Small nucleolar RNAs in chondorgenic differentiation and osteoarthritis

Valorization

snoRNA biomarkers in osteoarthritis and ageing

Cartilage is an important structural component of the body; essential during walking, sports and everyday life functioning. Due to the lack of vascularization, cartilage is largely hampered in its reparative capacity[1]. This has major implications for cartilage diseases such as osteoarthritis (OA), the most prevalent age-related degenerative joint disease[2,3]. OA affects over 300 million people globally[4] and is a common cause of chronic disability worldwide[2]. In addition it is a significant contributor to both individual and socioeconomic burden and the number of disability adapted life years globally[5]. If the deterioration in musculoskeletal health and development of OA can be identified and treated early, serious life impairment may be abrogated. The development of effective treatments for OA and the ability to predict disease progression has been hampered by the lack of biomarkers able to demonstrate pathological disturbances preceding identifiable tissue alterations. **Chapter 5** of this thesis has contributed to expanding this knowledge by the identification of snoRNA-based biomarkers that are indicative for ageing and/or for OA. For example, SNORA73 was increased in old joint and serum and thus represents a potential joint 'biological ageing' marker[6]. SNORA64 was increased and SNORD46 was decreased in serum in a mouse OA model, where OA was established by destabilization of the medial meniscus (DMM), but both were not differentially expressed in young versus old serum, highlighting these snoRNAs as possible OA markers[6]. SNORD18 was increased in serum both in ageing and following DMM, suggesting that it is affected in both ageing and OA. The most differentially expressed snoRNA in mouse DMM serum was SNORD116[6]. This increase was confirmed in serum of horses with metacarpophalangeal (MCP; joint with similarities to the human knee joint[7]) OA. Additionally, SNORD116 has previously been identified as increased in OA compared to normal human cartilage in a micro-array study[8], indicating that SNORD116 function might be conserved between species. Ideally, biomarkers should be assessable via low-invasive methods and serum is thus a good source for biomarker measurement. The snoRNA biomarkers identified in our work represent a class of bio-molecules for which quantitative assays can be set-up with relative ease. Following our findings it would thus be a possibility that snoRNAs could add to a diagnostic set of serum-based bio-molecules

indicative for biological age and OA status of the patient. Given the fact that we used a mouse model, effort should be put in the validation of our findings for the human situation, as well as the set-up of large cohort studies, where OA is well-defined and a relation between the disease stage and the specific snoRNA in the serum may be deduced. In addition, the snoRNAs' specificity should be investigated further to determine whether the situation in the joint is represented in the serum and for example how to extrapolate the results in case of co-morbidities.

snoRNAs in chondrogenic development and Cartilage Hair Hypoplasia

Mutations in the *RMRP* gene are the cause of a severe form of dwarfism known as cartilage-hair hypoplasia[9] (CHH, McKusick-type metaphyseal chondrodysplasia[10]), which is part of the anauxetic dysplasia spectrum of disorders[11,12]. To date, more than hundred individual CHH-pathogenic mutations have been identified in the *RMRP* gene[9]. Although relatively rare in the general population, the disease prevalence is exceptionally high among the Amish and Finnish populations[13]. Moreover, the disease consequences for those identified with the disease can be considered severe; one predominant phenotypic hallmark of CHH is short-limbed dwarfism caused by abnormal growth plate development. Other symptoms include sparse thin hair, anaemia, Hirschsprung's disease, bronchiectasis, and impaired T-cell immunity. In addition, adult patients have a predisposition to certain cancers (i.e. squamous cell carcinoma, basal cell carcinoma and non-Hodgkin lymphoma)[12,14]. The data and insight generated through investigation of this human disease model may provide further insight in the role of *RMRP* RNA, RNase MRP functions and rRNA processing in chondrocyte cell development and function (**Chapter 2**). Our data showed that *RMRP* RNA expression is regulated during different stages of chondrogenic differentiation and indicate that *RMRP* RNA plays a pivotal role in chondrocyte hypertrophy, with consequences for CHH pathobiology[15]. Interestingly, the gene expression alterations observed in OA cartilage also indicate a replay of chondrogenic differentiation towards chondrocyte hypertrophy[16]. This holds promise that by investigating this rare disease model we could additionally generate findings that contribute to the understanding of the complex etiology of a highly prevalent disease such as OA. However, for CHH patients, a multi-causal approach will most-probably be required when selecting novel therapeutic targets. Considering the many functions *RMRP*

RNA and the RNase MRP complex are involved in, a one-fit-for-all therapy should not be expected, but rather a personalized therapy based on the molecular effects of the diverse RMRP mutations and the overall disease phenotype. Treatment of CHH with growth hormone to support skeletal growth has been reported[17], but efficacy and safety are under debate. Recently CRISPR genome editing is being put forward for treatment of cystic fibrosis[18]. Considering the isolated genomic nature of RMRP mutations leading to CHH, this disease might represent a good candidate for CRISPR genome editing, and recent advancements in CRISPR genome editing may shed light on the future treatment options for CHH. However, the tissue delivery of such genome editing tools are expected to be challenging in CHH and in particular in the case of targeting developing cartilage. In relation to this notion it will therefore be important to additionally investigate the deregulated molecular networks downstream of RMRP in tissues with a high CHH-burden. This will potentially allow counteracting of the pathological molecular aberrations caused by mutations in RMRP in a downstream (symptomatic) manner. Our finding that viperin regulates a CXCL10–TGF-β/SMAD2/3 axis during chondrogenic differentiation, which is deregulated in CHH due to abnormal expression of viperin (**Chapter 3**)[19], might provide a starting point for such an approach. For example by targeting factors downstream of viperin, such as CXCL10 and investigating the possibility of an interferon-related therapy. In **Chapter 4**, snoRNAs were found to be differentially expressed during ATDC5 chondrogenic differentiation, with impact on the translational capacity of the cell. Even though here we have not yet identified isolated snoRNAs and their specific function, they appear to be vital for the overall outcome of chondrogenesis. Understanding and influencing chondrogenesis will be key for cartilage regenerative therapies (e.g. cartilage tissue engineering using MSCs) in the context of, for example, OA. Our data indicate that snoRNAs might be good candidates to target and influence the course of chondrogenesis. In this respect antisense oligonucleotides (ASOs) may proof a valuable means in targeting these snoRNAs in the future[20]. A wide range of modifications to ASOs have already been investigated to improve their stability, influence their mechanism of action or steer their delivery[20]. ASOs have been investigated in preclinical studies such as *in vitro* and small animal *in vivo* studies and several formulations were already regulatory (FDA / EMA) approved for use in the clinic[20-23]. Our group has recently been successfully using second generation ASOs for targeting snoRNA expression levels in chondrocytes[24,25]. These ASOs are designed in a 5-10-5 gapmer

configuration and have a phosphorotioate backbone, containing a core of 10 DNA nucleotides, flanked on both sides by modified (2'O-ribose methyl) RNA nucleotides. These ASOs are RNase H1 dependent and effective in targeting snoRNAs[26]. Therefore, using ASOs to target snoRNAs in the context of chondrogenic development would be a promising perspective for future OA therapies as well. However, before we can enter this stage, functional studies will be needed to investigate the function of specific snoRNAs in the context of chondrogenesis to select promising snoRNA targets.

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