

# MicroRNAs 125b, 199a-5p and 214 as modulators of bone homeostasis

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**Disclosures:** Virginie Joris (N), Elizabeth Rosado Balmayor (8- Section Editor EJMR, Associate Editor Frontiers, and Speciality Editor Bone & Joint Research and 9- treasurer executive board of TERMIS EU, Chair of DEI E-ORS board), Martijn van Griensven (7B-Springer Nature for editor in Chief Eur J Med Res, 8- Editor in Chief: Eur J Med Res, Field chief Editor: Frontiers in Regenerative Medicine, Section editor: Eur J Trauma Emer Surg, Associate Editor: PlosOne, Frontiers in Bioengineering and Biotechnology, specialty section Tissue Engineering and Regenerative Medicine, Editorial board: Biomaterials and Biosystems, European Cells and Materials, International Review Panel, Innovative Surgical Sciences, Journal of Materials Science: Materials in Medicine, Journal of 3D Printing in Medicine, European Surgical Research, Journal of Orthopaedic Translation, Archives of Orthopaedic and Trauma Surgery, 9- president elect of the Research Section of the German Society for Orthopaedic and Trauma Surgery, council member TERMIS-EU (until 2022))

**INTRODUCTION:** Bone homeostasis is a highly regulated process involving pathways in bone as WNT, FGF or BMP, but also requiring support from surrounding tissues as vessels and nerves. In bone diseases, the bone-vessel-nerve triad is impacted. Recently, new players appeared as regulators of bone homeostasis: microRNAs (miRNA). Five miRNAs associated with osteoporotic fractures are already known [1, 2], among which miR-125b is decreasing bone formation by downregulating human mesenchymal stem cells (hMSCs) differentiation. Other miRNAs, as miR-214 (in cluster with miR-199a), are secreted by osteoclasts to regulate osteoblasts and inhibit bone formation. This forms a very complex regulatory network.

**METHODS:** hMSC and osteoblasts were obtained during operations on fractures of long bones. The local ethical committee approved the study. Patients gave informed consent before obtaining the samples. Bone marrow was collected and hMSC were isolated using a standard protocol. Spongy bone was collected and osteoblasts were isolated using an outgrowth technique. Cells in the third passage were used. hMSCs and osteoblasts (n=3) were transfected with mimic/antagomiR of miR-125b, miR-199a-5p or miR-214, or with a scrambled miRNA (negative control) in osteogenic differentiation calcium-enriched medium (Ca<sup>++</sup>). Mineralization was assessed by Alizarin Red/CPC staining, miRNA expression by qPCR and protein by western blotting.

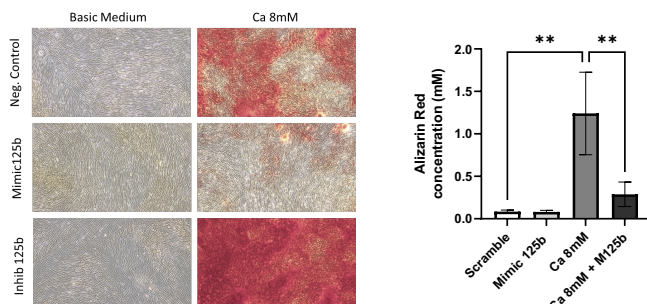
**RESULTS SECTION:** Exposure of hMSCs or osteoblasts to Ca<sup>++</sup> increased mineralization compared to basal medium. hMSCs transfected with miR-125b mimic in Ca<sup>++</sup> presented less mineralization compared to scramble (figure 1). This correlated with decreased levels of BMPR2 and RUNX2 (figure 2). hMSCs transfected with miR-125b inhibitor presented higher mineralization. Interestingly, hMSCs transfected with miR-214 mimic in Ca<sup>++</sup> presented no mineralization while miR-214 inhibitor increased mineralization. No differences were observed in hMSCs transfected with miR-199a-5p modulators. On the contrary, osteoblasts transfected with miR-199a-5p mimic present less mineralization than scrambled-transfected and same was observed for miR-214 and miR-125b mimics.

**DISCUSSION:** We highlight that miR-125b and miR-214 decrease mineralization of hMSCs in calcium-enriched medium. We noticed that miR-199a-5p is able to regulate mineralization in osteoblasts but not in hMSCs suggesting that this effect is cell-specific. Interestingly, the cluster miR-199a/214 is known as modulator of vascular function and could thus contribute to bone remodeling via different ways. With this work we slightly open the door to possible therapeutic approaches for bone diseases.

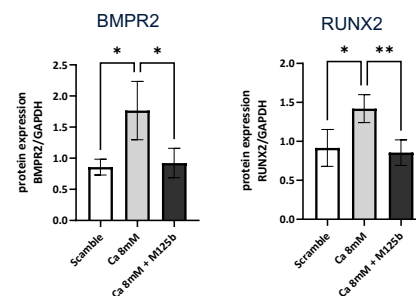
**SIGNIFICANCE:** MicroRNAs regulate bone regeneration and disturbance can lead to impaired fracture healing or bone formation such as in osteoporosis. Counteracting or mimicking those miRNAs may be a viable therapy option and is tested here in an in vitro study using two cell types important for bone regeneration.

## REFERENCES:

- [1] C. Seeliger, K. Karpinski, A.T. Haug, H. Vester, A. Schmitt, J.S. Bauer, M. van Griensven, Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 29 (2014) 1718-1728.
- [2] S. Kelch, E.R. Balmayor, C. Seeliger, H. Vester, J.S. Kirschke, M. van Griensven, miRNAs in bone tissue correlate to bone mineral density and circulating miRNAs are gender independent in osteoporotic patients, Sci Rep, 7 (2017) 15861.



**Figure 1:** alizarin red staining (left) and quantification (right) after 14 days



**Figure 2:** target protein expression with scrambled miRNA, Ca<sup>++</sup> medium, or with mimic 125b