

BMP7 as a multifactorial growth factor for cartilage homeostasis

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Impact Summary

IMPACT SUMMARY

Cartilage is a highly specialized connective tissue, best known in the form of articular cartilage¹. One of the most common chronic joint conditions compromising the articular cartilage is osteoarthritis (OA). OA development is highly associated with increasing age^{2,3} and is accompanied by a high economic and societal burden⁴⁻⁶. Currently, treatment options for OA patients are mainly focused on alleviating the associated symptoms, and in most cases (total) joint arthroplasty⁷ is the only long-term treatment option. On the cellular level, OA is characterized by the presence of different chondrocyte phenotypes in the articular cartilage, each contributing to OA pathophysiology⁸. With the current knowledge regarding molecular pathways that play a role in OA disease progression (described in *Chapter 2*), many attempts have been undertaken to develop disease-modifying drugs targeting the disrupted joint-tissue homeostasis in OA⁹⁻¹². Until today this has not led to any clinically implemented therapy¹³. Hence, continuing to unraveling and exploit molecular pathways driving these pathophysiological changes, as described in this thesis, is of great importance to enable the development of novel disease-modifying drugs targeting the pathological chondrocyte phenotypes in OA articular cartilage.

Although the chondrocyte hypertrophic- as well as the fibrocartilage chondrocyte phenotype are the most predominant chondrocyte phenotype subpopulations in late-stage OA cartilage⁸, most current studies focus primarily on targeting the hypertrophic chondrocyte phenotype in OA. However, the work described in *Chapter 7* demonstrated for the first time that BMP7, similar to its actions in organ fibrosis¹⁴, was able to attenuate the fibrocartilage chondrocyte phenotype and COL1A1 protein levels by increasing MMP2 activity. An important upstream regulator of (organ) fibrosis is SMAD3^{15,16}, which may be a potential target for reducing the fibrocartilage chondrocyte phenotype. In *Chapter 7* we have demonstrated that the BMP7-dependent reduction of the fibrocartilage chondrocyte phenotype is also associated with reduced SMAD3 activity. Interestingly, SMAD3-signaling is normally regarded as protective against OA-related changes in articular cartilage, as it inhibits the development of the chondrocyte hypertrophic phenotype¹⁷. Hence, the exact working mechanism by which the BMP7-dependent reduction of SMAD3 activity reduces the chondrocyte fibrocartilage phenotype in OA still needs to be elucidated. The ability to target the fibrocartilage chondrocyte phenotype may be of important clinical relevance as this chondrocyte phenotype expresses a high ratio of genes related to a negative OA disease outcome⁸. This additional target for BMP7 also broadens the treatment scope for joint pathologies in which BMP7 is a potential treatment option, for example, non-OA related arthrofibrosis¹⁸.

While one could envision the use of full-length BMP7 in the treatment of OA, the advancement of its use in the clinic might have been hampered due to drawbacks associated with the use of full-length recombinant BMP7. Challenges encountered in the use of full-length BMP7 are the harsh proteolytic environment in the synovial fluid of OA joints, potentially causing rapid and uncontrollable degradation of full-length BMP7¹⁹. This demands frequent intra-articular injections to maintain therapeutically relevant intra-articular concentrations of BMP7^{20,21}. However, frequent intra-articular injections cause discomfort and risk important adverse effects in patients²², such as septic arthritis²³. To potentially avoid these, we identified peptides (p[63-82] and p[113-132]) harbored within the full-length BMP7 amino acid sequence with OA

disease-modifying properties that are similar to full-length recombinant BMP7 (*Chapter 5*). These peptides reduced the unfavorable gene expression associated with the hypertrophic chondrocyte phenotype and attenuated *in vivo* cartilage degeneration in models for post-traumatic OA (*Chapter 5 and 6*). Intra-articular administration of these peptides may be exploited to fill the current treatment gap for OA patients, by preventing, or at least delaying, the necessity of (total) joint arthroplasty. Particularly the patient group <60 years of age (where a prosthesis has a limited survival²⁴) may benefit from this. In addition, intra-articular use of peptide p[63-82] in rats demonstrated improvement in static weight-bearing, which is likely attributable to a reduction of pain sensation (*Chapter 6*). Pain is an important hallmark of osteoarthritis^{2,6} and an important criterion to be eligible for (total) joint arthroplasty²⁵. However, efficient OA pain-management is currently unavailable^{6,26}. Hence, the effect of our identified peptides on knee joint pain related behavior may be an attractive clinically relevant feature. Furthermore, considering the chondrocyte hypertrophy as well as the fibrocartilage chondrocyte suppressive action of full-length BMP7, we hypothesize that our identified BMP7 peptides also may have the potential of a double mode-of-action, targeting both the hypertrophic, as well as the fibrocartilage chondrocyte phenotype in OA. However, a fibrocartilage phenotype-attenuating action still needs to be confirmed and would further progress the potential clinical use of BMP7-mimicking peptides as OA disease-modifying molecules. Currently, our research only focused on osteoarthritis of the knee joint. However, our BMP7 peptides could potentially also be used in osteoarthritis of other synovial joints, such as the facet joints in the spine, which undergo similar pathological changes as OA of the knee joint²⁷. In addition to OA, another potential application for our BMP7 peptides would comprise the field of cartilage regenerative medicine, where current attempts in the treatment of focal cartilage defects are generally being hampered by the development of the inferior hypertrophic cartilage or fibrocartilage²⁸. Our BMP7 peptides might be used to improve the outcome of articular cartilage repair by regenerative medicine approaches, as has previously been demonstrated for micro fracturing combined with full-length BMP7²⁹. Currently, our pre-clinical experimental OA models still made use of once- or twice-weekly intra-articular injections with BMP7 peptides. For potential clinical use this frequency needs to be reduced²², and future work that aims to increase the long-term intra-articular bioactivity of the peptides should enable this. Formulating these peptides with macromolecular drug delivery systems has important benefits over formulating full-length recombinant BMP7. The development of a local drug delivery system for these peptides could thus lower the frequency of intra-articular injections³⁰.

An important part of the cartilage ECM consists of proteins^{1,31,32} which are translated from specific mRNAs by the ribosome^{33,34}. In *Chapter 4* we demonstrated that ribosome biogenesis and protein translational capacity is affected in chondrocytes as a result of OA. This opens up an exciting new research area within the field of osteoarthritis and potentially provides a plethora of novel molecular targets that may aid in the development of new OA disease-modifying drugs. One such target could be the reduced U3 snoRNA expression levels in OA cartilage, which we demonstrated to be associated with reduced ribosomal RNA (rRNA) expression and reduced chondrocyte protein translation capacity, associated with aberrant chondrocyte homeostasis (*Chapter 4*). Approaches that allow the restoration of cellular levels of U3 snoRNA up to levels associated with healthy articular chondrocytes may be able to counteract reduced rRNA levels and the detrimental impact of reduced protein translational

capacity on the OA chondrocyte pathological phenotype. In *Chapter 4* we demonstrated that U3 snoRNA expression is influenced by external cues, such as BMP7. Here, BMP7 was able to enhance U3 snoRNA expression levels as well as rRNA levels in chondrocytes. Furthermore, in *Chapter 3* we discovered that BMP7 induced ribosomal RNA levels by stimulating transcription from the rDNA promoter in an NKX3-2-dependent manner. Interestingly, NKX3-2 is a key molecular switch of the hypertrophic chondrocyte phenotype in OA chondrocytes by suppressing the expression of RUNX2. Besides a major role in driving chondrocyte hypertrophy, RUNX2 is also known to suppress rDNA transcription. This provides a clue to the potential molecular mechanism of how BMP7 can stimulate chondrocyte rDNA transcription. This means that BMP7 is able to change chondrocyte rRNA levels and protein translation in at least two manners (U3 snoRNA and rDNA transcription), uncovering potential new molecular targets to improve chondrocyte homeostasis in OA.

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