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A NOVEL 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 (10m/min) or control (0m/min) for 45 min. Explants of IPFPs from mice subjected to or not subjected to treadmill running were co-cultured with cartilage from naïve 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 *adipsin* and *leptin*, adipo-regulatory transcription factor *Cebpa*, 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, *Cebpa*, *Pparg*, *Mmp13* and *Adamts5* in the IPFP in WT mice, but not in *Cited2*+/- mice. IPFP from treadmill-run WT mice, co-cultured with naïve WT cartilage, markedly inhibited the expression of *Mmp1*, *Mmp13*, *Adamts5*, and *Il1b* in the cartilage. Such effects were not observed in the cartilage co-cultured with IPFP from *Cited2*+/- mice. *In vitro*, C3H10T1/2 cells transfected with *Cited2* wild type cDNA (WT-Cited2) exhibited significantly reduced adipocyte formation, and reduced expression of adipocyte marker *aP2*, adipokines (i.e. *adipsin*, *leptin*), and transcriptional regulators (*Cebpa* and *Pparg*), while the cells transfected with dominant negative *Cited2* mutant defective in p300 binding (DN-Cited2) exhibited no such changes. WT-Cited2, but not DN-Cited2 transfected cells also reduced Cebpa proteins in a p300 immuno-pull-down complex, while Pparg proteins in the complex were not detected. This data indicates Cited2 represses adipogenic differentiation by, at least partly, competing with Cebpa to bind to the limited amount of co-factor p300.
Discussion and Conclusion: We identified a novel role of CITED2 in chondroprotection by suppressing expression of adipokine and pro-inflammatory mediators in the IPFP. CITED2 plays this role, at least in part, by repressing transactivity of C/ebpα. Furthermore, we demonstrated that loading-induced CITED2 contributed to the anti-inflammatory and anti-obesity effects of exercise.
http://dx.doi.org/10.1016/j.jot.2016.06.062

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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 β-catenin are essential for the skeletal cell proliferation and bone formation. Low-density lipoprotein receptor-related protein (LRP) 5 and LRP6 are two important co-receptors of the Wnt signaling 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 MC3T3-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 cell 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 8A Member 2 (ATP8A2), and Eukaryotic Translation Initiation Factor 4E Binding Protein 1 (EIF4EBP1), respectively. LRP5 promoted skeletal cell proliferation through negatively regulating miR-675-5p and miR-9-5p. Loss of function of LRP5 resulted in drastic cell apoptosis, increased negative regulators of osteogenesis, and impaired cell adhesion. Strontium stabilized β-catenin through increasing phosphorylation of LRP6 rather than the levels of LRP6.
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 LRP5 resulted in impaired osteogenesis.
Funding/support: This study was supported by NSFC (81371989, 81270967).
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http://dx.doi.org/10.1016/j.jot.2016.06.063

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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 in a variety of tissue and organ injuries. In this study, we employed *in vitro* cell culture using primary cultured chondrocytes from human knee OA and *in vivo* mouse models of post-traumatic osteoarthritis 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, TIMP3, bFGF, and TGF were estimated using real-time PCR (between six to nine samples per gene). 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 ligament 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, and MMP13 were significantly down-regulated after the treatment with 500μM and 2500μM of Rebamipide. MMP3 and 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