration of articular cartilage [4]. OA is an active pathological process in which chondrocytes residing in the articular cartilage undergo phenotypic changes fueled by pivotal alterations in their protein expression programs [5]. While gene transcription regulation behind these phenotypic and proteomic changes has been extensively studied in OA, mechanisms of protein translation regulation received only very limited attention. Even though ribosomes catalyze protein synthesis and as such, they are the core of cellular translation, ribosome-based mechanisms of translation regulation have not been addressed in OA. Therefore, the aim of the work carried out in this thesis was to explore this so far relatively obscure, but pivotal, area of OA research, and broaden the understanding of molecular mechanisms behind translation and ribosome (de)regulation in osteoarthritic chondrocytes. A comprehensive understanding of these processes can provide new opportunities for the development of molecular OA therapies.

Idiopathic OA is a chronic condition with multifactorial aetiology, which develops gradually over decades in response to various intrinsic and extrinsic factors [6]. OA is most commonly diagnosed using image-based methods, such as radiography. Unfortunately, at the stage when the joint damage is detectable by these methods, OA is already in its advanced stages when the damage to the joint is very severe and disease-modifying opportunities are very limited, if not impossible. Early detection of OA is therefore crucial for the timely administration of disease-modifying OA drugs (DMOADs), potentially diverging the course of OA and its clinical outcomes. Many studies showed that snoRNAs are stably expressed and can be reliably measured in body fluids including synovial fluid, blood plasma, and serum. Importantly, several studies highlighted the potential of snoRNAs to serve as diagnostic and prognostic markers in human diseases such

as cancer [7,8]. In **Chapter 3** we identified a panel of snoRNAs differentially expressed in human chondrocytes in cartilage ageing and OA. Articular chondrocytes are a likely source of snoRNAs present in synovial fluid. In line with that, several snoRNAs have been found differentially expressed in equine synovial fluid as a sign of early OA [9]. Our data thus indicate that snoRNAs carry the clinical potential as OA biomarkers. In **Chapters 3** and **4** we also uncovered that snoRNAs have functions in regulating chondrocyte gene expression with consequences for the pathological chondrocyte phenotype and signalling in OA and during chondrogenic differentiation. These results highlight the multifaceted roles of snoRNAs in chondrocyte (patho)biology and imply that targeting snoRNA expression in articular cartilage could be used in OA therapy to affect the chondrocyte phenotype and modulate ribosome heterogeneity. Antisense oligonucleotides (ASO)-based therapy is an emerging area in the field of drug development. Based on sequence complementarity, ASO binds the target RNA and thus regulates its levels. The technology is still developing and various chemical modifications, as well as delivery strategies, are being tested to minimize off-target side effects and optimize efficacy, enzymatic stability and biological activity [10]. ASO-based therapy has been used in the OA field already [11], where ASOs targeting miRNAs have shown promising results in preclinical OA animal models [12-15]. Intra-articular administration of ASOs seems to be the method of choice, as studies have shown that ASOs can penetrate the articular cartilage [14,15]. Of course, one can envision that a repeated injection scheme can be demanding if sustained downregulation of specific snoRNAs is needed. Injectable and thermoresponsive materials could be also used to help retain ASOs within the joint and optimize the cellular uptake of ASOs [16]. Another option is using viral vectors carrying ASOs to achieve more stable expression of ASOs in cells. Of course, a similar approach could be used to overexpress OA-protective snoRNAs. The potential of viral vector-based modalities in OA was highlighted by successful knockdown and overexpression of miR-128a in an OA animal model using intra-articularly administrated lentiviral constructs [15]. In 2017, the first OA viral gene therapy product that induces expression of TGF-β1, TissueGene C (Invossa), was approved for the treatment of OA in South Korea [17]. At this moment, it is in Phase III clinical trial in the USA (Clinical Trial ID: NCT03203330).

Modulating the expression of snoRNAs might be also used to regulate rRNA PTM-based ribosome heterogeneity. In **Chapters 2**, **5** and **6** we demonstrated ribosomal compositional changes in chondrocytes in response to OA-mimicking conditions. Importantly, this in turn

affected ribosome function, translation of specific OA-related mRNAs and alterations in the chondrocyte cellular proteome. We demonstrated that chondrocytes respond to OA-associated microenvironmental changes by functionally adapting their ribosome pool. The canonical function of the majority of snoRNAs is to guide rRNA PTMs [18]. Therefore, by modulating snoRNA expression we would be able to affect ribosome heterogeneity and manipulate the chondrocyte ribosome pool. By promoting the production of "healthy" ribosomes that efficiently translate mRNAs encoding e.g. collagen type 2, aggrecan or other cartilage ECM proteins, we might stimulate cartilage regenerative processes. In conjunction with the elimination of OA-promoting ribosomes, this may be a way how to counteract OA development.

In **Chapters 2** and **6** we also uncovered ribosome heterogeneity in terms of ribosomal protein composition in OA chondrocytes. This mostly concerned ribosome-associated factors, many of which are known regulators of IRES-mediated translation. Although first discovered in viruses, IRES-mediated translation is now increasingly recognized as a mechanism of eukaryotic translation regulation, which is activated under pathological stress conditions [19]. In line with that, putative IRES elements have been recently identified in several OA-relevant genes [20]. In **Chapters 2**, **5** and **6** we provided evidence that IRES-mediated translation regulation plays a role in OA pathobiology. By preventing the interaction of specific ribosome-associated factors with ribosomes, using inhibitors of protein-protein interactions, or downregulating the expression of these factors, the expression of specific pathological mRNAs containing IRES elements could be repressed.

Single-cell-based analysis of OA cartilage identified at least seven phenotypically (and presumably also functionally) distinct chondrocyte subpopulations in human OA cartilage [21,22]. So far, these subpopulations have been characterized at the level of gene transcription by RNA sequencing. However, we can expect that the gene expression profiles will soon be supplemented with data on their cellular proteome, secretome and even ribosome heterogeneity and specialization. Rapidly-advancing spatial gene expression [23,24] and proteomic [25-29] analysis technologies will provide valuable information about transcription and translation dynamics in cartilage and chondrocytes with spatial context. This will be important for deciphering the roles of individual subpopulations in OA (patho)biology and might help identify the critical changes that drive OA progression. Furthermore, ribosome profiling and thorough

analysis of 5' and 3' UTR sequences of translationally-regulated mRNAs will help to characterize the transcripts that are (dis)favoured by OA-specific chondrocyte ribosomes. This knowledge could be used to improve the design of synthetic therapeutical mRNAs for regenerative medicine and tissue engineering [30].

Ribosome-targeting therapy might be novel for the OA field, but it is recognised as a promising avenue in the treatment of other diseases. What started with antibiotics targeting prokaryotic ribosomes, is now expanding to ribosome-targeted treatments of non-infectious human diseases such as cancer or Duchenne muscular dystrophy [31,32]. A compelling example of successful and targeted ribosome-focused therapy is Ataluren, a small molecule developed by PTC Therapeutics for the treatment of Duchenne muscular dystrophy which is a disease caused by nonsense mutations in the dystrophin gene causing a premature stop in translation of its mRNA [32,33]. This small molecule compound interacts with ribosomes and facilitates the recruitment of near-cognate tRNAs thus allowing readthrough of the premature stop codons [34]. Ataluren is now approved for use by the European Medicine Agency. Ribosomes play important roles also in cancer biology, as they facilitate increased demands of proliferating cancer cells for protein synthesis and help them cope with metabolic stress [35]. Moreover, specific "onco-ribosomes" seem to facilitate oncogenic translation [36]. Currently, many potential therapeutic agents and small molecule inhibitors targeting ribosome biogenesis, translation initiation, or specific "onco-ribosomes" are being tested in cancer clinical trials [37,38]. These interventions were shown to activate anti-tumour pathways, affect cancer translation profiles, halt tumour proliferation, restore chemosensitivity, or activate anti-tumour immune responses [37,39]. As demonstrated by our work presented in this thesis (especially **Chapters 2**, **5** and **6**), translation regulation plays a role in OA. In fact, in terms of translation (de)regulation, there are many parallels between OA and cancer. Therefore, OA patients might also greatly benefit from translation- and ribosome-targeting therapy. In OA cartilage, translation is regulated in a phasic fashion. The rate of cap-mediated protein synthesis increases in early OA. However, unfavourable and pathological mRNAs are translated and fuel OA development (**Chapter 2**, [40]). In later stages, the translation rate decreases, preventing cartilage ECM deposition and thus further promoting cartilage degeneration [41]. We could focus on tuning the global translation rate by targeting specific signalling pathways (*e.g.* mTOR) or using inhibitors of cap-mediated translation initiation. The mTORC1 signalling pathway has been previously investigated in OA in

relation to its role in regulating autophagy [42-44]. However, it is also important in regulating translation [45]. Intra-articular injections of rapamycin, an mTOR-specific inhibitor, were shown to reduce the severity of OA in a murine OA model [46]. This function was attributed to the mTOR-dependent activation of autophagy. We might speculate that translation regulation might have been involved in that particular study. These data show that some of the already existing OA-targeting drugs might be linked to translation. Knowledge of their precise functions in chondrocytes will be important when considering case-specific therapeutic strategies in the future. In later stages of OA, patients might benefit from boosted protein synthesis. Previous work from our group showed that intra-articular injections of bioactive BMP7-derived peptides attenuate cartilage degeneration in rodent OA model [47]. These kinds of treatment strategies could be extra powerful if, we would promote the cellular pool of "healthy" ribosomes in chondrocytes in advance, for example by pre-treatments with snoRNA-targeting ASOs. Early OA diagnosis as well as appropriate timing and order of these interventions will be absolutely crucial for their success.

Considering the patient burden related to late-stage OA, such as pain, inactivity and work limitations, social isolation and depression as well as limitations of joint replacement surgery (which is currently the only treatment option for end-stage OA), slowing down OA progression and postponing joint replacement surgery is a major goal to pursue.

In this thesis, we provided evidence for translation regulation and ribosome heterogeneity in articular chondrocytes. However, considering the profound changes in proteomic profiles of other tissues of the joint [48,49], we might expect that similar mechanisms of translation and ribosome regulation take place in all joint tissues. Further research should address this knowledge gap to better understand the translation deregulation in OA joint as a whole. Our data provide the foundation for future translation and ribosome-based research in the OA field. We identified pivotal evidence for ribosomes as drugable targets in the search for DMOADs. Overall this opens up new, exciting opportunities in developing OA treatment strategies.

References

1 Long, H. *et al.* Prevalence Trends of Site-Specific Osteoarthritis From 1990 to 2019: Findings From the Global Burden of Disease Study 2019. *Arthritis Rheumatol* 74, 1172-1183, doi:10.1002/art.42089 (2022).

2 Vos, T. *et al.* Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380, 2163-2196, doi:10.1016/S0140-6736(12)61729-2 (2012).

3 Colombo, G. L., Heiman, F. & Peduto, I. Utilization of Healthcare Resources in Osteoarthritis: A Cost of Illness Analysis Based on Real-World Data in Italy. *Ther Clin Risk Manag* 17, 345-356, doi:10.2147/TCRM.S301005 (2021).

4 Loeser, R. F., Goldring, S. R., Scanzello, C. R. & Goldring, M. B. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* 64, 1697-1707, doi:10.1002/art.34453 (2012).

5 Sandell, L. J. & Aigner, T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res* 3, 107-113, doi:10.1186/ar148 (2001).

6 Blagojevic, M., Jinks, C., Jeffery, A. & Jordan, K. P. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis Cartilage* 18, 24-33, doi:10.1016/j.joca.2009.08.010 (2010).

7 Chen, W. D. & Zhu, X. F. Small nucleolar RNAs (snoRNAs) as potential non-invasive biomarkers for early cancer detection. *Chin J Cancer* 32, 99-101, doi:10.5732/cjc.012.10132 (2013).

8 Liang, J. *et al.* Small Nucleolar RNAs: Insight Into Their Function in Cancer. *Front Oncol* 9, 587, doi:10.3389/fonc.2019.00587 (2019).

9 Castanheira, C. *et al.* Equine synovial fluid small non-coding RNA signatures in early osteoarthritis. *BMC Vet Res* 17, 26, doi:10.1186/s12917-020-02707-7 (2021).

10 Dhuri, K. *et al.* Antisense Oligonucleotides: An Emerging Area in Drug Discovery and Development. *J Clin Med* 9, doi:10.3390/jcm9062004 (2020).

11 Nakamura, A., Ali, S. A. & Kapoor, M. Antisense oligonucleotide-based therapies for the treatment of osteoarthritis: Opportunities and roadblocks. *Bone* 138, 115461, doi:10.1016/j.bone.2020.115461 (2020).

12 Endisha, H. *et al.* MicroRNA-34a-5p Promotes Joint Destruction During Osteoarthritis. *Arthritis Rheumatol* 73, 426-439, doi:10.1002/art.41552 (2021).

13 Nakamura, A. *et al.* microRNA-181a-5p antisense oligonucleotides attenuate osteoarthritis in facet and knee joints. *Ann Rheum Dis* 78, 111-121, doi:10.1136/annrheumdis-2018-213629 (2019).

14 Baek, D. *et al.* Inhibition of miR-449a Promotes Cartilage Regeneration and Prevents Progression of Osteoarthritis in In Vivo Rat Models. *Mol Ther Nucleic Acids* 13, 322-333, doi:10.1016/j.omtn.2018.09.015 (2018).

15 Lian, W. S. *et al.* MicroRNA-128a represses chondrocyte autophagy and exacerbates knee osteoarthritis by disrupting Atg12. *Cell Death Dis* 9, 919, doi:10.1038/s41419-018-0994-y (2018).

16 Yang, H. Y. *et al.* A novel injectable thermoresponsive and cytocompatible gel of poly(N-isopropylacrylamide) with layered double hydroxides facilitates siRNA delivery into chondrocytes in 3D culture. *Acta Biomater* 23, 214-228, doi:10.1016/j.actbio.2015.05.018 (2015).

17 Kim, M. K. *et al.* A Multicenter, Double-Blind, Phase III Clinical Trial to Evaluate the Efficacy and Safety of a Cell and Gene Therapy in Knee Osteoarthritis Patients. *Hum Gene Ther Clin Dev* 29, 48-59, doi:10.1089/humc.2017.249 (2018).

18 Jorjani, H. *et al.* An updated human snoRNAome. *Nucleic Acids Res* 44, 5068-5082, doi:10.1093/nar/gkw386 (2016).

19 Godet, A. C. *et al.* IRES Trans-Acting Factors, Key Actors of the Stress Response. *Int J Mol Sci* 20, doi:10.3390/ijms20040924 (2019).

20 Weingarten-Gabbay, S. *et al.* Comparative genetics. Systematic discovery of cap-independent translation sequences in human and viral genomes. *Science* 351, doi:10.1126/science.aad4939 (2016).

21 Chou, C. H. *et al.* Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci Rep* 10, 10868, doi:10.1038/s41598-020-67730-y (2020).

22 Ji, Q. *et al.* Single-cell RNA-seq analysis reveals the progression of human osteoarthritis. *Ann Rheum Dis* 78, 100-110, doi:10.1136/annrheumdis-2017-212863 (2019).

23 Aldridge, S. & Teichmann, S. A. Single cell transcriptomics comes of age. *Nat Commun* 11, 4307, doi:10.1038/s41467-020-18158-5 (2020).

24 Marx, V. Method of the Year: spatially resolved transcriptomics. *Nat Methods* 18, 9-14, doi:10.1038/s41592-020-01033-y (2021).

25 Doerr, A. Single-cell proteomics. *Nat Methods* 16, 20, doi:10.1038/s41592-018-0273-y (2019).

26 Kelly, R. T. Single-cell Proteomics: Progress and Prospects. *Mol Cell Proteomics* 19, 1739-1748, doi:10.1074/mcp.R120.002234 (2020).

27 Vistain, L. F. & Tay, S. Single-Cell Proteomics. *Trends Biochem Sci* 46, 661-672, doi:10.1016/j.tibs.2021.01.013 (2021).

28 Mund, A., Brunner, A. D. & Mann, M. Unbiased spatial proteomics with single-cell resolution in tissues. *Mol Cell* 82, 2335-2349, doi:10.1016/j.molcel.2022.05.022 (2022).

29 Lundberg, E. & Borner, G. H. H. Spatial proteomics: a powerful discovery tool for cell biology. *Nat Rev Mol Cell Biol* 20, 285-302, doi:10.1038/s41580-018-0094-y (2019).

30 Balmayor, E. R. Synthetic mRNA - emerging new class of drug for tissue regeneration. *Curr Opin Biotechnol* 74, 8-14, doi:10.1016/j.copbio.2021.10.015 (2022).

31 Temaj, G. *et al.* Ribosome-Directed Therapies in Cancer. *Biomedicines* 10, doi:10.3390/biomedicines10092088 (2022).

32 Haas, M. *et al.* European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscul Disord* 25, 5-13, doi:10.1016/j.nmd.2014.11.011 (2015).

33 Michael, E., Sofou, K., Wahlgren, L., Kroksmark, A. K. & Tulinius, M. Long term treatment with ataluren-the Swedish experience. *BMC Musculoskelet Disord* 22, 837, doi:10.1186/s12891-021-04700-z (2021).

34 Siddiqui, N. & Sonenberg, N. Proposing a mechanism of action for ataluren. *Proc Natl Acad Sci U S A* 113, 12353-12355, doi:10.1073/pnas.1615548113 (2016).

35 Pelletier, J., Thomas, G. & Volarevic, S. Ribosome biogenesis in cancer: new players and therapeutic avenues. *Nat Rev Cancer* 18, 51-63, doi:10.1038/nrc.2017.104 (2018).

36 Kampen, K. R., Sulima, S. O. & De Keersmaecker, K. Rise of the specialized onco-ribosomes. *Oncotarget* 9, 35205-35206, doi:10.18632/oncotarget.26231 (2018).

37 Jiang, S. L. *et al.* Targeting translation regulators improves cancer therapy. *Genomics* 113, 1247-1256, doi:10.1016/j.ygeno.2020.11.011 (2021).

38 Gilles, A. *et al.* Targeting the Human 80S Ribosome in Cancer: From Structure to Function and Drug Design for Innovative Adjuvant Therapeutic Strategies. *Cells* 9, doi:10.3390/cells9030629 (2020).

39 Pellegrino, S. *et al.* The Amaryllidaceae Alkaloid Haemanthamine Binds the Eukaryotic Ribosome to Repress Cancer Cell Growth. *Structure* 26, 416-425 e414, doi:10.1016/j.str.2018.01.009 (2018).

40 Katsara, O. & Kolupaeva, V. mTOR-mediated inactivation of 4E-BP1, an inhibitor of translation, precedes cartilage degeneration in rat osteoarthritic knees. *J Orthop Res* 36, 2728-2735, doi:10.1002/jor.24049 (2018).

41 Ripmeester, E. G. J. *et al.* Impaired chondrocyte U3 snoRNA expression in osteoarthritis impacts the chondrocyte protein translation apparatus. *Sci Rep* 10, 13426, doi:10.1038/s41598-020-70453-9 (2020).

42 Carames, B. *et al.* Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. *Ann Rheum Dis* 71, 575-581, doi:10.1136/annrheumdis-2011-200557 (2012).

43 Pal, B., Endisha, H., Zhang, Y. & Kapoor, M. mTOR: a potential therapeutic target in osteoarthritis? *Drugs R D* 15, 27-36, doi:10.1007/s40268-015-0082-z (2015).

44 Zhang, Y. *et al.* Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. *Ann Rheum Dis* 74, 1432-1440, doi:10.1136/annrheumdis-2013-204599 (2015).

45 Ma, X. M. & Blenis, J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* 10, 307-318, doi:10.1038/nrm2672 (2009).

46 Takayama, K. *et al.* Local intra-articular injection of rapamycin delays articular cartilage degeneration in a murine model of osteoarthritis. *Arthritis Res Ther* 16, 482, doi:10.1186/s13075-014-0482-4 (2014).

47 Caron, M. M. J. *et al.* Discovery of bone morphogenetic protein 7-derived peptide sequences that attenuate the human osteoarthritic chondrocyte phenotype. *Mol Ther Methods Clin Dev* 21, 247-261, doi:10.1016/j.omtm.2021.03.009 (2021).

48 Belluzzi, E. *et al.* Infrapatellar Fat Pad Gene Expression and Protein Production in Patients with and without Osteoarthritis. *Int J Mol Sci* 21, doi:10.3390/ijms21176016 (2020).

49 Ren, X. *et al.* Quantitative Proteomic Analysis of Synovial Tissue Reveals That Upregulated OLFM4 Aggravates Inflammation in Rheumatoid Arthritis. *J Proteome Res* 20, 4746-4757, doi:10.1021/acs.jproteome.1c00399 (2021).