A Novo Role of CITED2 in Cartilage Protection by Suppressing Adipogenesis and Pro-Inflammatory Mediators in the Infrapatellar Fat Pad

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Introduction: Obesity generates a chronic, low-grade inflammation that is suggested to contribute to cartilage degeneration. The infrapatellar fat pad (IPFP), an adipose tissue located within the knee joint synovial capsule, may participate in this process. However, regulatory mechanisms of pro-inflammatory mediator expression in the IPFP are not fully understood. Cbp/p300-interacting transactivator 2 (CITED2) is a mechanically sensitive transcriptional regulator that exerts chondroprotective actions on cartilage by repressing expression of matrix metalloproteinases (MMPs) in chondrocytes. CITED2 deficiency is associated with increased differentiation of aged tendon/stem/progenitor cells into adipocytes. As adipokines and pro-inflammatory mediators produced in the IPFP exert significant impact on joint inflammation and cartilage breakdown in osteoarthritis (OA), we tested the hypothesis that CITED2 plays a chondroprotective role by, at least in part, suppressing adipogenesis and expression of adipokines and pro-inflammatory mediators in the IPFP.

Subjects and Methods: Cited2+/− and wild-type (WT) littermates (18 weeks-old, male, n = 6/group) were subjected to normal (13.5% calories from fat) or high fat diet (60% calories from fat) and/or subjected to moderate treadmill running (10 km/min) or control (0 km/min) for 45 min. Explants of IPFP from mice subjected to or not subjected to treadmill running were co-cultured with cartilage from naive WT mice. Effects of CITED2 manipulation on adipogenesis was investigated using murine C3H10T1/2 pluripotent stem cells cultured in adipogenic induction medium.

Results: Cited2 expression decreased following a two week high fat diet in wild-type mice, which was associated with increased expression of adipokines adipin, adipin and leptin, adipose-regulatory transcription factor Cebp, proteolytic enzymes Mmp13 and Adamts5. Cited2+/− mice on a normal diet mimicked the altered gene expression changes observed in WT mice on a high fat diet, while Cited2−/− mice on high fat diet further exaggerated the altered expression of these genes. Treadmill running led to suppressed expression of adipokines, Cebp, Pparg, Mmp13 and Adamts5 in the IPFP in WT mice, but not in Cited2−/− mice. IPFP from treadmill-run WT mice, co-cultured with naive WT cartilage, markedly inhibited the expression of Mmp1, Mmp3, Adamts5, and Adamts11 in the cartilage. Such effects were not observed in cartilage co-cultured with IPFP from Cited2+/− mice, and a significant upregulation of Mmp13, Adamts11, and Cited2−/− in the cartilage. Effects of CITED2 manipulation on adipogenesis was investigated using murine C3H10T1/2 pluripotent stem cells cultured in adipogenic induction medium.

Discussion and Conclusion: We identified a novel role of CITED2 in chondroprotection by suppressing expression of adipokines and pro-inflammatory mediators in the IPFP. CITED2 plays this role, at least in part, by repressing transactivity of Cebp, which is associated with increased expression of adipokines adipin and leptin, adipose-regulatory transcription factor Cebp, proteolytic enzymes Mmp13 and Adamts5, while Pparg protein in the p300 immuno-pull-down complex, while Pparg protein.

61 miR-9-5p, miR-675-5p and miR-138-5p Regulate Wnt Signalling Pathways and Strontium-Mediated Osteogenesis

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Introduction and Subjects: The microRNAs (miRNAs) play important roles in many biological processes such as cell differentiation and apoptosis, so they may be used in the diagnosis and treatment of many diseases like osteoporosis. Wnt signalling pathways including G-protein-coupled ligand and receptor. Common ligand is Wnt. Wnt signalling pathways including β-catenin are essential for the skeletal cell proliferation and bone formation. Using the p300 immuno-pull-down complex, the protein (LRP 5) and LRPS are two important co-receptors of the Wnt signalling pathways. However, the exact roles of these two co-receptors in the skeletal cell proliferation and bone formation are not explicit. This study was designed to evaluate the effects of strontium on the expression levels of miRNAs and to explore their effects on skeletal cell proliferation, differentiation, adhesion, and apoptosis. The targets of these miRNAs were also studied.

Methods: Molecular cloning, cell proliferation assay, cell apoptosis assay, quantitative real-time PCR, luciferase reporter assay, immunofluorescence, western blot, and other methods were used.

Results: Strontium altered the expression levels of miRNAs in vitro and in vivo. miR-9-5p, miR-675-5p, and miR-138-5p impaired skeletal cell proliferation and cell differentiation, and altered cell adhesion. miR-9-5p and miR-675-5p induced MCT3-E1 cell apoptosis more specifically than miR-138-5p. In addition, miR-675-5p induced skeletal cell apoptosis by upregulating Nemo-like kinase (NLK) while miR-9-5p inhibited ATDC5 cells apoptosis by downregulating NLK. miR-9-5p, miR-675-5p, and miR-138-5p targeted glycogen synthase kinase 3 β (GSK3β), Atpase Aminophospholipid Transporter Class I Type A2 Member 2 (ATPB2A), and Eukaryotic Translation Initiation Factor 4E Binding Protein 1 (EIF4EBP1), respectively. LRPS promoted skeletal cell proliferation through negatively regulating miR-675-5p and miR-9-5p. Loss of function of LRPS resulted in drastic cell apoptosis, increased negative regulators of osteogenesis, and impaired cell adhesion. Strontium stabilized β-catenin through increasing phosphorylation of LRPS rather than the levels of LRPS.

Discussion and conclusion: miR-9-5p, miR-675-5p, and miR-138-5p impaired skeletal cell proliferation and differentiation, and altered cell adhesion and apoptosis. Loss of function of LRPS resulted in impaired osteogenesis.

Funding/support: This study was supported by NSFC (81371989, 81270967).

References

62 Chondroprotective Effect of Rebamipide on Articular Cartilage

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Introduction: Osteoarthritis (OA) is the most common degenerative joint disease. However, few drugs are available to effectively prevent or treat cartilage degeneration. So, as a new agent for chondroprotection, we expect Rebamipide to have a protective effect on cartilage. Rebamipide is a protective drug used for gastric mucosal injuries such as gastric ulcer and gastritis and also has protective effects on the articular cartilage. Thus far, there is no evidence that Rebamipide has a protective effect on cartilage. Therefore, it is expected to have a new effect for chondroprotection. In this study, we investigated the effects of Rebamipide on articular cartilage in vivo by culturing chondrocytes from human knee OA and in vivo mouse models of post-traumatic arthritis to examine the effects of Rebamipide on articular cartilage degeneration.

Subjects and Methods: Chondrocytes from patients who underwent total knee arthroplasty were isolated and plated on 6-well tissue culture plates. Cells were stimulated with recombinant human IL-1β, and then treated with or without Rebamipide for 24h. The levels of mRNA expression of COL2A, IL-1β, TNF, NF-κB, MMP3, MMP13, ADAMTS5, TGF, bFGF, and TGF were estimated using real-time PCR (between six to nine samples per group). The mRNA levels were normalized by GAPDH levels of each sample. Statistical significance was determined using the Kruskal-Wallis test. Forty eight-week-old male BALB/c strain mice were used. The anterior cruciate liga-ment and medial collateral ligament were transected in both knees (PTOA). The knees were divided into four groups. The concentrations of Rebamipide were 0 (A), 0.1mg/ kg (B), 1mg/kg (C), and 10mg/kg (D). Mice were injected with Rebamipide into the knee joint every week. Mice were sacrificed at six weeks after operation. All samples were underwent haematoxylin and eosin (H&E) staining and safranin-O staining, and evaluated using the Mankin scoring system and OARSI grading system.

Results: The mRNA expression of COL2A was significantly up-regulated after the treatment with 500μM and 2500μM of Rebamipide. TIMP3, TGF, bFGF were also significantly up-regulated after the treatment with 2500μM of Rebamipide. IL-1β, TNF, NF-κB, MMP3, MMP13, ADAMTS5 were also significantly down-regulated after the treatment with 2500μM of Rebamipide. Proteoglycan loss and alterations in surface structure were observed in A and B, but the articular cartilage had a smooth surface in C and D. In Mankin scoring system and OARSI grading system, average histological scores were significantly better in C and D than in A.

Discussion and Conclusion: This study demonstrated that Rebamipide up-regulated the mRNA expression of COL2A and anabolic factors (TIMP3, TGF and bFGF) in...