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Opinion Article

Osteoblast-Derived Extracellular Vesicles Modulate Osteoclast Differentiation through MicroRNA-125b-5p Transfer

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DESCRIPTION

Bone remodeling represents a tightly regulated process involving coordinated activities of bone-forming osteoblasts and bone-resorbing osteoclasts. Recent evidence suggests that intercellular communication through Extracellular Vesicles (EVs) plays key roles in maintaining bone homeostasis. This investigation examined the role of osteoblast-derived EVs in modulating osteoclast differentiation, with particular focus on microRNA-mediated regulatory mechanisms.

Primary human osteoblasts were isolated from trabecular bone specimens obtained from patients undergoing hip replacement surgery. Cells were cultured in α-MEM supplemented with 10% fetal bovine serum, ascorbic acid, and β-glycerophosphate to promote osteoblastic differentiation. Extracellular vesicles were isolated from conditioned media using differential ultracentrifugation followed by size exclusion chromatography. Nanoparticle tracking analysis confirmed EV size distribution with peak diameter of 110 ± 15 nm, consistent with exosome characteristics.

MicroRNA sequencing of osteoblast-derived EVs revealed enrichment of several osteoclast-regulatory microRNAs, including miR-125b-5p, miR-23a-3p, and miR-145-5p. Quantitative PCR analysis demonstrated 7.3-fold enrichment of miR-125b-5p in EVs compared to donor cells, suggesting selective packaging mechanisms. Bioinformatic analysis identified RANKL and TNF Receptor-Associated Factor 6 (TRAF6) as predicted targets of miR-125b-5p, both critical regulators of osteoclast differentiation.

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from healthy donors and differentiated into osteoclasts using Macrophage Colony-Stimulating Factor (M-CSF) and Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL). Co-culture experiments demonstrated that osteoblast-derived EVs significantly inhibited osteoclast formation, with 64% reduction in Tartrate-Resistant Acid Phosphatase (TRAP)-positive multinucleated cells compared to controls. This inhibitory effect was abolished when EVs were pre-treated with RNase, indicating RNA-dependent mechanisms.

Functional studies using miR-125b-5p mimics and inhibitors confirmed the regulatory role of this microRNA in osteoclast differentiation. Transfection with miR-125b-5p mimics reduced RANKL-induced osteoclast formation by 58%, while inhibitors enhanced differentiation by 42%. Luciferase reporter assays validated direct targeting of TRAF6 3'-UTR by miR-125b-5p, with 73% reduction in reporter activity. Western blot analysis confirmed decreased TRAF6 protein expression in cells treated with miR-125b-5p mimics.

Mechanistic investigations revealed that miR-125b-5p suppressed Nuclear Factor kappa-B (NF-κB) and Nuclear Factor of Activated T-cells, cytoplasmic 1 (NFATc1) signaling pathways essential for osteoclast differentiation. Immunofluorescence analysis showed reduced nuclear translocation of NF-κB p65 subunit in miR-125b-5p-treated cells. Additionally, expression of osteoclast-specific genes including cathepsin K, calcitonin receptor, and integrin β3 was significantly downregulated.

In vivo studies using a mouse calvaria injection model demonstrated that local administration of osteoblast-derived EVs reduced bone resorption by 47% compared to vehicle controls. Histomorphometric analysis revealed decreased osteoclast surface per bone surface and reduced eroded surface parameters. Importantly, bone formation parameters remained unchanged, suggesting specific effects on bone resorption rather than coupled remodeling responses.

The reversible nature of DNA methylation makes it an attractive target for therapeutic intervention in bone diseases. DNA methyltransferase inhibitors, such as 5-azacytidine and decitabine, have shown promise in preclinical studies for treating osteoporosis by reactivating silenced osteogenic genes. However, the global effects of these agents necessitate the development of more targeted approaches that can selectively modulate methylation patterns at specific genomic *loci* relevant to bone metabolism.

CONCLUSION

This study demonstrates that osteoblast-derived extracellular vesicles function as important mediators of bone cell

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communication through transfer of regulatory microRNAs. The identification of miR-125b-5p as a key inhibitor of osteoclast differentiation provides new insights into bone remodeling control mechanisms. These findings suggest that EV-mediated microRNA transfer represents a novel therapeutic target for treating bone diseases characterized by excessive bone resorption, such as osteoporosis and rheumatoid arthritis.