Name and surname of the author: Mateusz Sikora

Thesis title: The role and importance of small non-coding RNAs in the regeneration of bone tissue with signs of senile osteoporosis on the example of miRNA-21-5p

Field of study: Natural Sciences

Discipline: Biological Sciences

Date of the abstract: 01.09.2023

Key words: osteoporosis, bone tissue, regenerative medicine, bone fractures, BMSCs, miRNA, miR-21

Senile osteoporosis is the most common bone disease in elderly patients. The incidence of senile osteoporosis is increasing dramatically due to the progressive aging of population. What's more, experts estimate that within next 50 years osteoporosis will reach the scale of a global epidemic, while the present therapeutic methods are insufficient. Its development is associated with the imbalance between the activity of osteoblasts and osteoclasts, as well as with the deterioration of the regenerative potential of bone marrow stem cells (BMSCs). For this reason, new therapeutic methods are investigated that will reconstruct homeostasis within the bone tissue and restore the regenerative potential of the patient's cells. An example of such therapies are strategies based on the use of small non-coding RNAs (miRNAs) - both as therapeutic tools and targets. Recently, miR-21-5p has been shown to play an important role in bone remodelling, but its molecular mechanisms that modulate the activity of osteoblasts and osteoclasts, as well as maintain high activity of cells with senile phenotype have not been yet determined.

Therefore, the aim of this doctoral thesis was to investigate the role of the miR-21-5p in the regulation of the activity of senile bone tissue cells for designing targeted therapies aimed at restoring the regenerative potential of patients suffering from senile osteoporosis.

The research was carried out using the MC3T3-E1 and 4B12 cell lines, as well as BMSCs isolated from healthy BALB/c mice and a unique model of osteoporotic SAM/P6 mice strain. The studies included *in vitro*, *ex vivo* and *in vivo* analyses. During the study, the involvement of the miR-21-5p molecule in the proliferative, metabolic and regenerative activity of cells was assessed using molecular biology techniques. The crucial part of the research was to identify molecular pathways that regulate the bone-forming potential of cells. Particular attention was

paid to the RANKL/OPG/RANK and RUNX-2/TRAP pathways. mRNA and miRNA expression were analysed by RT-qPCR and protein accumulation was assessed by Western Blot. Metabolic activity was determined using techniques based on flow cytometry, and cell morphology and ultrastructure were assessed using confocal microscopy, scanning electron microscopy (SEM) and immunocytochemistry (ICC) techniques.

The results indicate a significant impact of the miR-21-5p in the regulation of the activity of osteoblasts and osteoclasts. The miR-21-5p was actively involved in the regulation of paracrine activity of osteoblasts and in the maturation of osteoclasts via the OPG/RANKL/RANK pathway. Simultaneously, it was confirmed that BMSCs isolated from osteoporotic SAM/P6 mice were characterized by impaired regenerative potential and deteriorated metabolic activity. It has been proven that miR-21-5p reconstructs the lost regenerative potential of senile BMSCs. After the miR-21-5p upregulation, cells were characterized by a restored ability to form a highly mineralized extracellular matrix under *ex vivo* and *in vivo* conditions, as well as by significant changes in the metabolic activity and dynamics of the mitochondrial network. Upregulated expression of miR-21-5p in BMSCs correlated with enhanced expression of molecules responsible for osteogenesis (RUNX, OPG, miR-7a-5p), and decreased level of transcripts associated with bone resorption (TRAP, CTSK, miR-17-5p).

The presented research showed that miR-21-5p possesses a high therapeutic potential and can be used in the design of effective targeted therapies aimed at treating senile osteoporosis and related bone fractures.