**Session: Disease & Treatment — Osteoarthritis**

**108 ANTI-APOPTOTIC EFFECT OF mmu-miR-214-3p ON CHONDROCYTES FROM IL-1β-INDUCED APOTOTIS**

Qiushi Wang a, Simin Luo a,b, Huantian Zhang a,b, Ning Liu a,b, Jieruo Li a,b, Songwei Huan a,b, Guorong She c,d, Jie Yang e, Zhengang Zhao e

1Department of Orthopaedic Disease Research, Jiaon University, Guangzhou, China  
2Department of Joint Surgery, The First Affiliated Hospital, Jiaon University, Guangzhou, China

**Background:** Osteoarthritis is a multifactorial degenerative disease that generally affected synovial joints. The apoptosis of chondrocytes play an important role in the pathogenesis of OA. Here we study the effect of mmu-miR-214-3p on the apoptosis and proliferation of mouse chondrocytes.

**Subjects and Methods:** Chondrocytes of C57/Bl6 mice were exacted and cultured in vitro. Chondrocytes were then transfected with mimics or inhibitor of mmu-miR-214-3p with or without IL-1β (10ng/ml) treatment for 24 to 48 hours. The proliferation and cell viability were evaluated by CCK-8 test. The apoptosis rate was determined by Annexin-V/7-AAD flow cytometry and tunnel staining. A quantitative colorimetric assay was used to determine activated caspase-3, mRNA expression of Bim, Bcl-2, Bad, AIF, PTEN were evaluated by real-time PCR, and the total Akt, phosphorylated Akt (p-Akt), Bcl-2, Bad, cytochrome C (Cyto c) were determined by Western blotting.

**Results:** There were no significant differences in absorbance between the miR-214-3p mimics group (50nM, 100nM, 200nM), miR-214-3p Inhibitor group (100nM, 200nM), mimics NC and inhibitor NC group for cell proliferation. Cell viability was significantly reduced in IL-1β treated group. However the over expression of mmu-miR-214-3p (50nM, 100nM) decreased cell damage caused by IL-1β (P < 0.05). There were no significant differences in apoptosis rate between over expression or knock down of mmu-miR-214-3p and NC. Over expression of mmu-miR-214-3p (50nM) can significantly reduce the apoptotic rate of chondrocytes induced by IL-1β (P = 0.003). Expression of activated caspase-3 was lower for mmu-miR-214-3p mimics+IL-1β group than IL-1β group and mimics NC-IL-1β group. Mmu-miR-214-3p mimics transfection could reduce the mRNA expression of Bim, Bcl-2 and PTEN (P < 0.05, P < 0.05and P < 0.01), whereas mmu-miR-214-3p knock down could improve the mRNA expression of Bim, AIF and PTEN (P < 0.01, P < 0.05and P < 0.05), mmu-miR-214-3p mimics transfection improved p-Akt and Bcl-2 protein expression (P < 0.05, P < 0.05) and reduced Bad, Cyto c expression (P < 0.01, P < 0.05).

**Discussion and Conclusion:** The over expression or knock down of mmu-miR-214-3p alone did not exhibit a significant influence on the proliferation and apoptosis of chondrocytes. However, over expression of mmu-miR-214-3p showed an anti-apoptotic effect on IL-1β induced apoptosis, which indicated that mmu-miR-214-3p may be related to the inflammatory course of OA. The protective effect of mmu-miR-214-3p against apoptosis probably via PI3K/Akt pathway.

**Session: Regenerative Medicine — Growth Factors**

**117 FIBROBLAST GROWTH FACTOR-2 IMPROVES THE TENDON-TO-BONE HEALING BY STIMULATING THE GROWTH OF TENOGNIC PROGENITORS IN A RAT ROTATOR CUFF REPAIR MODEL**

Takuya Tokunaga a, Yuji Hirakata a, Chiesa Shukunami b, Hitoshi Arimura a, Ryuji Yonemitsu a, Junji Ide a,b, Hiroshi Mizutani a

1Department of Orthopaedic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan  
2Department of Cellular Differentiation, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan  
3Department of Molecular Biology and Biochemistry, Division of Basic Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan  
4Department of Advanced Joint Reconstructive Surgery, Kumamoto University Hospital, Kumamoto University, Kumamoto, Japan

**Background:** Certain growth factors are noted to enhance healing after rotator cuff (RC) surgical repair. Fibroblast growth factor (FGF)-2 has the potential to promote tendon-to-bone healing after CR repair in rats; however, this mechanism remains unclear. FGF-2 stimulates self-renewal of mesenchymal stem- or progenitor cells; thus, we hypothesized that FGF-2 administration to reparative sites may promote tenogenic-progenitor growth, resulting in biomechanical and histological improvement of repaired rat RCs. This was tested in a rat CR repair model by analyzing early, scleraxis (Scx)—a basic helix-loop-helix family transcription factor—and late, tenomodulin (Tnmd)—a type II transmembrane protein—tenogenic marker expression. Histological evaluation and biomechanical testing were also performed.

**Methods:** Adult male Sprague-Dawley rats (N = 156) underwent unilateral surgery to reattach the supraspinatus tendon to its insertion site. We used biodegradable gelatin hydrogel sheets as carriers for FGF-2, placed between the supraspinatus tendon and bone. Two groups were assessed for the effect of FGF-2 on RC healing: an FGF-2 (5 µg)-treated group and a control group (carrier only). At 2, 4, 6, 8, and 12 weeks post-operation, tenogenic-marker (Scx and Tnmd) expressions in reparative tissues were evaluated using real-time reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization (ISH). Healing sites were evaluated using semi-quantitative histology. At 12 weeks, biomechanical testing was performed. Statistical significance was estimated using the Mann-Whitney U-test; p < 0.05 was considered statistically significant.

**Results:** Real-time RT-PCR analysis demonstrated that Scx and Tnmd expression levels increased significantly in the FGF-2—treated group from 4–8 and 4–12 weeks postoperatively, respectively. ISH analyses showed that Scx and Tnmd transcripts were mainly observed in the spindle-shaped cells of the tendon mid-substance in both groups, and these signals tended to expand into reparative tissue; a greater extent in the FGF-2—treated group than in the control group at ≥6 weeks. The FGF-2—treated group demonstrated a tenod-like tissue with oriented collagen fibers between tendon and bone compared to the control group; consequently, histological scores were significantly higher in the FGF-2—treated group than in the control group at ≤4 weeks. At 12 weeks, biomechanical testing showed that the ultimate load-to-failure (ultimate load) and ultimate stress-to-failure (ultimate stress) were significantly higher in the FGF-2—treated group than in the controls (ultimate load: 23.8 ± 4.6 N vs. 13.8 ± 4.7 N; ultimate stress: 6.9 ± 1.1 N/mm² vs. 3.9 ± 1.1 N/mm²). No differences were observed in the stiffness and cross-sectional area of the repair site.

**Discussion and Conclusion:** In the FGF-2—treated group, expression of Scx and Tnmd increased at ≥4 weeks, indicating that more tenogenic progenitors were included in the reparative site. Improvement of histological parameters, such as fibrovascular tissue reduction and collagen orientation at the insertion site, was observed at ≥4 weeks. Biomechanical strength also increased at 12 weeks. Collectively, this suggests that FGF-2 promotes growth of tenogenic progenitors at the reparative site, which contribute to tendon-to-bone healing. These findings provide insight into the clinical development of biological enhancement strategies for RC healing.

**Session: Disease & Treatment — Pharmaceutical Interventions**

**122 LOCAL INTRA-ARTICULAR INJECTION OF RESVERATROL DELAYS ARTICULAR CARTILAGE DEGENERATION BY REGULATING HIF AND THEREBY PROMOTING CHONDROCYTE AUTOPHagy**

Na Qin

Luoyang Orthopedic Hospital, China

**Objective:** Autophagy is an essential cellular homeostasis mechanism that was found to be compromised in aging and osteoarthritis (OA) cartilage. Previous studies showed that resveratrol can effectively regulate autophagy in other cells. The purpose of this study was to determine whether the chondroprotective effect of resveratrol was related to chondrocyte autophagy and to elucidate underlying mechanisms.

**Methods:** OA model was induced by destabilization of the medial meniscus (DMM) in 10-week-old male mice. OA mice were treated with resveratrol with or without 3-MA for 8 weeks beginning 4 weeks after surgery. The changes of articular cartilage structure were examined by using semiquantitative scoring systems and Safranin O-fast green. The protein expressions of ULK1, LC3, Beclin1, HIF-1α, HIF-2α, AMPK, p-AMPK, mTOR, p-mTOR, COL2A1, Aggrecan, MMP13, ADAMTS5 expressions were analyzed by Western blot. COL2A1, Aggrecan, MMP13, ADAMTS5 mRNA expressions were analyzed by Real-time PCR.

**Results:** The local intra-articular injection of resveratrol delayed articular cartilage degradation in DMM-induced OA. Resveratrol treatment increased LC3, Beclin1, HIF-1α, p-AMPK, COL2A1, Aggrecan expressions, but decreased HIF-2α, p-mTOR, MMP13and ADAMTS5 expressions. The effects of resveratrol were obviously blunted by 3-MA except HIF and AMPK.

**Conclusion:** The study demonstrated that resveratrol intra-articular injection delayed articular degeneration and promoted chondrocyte autophagy in an experimemtal model of surgical DMM-induced OA, in part via activating AMPK signaling.