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MIR-21 REGULATES OSTEOGENESIS AND CHONDROGENESIS OF MESENCHYAL STEM CELLS

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Background: MicroRNAs are noncoding, small RNAs, 21–25nt in length, encoded in the genome, which can regulate the gene expression by targeting the 3' untranslated region (UTR) of mRNAs at the post-transcriptional level. Emerging evidences suggest that microRNAs play important roles in osteogenesis and skeletal homeostasis. Recent studies indicated the significant regulatory function of mir-21 in osteogenesis in vitro, but the effect of mir-21 on multi-lineage differentiation ability of mesenchymal stem cells is not fully revealed. In the present study, we aimed to investigate the effect of mir-21 intervention on osteogenic and chondrogenic differentiation of rat bone marrow derived MSCs in vitro and in vivo.

Results: The results showed that the up-regulation of mir-21 promoted osteogenesis and chondrogenesis of MSCs. Not only did mir-21 increase the expression of osteopontin and alkaline phosphatase in rBMSCs, but also promote mineralisation in the condition of osteogenic induction. Furthermore, using the open femur fracture model, we found that the bone healing properties were also improved by mir-21 overexpressing MSCs according to the results of microCT, mechanical test, and histological analysis.

Discussion and Conclusion: In conclusion, this study demonstrated that the over expression of mir-21 could promote osteogenesis and chondrogenesis. In addition, mir-21 overexpressing MSCs could accelerate bone fracture healing, which may contribute to a new therapeutic way for fracture repair.

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STAPHYLOCOCCAL ENTEROTOXIN C2 EXPEDITES BONE CONSOLIDATION DURING DISTRACTION OSTEOGENESIS

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Distraction osteogenesis (DO) could successfully induce large-size bone defect regeneration, but DO usually requires a long duration of bone consolidation. Developing innovative approaches to augment bone consolidation during DO is in burning need. Staphylococcal enterotoxin C2 (SEC2) has been developed and found to suppress osteoastogenensis of mesenchymal stem cells in vitro. In this study, we investigated the effect of SEC2 on the proliferation of rat bone marrow derived mesenchymal stem cells (rBMSCs) and osteogenic differentiation of rBMSCs. Furthermore, we locally administrated SEC2 (10ng/ml) or PBS into the gap in a rat DO model every three days until termination. The distraction regenerates were subjected to X-rays, micro-computed tomography (microCT), and mechanical testing. Histology and immunohistochemistry examinations were used to assess new bone quality. For the results, SEC2 had no effect on cell viability. The calcium testing. Histology and immunohistochemistry examinations were used to assess new bone quality. For the results, SEC2 had no effect on cell viability. The calcium

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THE EFFECT OF POROUS HYDROXYAPATITE SCAFFOLD COATED WITH COLLAGEN/rhBMP-2 CHITOSAN MICROSPHERES ON THE ADHESION AND OSTEOGENIC DIFFERENTIATION OF MSCs

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Introduction: Three dimensional printing (3DP) technique could be used for fabricating interconnected porous hydroxyapatite (HA) scaffolds. However, the initial product of HA scaffolds lacked osteoinduction bioactivity. The objective of the study was to construct a novel 3D porous HA scaffold coated with collagen/rhBMP-2 chitosan microspheres (CMS) and assess its effect on the adhesion and osteogenic differentiation of MSCs.

Subjects and Methods: Porous HA scaffolds were fabricated via 3DP technique first and then rhBMP-2 chitosan microspheres were coated on the surface of the scaffolds. Scanning electron microscopy was used to observe the scaffolds. Mesenchymal stem cells (MSCs) were used to determine the biocompatibility of the composite scaffolds. A soaking method was used to study the in vitro release kinetics of rhBMP-2. The bioactivity of the released rhBMP-2 was further assessed.

Results: Scanning electron microscopy observations revealed that the rhBMP-2 CMS adhered to the surface of collagen coating. The scaffold did not show cytotoxicity and could support the proliferation of MSCs in vitro. The collagen/rhBMP-2 CMS coating facilitated the attachment of MSCs to the surface of the scaffold. In vitro rhBMP-2 release experiment showed that the protein could be released for over 3 weeks and the concentrations were all over 112.8ng/ml at each tested time point. ALP activity of MSCs demonstrated that the released rhBMP-2 was still bioactive and could induce the differentiation of MSCs.

Discussion and Conclusion: The collagen/rhBMP-2 CMS coating was an effective way to introduce rhBMP-2 to the porous scaffold fabricated by the 3DP technique. The scaffolds had good biocompatibility and rhBMP-2 CMS could control the release source for RC repair. Our team has fabricated a scaffold-free TDSC sheet [5]. However, the TDSC sheet just improves the early graft healing in ACLR [6]. Thus, for better use of this cell-based strategy, differentiation factors would help in the biological repair of tendon-bone insertion. Magnesium ions (Mg 2+) have been shown to have beneficial effects on both chondrogenesis and osteogenesis of stem cells [7, 8]. Based on these findings, we hypothesized that Mg 2+ treated TDSC cell sheet would promote the healing of injured RC and tested our hypothesis accordingly.

Methods: Rat TDSCs were isolated [5,6]. MgCl 2 was dissolved in neutralized culture medium. The effects of Mg 2+ on the adhesion and differentiation of TDSCs were investigated by specific staining. Western blotting was used to determine Mg transporter 1 (Magt1) and related signaling pathways. We also used the metafluor system to monitor intracellular Mg 2+ concentration. Rat RC repair model was adopted to test the efficacy of this novel cell sheet in vivo [1,2,9]. Results: The proliferation of TDSC was not affected by addition of Mg 2+ with various concentrations of 1–10 mM (n=6/group). 10mM supplementation of Mg 2+ significantly promoted the adhesion of TDSCs at early time points via up-regulating the expression of phosphorylated focal adhesion kinase (FAK) at the site of tyrosine 397. FAK is also suggested to be an osteogenic factor [10], and it helps to explain our observation that Mg 2+ (10 mM) could significantly promote the chondrogenic and osteogenic differentiation of TDSCs. Furthermore, above effects were mainly mediated by Mg transporter 1 (Magt1) as the effects of Mg 2+ were almost completely abrogated when the TDSCs with Magt1-knockdown or Magt1 blocker, Nitrendipine. Eight weeks post the transplantation of the Mg 2+ treated TDSC sheet, significantly more cartilage-like cells were observed at the regenerated transition. Conclusion: We identified that Magt1 mediates the activation of FAK signaling pathway, finally leading to the enhancement of chondro-osteogenic differentiation induced by Mg 2+.

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TENDON-DERIVED STEM CELL SHEET ENHANCES THE REPAIR OF INJURED ROTATOR CUFF

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Introduction: Rotator cuff (RC) tendons are often prone to lesions, as 30–50% of the population over 50 years of age suffer from partial- and full-thickness RC tears. A torn rotator cuff is a disruption in the integrity of the tendon at the insertion into the humeral head. Damaged RC heals very slowly and rarely attains the structural integrity and mechanical strength of normal, undamaged RC. It has been reported that addition of BMSCs to the injured RC insertion site did not improve the outcome [1]. Interestingly, the same authors found that BMSCs over-expressed with scleraxis significantly improve RC healing [2]. Tendon derived stem cells, with highly expressed scleraxis [3,4], may be an alternative cell
Discussion and Conclusion: The indexes of chondrogenesis in LiCl group were significantly increased. Addition decreased in the IL-1 group. However, in inflammatory conditions induced by IL-1, mRNA of Sox 9, Collagen 2a, Aggrecan, and the amount of GAG, were significantly decreased, and NF-κB protein was increased, p-NF-κB protein was significantly decreased, and NF-κB protein in the nucleus was significantly decreased. All of these in GSK-3b group were just opposite to the LiCl group. The possible mechanism was LiCl promotes the phosphorylation of the NF-κB protein, while IL-1 could enhance the ability although with IL-1. The possible mechanism was LiCl promotes the phosphorylation of the NF-κB protein and the other group with 10 nM GSK-3b was specifically inhibited. The next step was the NF-κB signaling pathway.

Introduction: It is a complex process to regulate bone marrow mesenchymal stem cells’ (BMSCs) chondrogenic differentiation, especially in inflammatory conditions. This study aimed to investigate the effect of LiCl in chondrogenic differentiation of BMSCs in inflammatory conditions and the possible mechanism in this process. Subjects and Methods: BMSCs were treated with IL-1 in the process of chondrogenic differentiation. Along with IL-1, one group was treated with 10 nM LiCl and the other group with 10 nM GSK-3b. For each group, the glycosaminoglycan (GAG) amount was quantitatively tested and the mRNA of Sox 9, Collagen 2a, and Aggrecan were tested by RT-qPCR. The total NF-κB protein, p-NF-κB protein, and the NF-κB protein in cytosol or nucleus were tested by Western blot. Results: Our results demonstrated that the index of chondrogenesis, such as the mRNA of Sox 9, Collagen 2a, and Aggrecan, and the amount of GAG, were significantly decreased in the IL-1 group. However, in inflammatory conditions induced by IL-1, the indexes of chondrogenesis in LiCl group were significantly increased. Addition, total NF-κB protein was increased, p-NF-κB protein was significantly decreased, and NF-κB protein in the nucleus was significantly decreased. All of these in GSK-3b group were just opposite to the LiCl group. Discussion and Conclusion: These results strongly suggest that the ability of BMSCs’ chondrogenic differentiation were decreased in inflammatory conditions induced by IL-1, but LiCl could enhance the ability although with IL-1. The possible mechanism was LiCl promotes the phosphorylation of the NF-κB protein and the transfer into nucleus, and then suppresses the NF-κB signaling pathway.