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Osteoprotegerin: a double-edged sword in osteoarthritis development

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ADDENDUM

English Summary
Nederlandse Samenvatting
List of Publications
Curriculum Vitae
Acknowledgements

English summary

Osteoarthritis (OA) is a degenerative disease of the joints characterized by degradation of cartilage, subchondral bone remodeling, osteophyte formation and synovial inflammation. Clinically OA is marked by chronic pain, joint stiffness and disability of patients. OA generally affects hands, hips and knees and can be influenced by multiple risk factors such as body mass index (BMI), bone mineral density (BMD), injury and genetics. Currently in the Netherlands there are approximately 1.2 million people affected by OA and this number is only expected to increase. As such, the world health organization expects that by 2050, 15% of the worldwide population over 60 years old will suffer from OA. As of yet, joint replacement is the only effective therapy, however this is a very costly procedure that does not guarantee complete recovery. As a result, it is estimated that 1-2.5% of gross national products is spent in OA related treatments.

To advance development of effective disease modifying OA treatments, a better understanding of its pathophysiological mechanisms is necessary. By studying a family with early onset OA and high cartilage mineralization, a likely causal mutation in the TNF receptor superfamily member 11b (*TNFRSF11B*) encoding for osteoprotegerin (OPG) was identified. This mutation causes a 19 amino acid extension in the C-terminal domain of OPG (OPG-XL). OPG is a decoy receptor that competes with receptor activator of the nuclear KB factor (RANK) for the binding of nuclear factor KB ligand (RANKL). This triad is known for regulating the formation of osteoclasts, hence playing a critical role in bone remodeling. Given that *TNFRSF11B* is also one of the highest upregulated genes in OA lesioned cartilage as compared to preserved, this gene is likely underlying OA development and progression but its implication in cartilage homeostasis is as of yet unknown.

To explore the role of *TNFRSF11B* in development of OA, in **Chapter 2**, *TNFRSF11B* was overexpressed in an *in vitro* cell culture model of neo-cartilage deposited by chondrocytes of nine patients that underwent joint replacement surgery due to OA. Subsequently, characteristic markers of OA development and bone formation were measured. This revealed an upregulation of genes commonly associated with OA development and matrix turnover, such as *MMP13*, *COL2A1* and *COL1A1*. Additionally, the consistent higher expression of genes associated with osteoblast formation and mineralization such as *RUNX2*, *ASPN* and *OGN*, suggested a chondrocyte to osteoblast transition. Of note, neo-cartilage showed no changes in expression of the other two members of the: *TNFSF11* (RANKL) and *TNFRSF11A* (RANK), indicating a difference in the OPG mechanism in cartilage when compared to its bone counterpart.

To obtain more information about the intrinsic mechanism of OPG in OA development, we proceeded to further study the OPG-XL family. For this, we used induced pluripotent stem cells (iPSCs). iPSCs are embryonic stem cell-like cells able to differentiate into different tissues, while maintaining the genetic background of the donor. This makes them an excellent approach for disease modeling and regenerative therapies. In this thesis, we applied iPSC technology to generate a stem cell line from skin fibroblasts of a carrier of the OPG-XL mutation. Since access to cartilage and bone tissue of members of this family is rare, availability of the iPSCs allowed us to have a renewable source of neo-cartilage and neo-osseous tissue carrying this mutation for further studies.

Several protocols to generate neo-cartilage from human iPSCs were available. These protocols followed different developmental routes either by mesenchymal stromal cell (hiMSC) formation or by generating chondroprogenitor cells (hiCPC). Yet, the quality of the generated neo-cartilage was unknown. As such, to determine the best method for neo-cartilage generation for our OPG-XL model, in **Chapter 3** we compared the neo-cartilage generated from both protocols to neo-cartilage deposited by respectively bone marrow mesenchymal stromal cells (hBMSCs) and human primary articular chondrocytes (hPACs). Based on a panel of 20 relevant genes, we showed a 53% similarity between hiMSCs and hBMSCs, and a 65% similarity between hiCPC and hPACs neo-cartilage. In addition, hiCPCs neo-cartilage showed a higher expression of markers associated with articular cartilage matrix deposition, while hiMSCs neo-cartilage was more prone to hypertrophy. With hypertrophy as a hallmark of OA, this would make hiMSCs more suited to study cartilage and bone related diseases.

By following the hiMSC approach, we differentiated the hiPSC-OPG-XL line into neo-cartilage and neo-bone and characterized the effects of the mutation in **Chapter 4**. To prove causality, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) technology was used to repair the OPG-XL mutation. This technology recognizes and cleavages specific strands of DNA complementary to the CRISPR sequence, generating an isogenic control to OPG-XL. This resulted in two CRISPR/Cas9 OPG-XL repaired hiPSC lines where neo-cartilage and neo-bone tissue were generated. Comparison between the OPG-XL repaired and OPG-XL tissues revealed that this mutation had a fibrotic effect in neo-cartilage, while neo-bone tissue had a stronger mineralization, likely via function of MGP and DIO2 respectively.

Immobilization of secreted OPG on the osteoblast membrane via binding to heparan sulphate is necessary for RANKL mediated osteoclast inhibition. Since immobilization results from the C-terminal binding of OPG to heparan sulphate and the OPG-XL mutant is changed at the C-terminus, we hypothesize that the effects on MGP and DIO2 might be triggered by interference with binding

between RANKL, heparan sulphate and OPG. Additionally, osteoclast formation was studied with monocytes from OPG-XL carriers and matched healthy controls. This revealed a higher osteoclast activity as measured by gene expression, despite similar bone resorption levels were observed in culture. To determine how these results were translated into the OPG-XL carriers phenotype, we performed MRI and DEXA scans. This revealed a pleiotropy of OPG-XL with cartilage calcification accompanied by low subchondral bone mineralization, both hallmarks of OA pathophysiology, and in consonance with the observed higher osteoclast activity.

Altogether, this thesis highlights the role of OPG in OA development by generating an OPG overexpression system in primary chondrocytes and by studying a rare mutation in *TNFRSF11B*. By further generating neo-cartilage, neo-bone and osteoclasts from the OPG-XL family members, we showed a bidirectional interplay of OPG-XL characterized by higher bone resorption and higher cartilage mineralization. Novel treatments for this family and extrapolation to common OA could be addressed on highly differentially expressed genes such as *MGP* and *DIO2*. Finally, the pleiotropy that OPG-XL showed indicates a beneficial or detrimental stage depending on the tissue, making OPG-XL, and likely OPG, a double-edged sword in OA development.