Session: Disease & Treatment — Bone and Cartilage Metabolism

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DOWNREGULATION OF FABP4 PROMOTES OSTEOCLAST FORMATION AND FUNCTION BY ACTING PPAR-γ SIGNALING

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Background: Fatty acid-binding proteins (FABPs) are critical mediators of metabolism and inflammatory processes both locally and systemically, and has been proved not only be responsible for the formation of mature adipocytes but also function as the key modulator in osteoblast formation and osteogenesis. It has been reported that FABP4 had the capacity to enhance the adipogenesis of BMSCs, and broke the balance between adipocyte and osteoblast differentiation in bone marrow system. Researches on the side effects of Methotrexate chemotherapy, such as osteopenia, osteoporosis and fractures, also show that the gene expression of FABP4 is up-regulated in the stromal population. However, the relationship between FABP4 and osteoclast formation remains poorly understood, our research is aimed to find out what role FABP4 takes in osteoclast formation and bone resorption.
Subjects and Methods: BMM was collected from 6-week-old C57/BL6 mice, and induced to the osteoclast in the presence of M-CSF and RANKL for 6 days, BMS were used as the inhibitor of FABP4, protein expression of RAP, CK, MMP9, NFATC1, c-fos and PPAR-γ were evaluated on day 1, day 3, and day 5 by Western Blot, RT-PCR gene expression analysis on TRAP, CK, and MMP9 were also performed on day 2. On day 6, the cells were fixed and stained for TRAP assay in both BMS and BMS+GW9962 groups. And Pit formation assays on BMS group were also performed on day 6.
Results: In Western Blot, BMS suppresses expression of FABP4. However, the expression of TRAP, CK, MMP9, NFATC1, c-fos and PPAR-γ were increased on day 1, day 3, and day. RT-PCR gene expression analyses showed TRAP, CK, and MMP9 to be up-regulated on day 2. TRAP assays and Pit formation assays also showed that osteoclast formation and function is promoted under BMS treatment. We also investigated whether FABP4 is correlated with PPAR-γ and have an effect on osteoclast genesis by TRAP assays and Western Blot. In Western Blot, the protein expression of PPARγ is decreased. The TRAP assays result also shows that the formation of osteoclast is suppressed with the additional presence of GW9662 (a PPARγ inhibitor).
Discussion and Conclusion: Our research shows that block FABP4 have a positive impact on osteoclast formation, which is produced by acting PPAR-γsignaling. These phenomenon highlighting that the overexpression FABP4 can be a new potential target for treating osteopenia related dysfunction.
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Session: Disease & Treatment — Tumors

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CXCL12/CXCR4 AXIS PROMOTES VASCULOGENIC MIMICRY VIA PI3K/AKT SIGNALING IN OSTEOSARCOMA

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Background: The term vasculogenic mimicry (VM) refers to the unique capability of aggressive tumor cells to mimic the pattern of embryonic vasculogenic networks. Although VM has been shown to occur in osteosarcoma, a degree of which correlates with a poor clinical outcome, the precise molecular events underlying the process of VM in osteosarcoma have not been well defined. In this study, we explored osteosarcoma as a VM model in order to investigate the role of CXCL12/CXCR4 axis in VM formation.
Subjects and Methods: The effect of SDF-1 with different concentrations (10,100μmol/L) and different action times (1 to 6 days) on the growth of osteosarcoma cell line MG-63 was detected by Cell Counting Kit-8(CCK-8) analysis. The VM abilities of osteosarcoma cells were detected by a well-established *in vitro* 3D model of VM formation. The expression of CXCR4, Akt and p-Akt were detected by RT-PCR or Western blot.
Results: Osteosarcoma MG63 cells could form patterned matrix VM or tubular VM in 3D cultures *in vitro* and CXCL12 effectively promoted the formation of VM structures. Inhibition of CXCR4 function by an antagonist AMD3100 blocked CXCL12-induced VM formations of MG63 cells. Furthermore, CXCL12 increased phosphorylation of Akt. Additionally, blocking PI3K/Akt Pathway using a PI3K inhibitor LY294002 decreased CXCL12-induced VM formations of MG63 cells.
Discussion and Conclusion: The results of this study indicate that CXCL12/CXCR4 axis plays an important role in osteosarcoma VM formation more through the PI3K/AKT signaling. Our findings also demonstrate a novel cogitation in inhibition of osteosarcoma angiogenesis and metastasis.
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Session: Disease & Treatment — Skeletal Growth & Development

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ALENDRONATE-MODIFIED LIPOSOME FOR BONE TARGETING GENE DELIVERY REGULATING MESENCHYMAL STEM CELL MIGRATION

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Introduction: Aging is usually related to an increase in metabolic skeletal disorders such as osteoporosis and bone metastases, accompanied with the decrease in mesenchymal stem cells (MSCs) number.[1] For bone regeneration, directing MSCs toward the bone surface is a key step for further osteogenic differentiation.[2] Stromal cell-derived factor-1 (SDF-1) has been reported with the effect in inducing MSC migration.[3] Construction of the bone targeted gene delivery system would be a promising way to locally express SDF-1 on bone surface for attracting MSC migration and differentiation. This study aimed to develop bone targeting liposome by alendronate (Aln) modification for modulating MSC migration.
Subjects and Methods: Aln was conjugated to DSPE-PEG2000-COOH via carbodiimide chemistry through the reaction between carboxyl and amino. Aln-liposome was prepared using the thin-film hydration method with the component of DOTAP, DOPE, cholesterol, DSPE-PEG2000 and DSPE-PEG2000-Aln. The morphology of liposomes was observed by TEM. The transfection efficiency was examined by transfection of COS-1 cell with GFP reporter gene and luciferase gene. Bone target ability was examined using fluorescence absorption test. In vitro cytotoxicity of Aln-liposome was evaluated by CCK-8 assay. Then Aln-liposome was applied to SDF-1 gene delivery to MC3T3 osteoblast cells for MSC migration. The expressed SDF-1 mRNA and protein in MC3T3 cells after transfection were examined by RT-PCR and Western blot.
Results: Bone targeting Aln-liposome was prepared by conjugating liposome with alendronate. The average diameter of liposome was around 104 nm measured by a dynamic light scattering detector. TEM image showed the sphere morphology of Aln-liposome. The critical DNA complex ratio examined by gel retardation was 1:1 (N/P). The transfection efficiency of Aln-liposome was even higher than that of Lipofectamine™ 2000 shown by luciferase and GFP reporter gene expression in COS-1 cell. The cytotoxicity of Aln-liposome investigated by cck-8 assay was lower than that of Lipofectamine™ 2000 in COS-1 cells. More than 90% of cell viability could be observed in the cells when the concentration of liposomes reached 50 μg/mL. Aln-liposome showed the bone targeting ability in vitro. The binded liposomes increased from 13.7% to 61.4% after modification with alendronate by examining the changes in fluorescence of NBD-labeled liposome after mixed with hydroxyapatite. Aln-liposome was then applied to deliver SDF-1 gene to MC3T3 osteoblast cells, and its expression was confirmed by PCR and western blot. The expressed SDF-1 showed the effect in attracting MSC migration in transwell culture.
Discussion and Conclusion: Alendronate modified liposome showed the bone targeting ability and high gene transfection efficiency. By delivery SDF-1 gene, this system could attract MSC migration. This study provides a novel potential technique for bone regeneration.
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Session: Regenerative Medicine — Cell Therapy

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CHEMICAL-DRIVEN LINEAGE CONVERSION FROM MOUSE FIBROBLASTS TO CARTILAGE

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Objective: To overcome the cell source limitation and scar tissue accumulation in cartilage repair, lineage conversion with transcription factors from fibroblasts to chondrocytes provides a promising approach. However, genetic manipulation raises safety concerns in clinical application, while small molecule compounds are more cost-effective and easily-standardized. This study aims to illustrate the achievement of a gene-free conversion from mouse fibroblasts to cartilage tissue by chemical cocktails.
Methods and Materials: In this study we showed that mouse embryonic fibroblasts (MEFs) could be successfully induced into chondrocytes by a two-stage protocol. Chemical cocktails and hypoxia atmosphere were essential in the first 6 days, to