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Report: Osteoporosis in mucopolidosis II: a potential corrective approach

Funding period (March 2017-February 2018)

Significance and aim

Major symptoms of MLII are severe skeletal defects summarized as *Dysostosis multiplex* resembled in an MLII knock-in mouse model generated in our laboratory. The progressive bone loss and osteoporotic phenotype in MLII is a consequence of an increased number of osteoclasts most likely induced by osteoblast-derived interleukin-6 (IL-6). Soluble gp130 (sgp130) functions as natural inhibitor of the IL-6 signaling pathway. Therefore sgp130-mediated IL-6 blocking represents a potential treatment in MLII and most likely in MLIII that aims to prevent the bone loss and skeletal deformities in these diseases.

Progress in the funding period

1. *Ex-vivo* analysis of MLII osteoblasts

Bone marrow cells were isolated from tibias and femora of 15 weeks old wild-type (WT) and MLII mice and cultured for 5 days. For osteoblast differentiation, ascorbic acid and β -glycerophosphate was added to bone marrow cells and cultured for 25 days. Total RNA were isolated from WT and MLII osteoblasts cultures and analysed by quantitative real-time PCR. MLII osteoblasts displayed reduced expression of osteoblast differentiation markers, such as alkaline phosphatase, osteocalcin and collagen. In contrast, the expression of IL-6 mRNA was 10-fold increased in MLII osteoblasts compared to WT cells. These data confirm our previous results and show that the impaired bone formation in MLII is caused by dysfunctional osteoblasts, which highly produce the osteoclastogenic cytokine IL-6.

2. Generation of MLII mice overexpressing the IL-6 inhibitor sgp130

For sgp130-mediated IL-6 blocking, we have crossed heterozygous MLII mice with transgenic mice overexpressing human sgp130 that were kindly provided by Dr. Stefan Rose-John (University of Kiel, Germany). For genotyping of the offspring from eight breeding pairs, genomic DNAs from tail biopsies of a total of 400 mouse pups were isolated. The subsequent PCR analysis was performed using gene-specific primers to amplify genomic fragments of wild-type (WT) or mutant murine *Gnptab* and human *sgp130*.

The high number of breeding pairs was required to obtain at least 10 animals of each genotype (WT, WT+sgp130, MLII, MLII+sgp130), which are necessary for the further experiments. The expression of human sgp130 was determined in the serum of all 40 mice at the age of 6 weeks by a specific enzyme linked immunosorbent assay (ELISA). As expected, in WT and MLII mice no human sgp130 was detectable. In WT+sgp130 and MLII+sgp130 concentrations between 19 and 22 µg/ml sgp130 was found confirming the results obtained by sgp130-specific PCR of genomic DNA. Analysis by cytokine multiplex ELISA revealed an IL-6 level in the range of 5-10 pg/ml in mouse serum. Since overexpressed sgp130 is in molar excess over IL-6, the increased secretion of IL-6 by MLII osteoblasts will be neutralized by circulating sgp130. These molecular and biochemical analyses demonstrate that the generation of sufficient MLII+sgp130 mice and respective control mice was successful.

3. Skeletal analysis of MLII+sgp130 mice

The bone analysis of 15 weeks old mice, i.e. WT, WT+sgp130, MLII and MLII+sgp130, will be started in May 2018 and performed in close collaboration with Dr. Thorsten Schinke (Institute for Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Germany). In particular, we will systematically analyse the skeleton of the animals, which includes contact radiography, cross-sectional microcomputed tomography, and dynamic histomorphometric quantification of the bone formation rate. Additionally, non-calcified histology and histomorphometry will be applied to quantify trabecular and cortical bone parameters as well as numbers of osteoclasts and osteoblasts. In addition, we will perform *in-situ* immunohistological IL-6 staining and electron microscopy to analyse bone marrow cells and growth plate chondrocytes of the tibia of transgenic and WT mice.

We expect an improvement of various bone-morphometric parameters in transgenic MLII+sgp130 mice in comparison with MLII mice.