Macrophage exosomes modified by miR-365-2-5p promoted osteoblast osteogenic differentiationby targeting OLFML1

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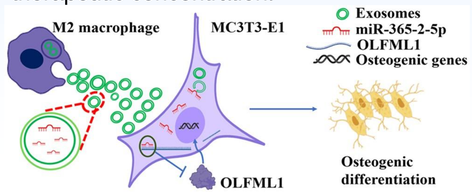
RATIONALE

In the bone immune microenvironment, immune cells can regulate osteoblasts through a complex communication network. Macrophages play a central role in mediating immune osteogenesis, exosomes derived from them have osteogenic regulation and can be used as carriers in bone tissue engineering. However, there are problems with exosomal therapy alone, such as poor targeting, and the content of loaded molecules cannot reach the therapeutic concentration.



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OBJECTIVE

The aim of this study was to investigate the effect of miRNA derived from M2 macrophage exosomes on osteogenic differentiation of mouse cranial osteoblasts (MC3T3-E1) and its potential therapeutic role in bone defect repair. With the insight of this process, we wish to prepare miRNA-modified M1 and M2 macrophage exosomes in combination with bone tissue engineering. To obtain biological substitutes that can remodel bone morphology and perform temporal immunomodulation to promote effective bone healing.

METHODS

The main points of the research are as follows:
Cell culture:
MC3T3-E1 osteoblasts and RAW264.7 macrophages were cultured in special media.
Macrophages were polarized into M0, M1, and M2 types using various cytokines.
Effect of macrophage conditioned medium:
Conditioned media from differently polarized macrophages (CM0, CM1, CM2) were prepared.
Their effect on osteogenic differentiation of MC3T3-E1 cells was studied using ALP and ARS staining, as well as qRT-PCR.
Exosome research:
Exosomes were isolated from M2 macrophages via ultracentrifugation.
Their effect on proliferation and osteogenic differentiation of MC3T3-E1 cells was investigated.
Role of miRNA:
MiRNA sequencing was performed to identify highly expressed miRNAs in M2 exosomes.
Special attention was paid to miR-365-2-5p, its effect on osteogenesis was studied.
Mechanisms of miR-365-2-5p action were investigated using Western blot and dual luciferase assay.
Methods used:
Quantitative real-time PCR
Alkaline phosphatase (ALP) and Alizarin Red S (ARS) staining
Western blot analysis
High-throughput sequencing
Dual luciferase reporter gene assay

RESULTS

Effect of macrophages on osteogenesis:
M2 macrophages significantly enhance osteogenic differentiation of MC3T3-E1 cells.
Increased ALP activity and formation of mineralized nodules are observed.
Expression of genes associated with osteogenesis (ALP, COL-1, Runx2) is increased.
Role of exosomes:
M2 macrophage exosomes stimulate MC3T3-E1 cell proliferation.
Optimal exosome concentration is 5 μg/ml.
Exosome size is approximately 71.75 nm, concentration is 2.08E+10 particles/ml.
Mechanisms of action:
Differences in microRNA expression were identified in exosomes of M1 and M2 macrophages.
A key role for miR-365-2-5p in stimulating osteogenesis was determined.
It was established that miR-365-2-5p affects the gene OLFML1, Promoting Bone Formation
The study demonstrates that macrophage M2 exosomes and the microRNAs they contain, particularly miR-365-2-5p, play a key role in stimulating bone formation through their effects on MC3T3-E1 cells.

CONCLUSIONS

In conclusion, miR-365-2-5p derived from M2 macrophage exosomes promotes osteogenesis of MC3T3-E1 by targeting OLFML1. The current findings provide new insights in clinical practice for treating bone defects with exosomes. The sequential release of miRNA-modified M1 and M2 macrophage exosomes from bone tissue-engineered scaffolds will be a possible way to treat diseases, such as bone defects in the future.

KEY FINDINGS

Immune regulation of bone healing:
The immune system influences the bone healing process by switching between inflammatory and anti-inflammatory cell phenotypes.
Macrophages play a key role in tissue repair after injury.
Macrophage types:
M1 macrophages are activated by LPS and proinflammatory cytokines, express iNOS and CD86.
M2 macrophages are activated by IL-4, promote tissue repair, and express Arg-1 and CD206.
M2 macrophages have been shown to stimulate osteogenic differentiation in MC3T3-E1 cells.
The role of exosomes:
Exosomes are vesicles measuring 30–200 nm that are involved in intercellular communication.
M2 exosomes are effectively taken up by MC3T3-E1 cells.
A concentration of 5 μg/ml of M2 exosomes maximally stimulates Cell proliferation
Mechanism of action:
MiR-365-2-5p plays a key role, which:
is transported via exosomes
suppresses OLFML1 expression
directly enhances the osteogenic capacity of cells
Research prospects:
Further studies are needed to examine the effect of miR-365-2-5p on bone defect healing in vivo.
It is important to study the effect of exosomes on osteoclasts.
Development of tissue-engineered scaffolds for exosome delivery is promising.
This study demonstrates that M2 macrophage exosomes, through miR-365-2-5p and OLFML1 suppression, promote osteogenesis, opening new possibilities for regenerative medicine.