

Fig. 2. GAG was stained by Picosirius Red in the central portion.

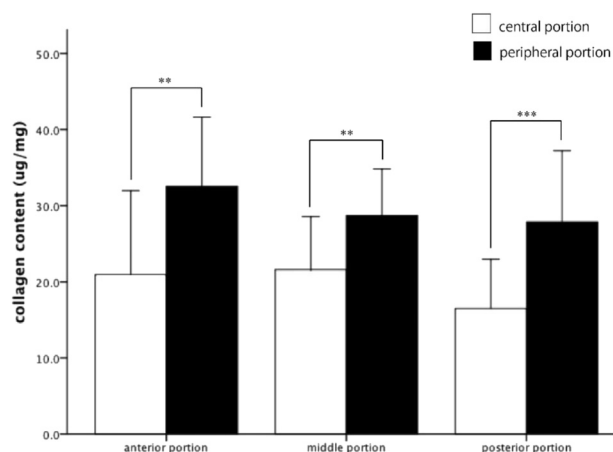


Fig. 3. In hydroxyproline assay, collagen content in the peripheral portion was more than in the central portion.

miRNA

554

MIR-193B-3P REGULATES CHONDROGENESIS OF ATDC5 CELLS VIA TARGETING TGFBR3

C. Hou, Z. Zhang, Y. Kang, Z. Zhang, W. Liao. First affiliated Hosp. of Sun Yat-Sen Univ., Guangzhou, China

Purpose: To investigate the biological effect of mmu-miR-193b-3p on chondrogenic differentiation.

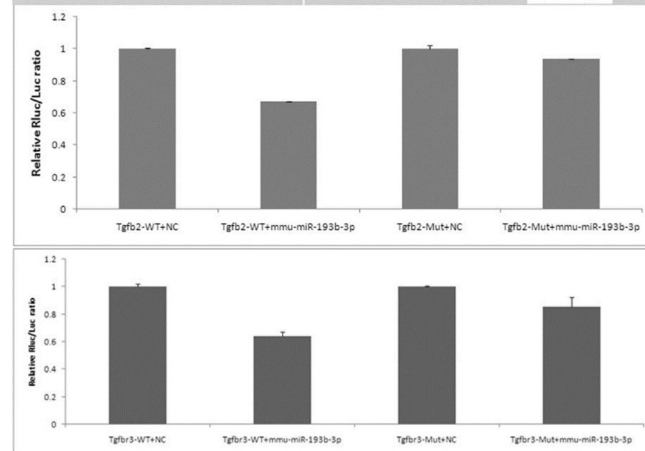
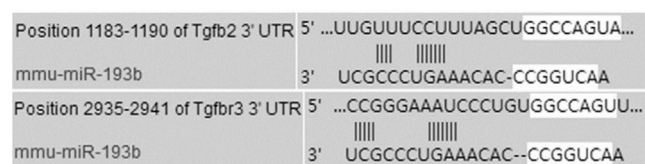
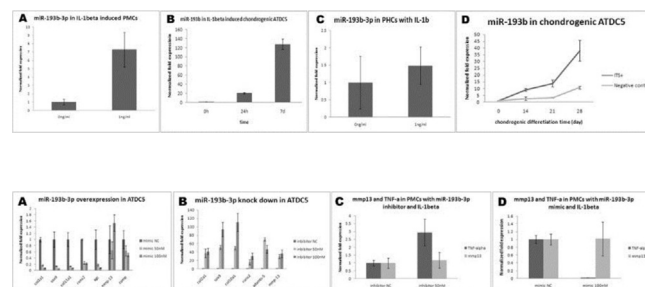
Methods: Chondrocyte-like ATDC5 cell line was stimulated with ITS+ premix to form cartilage nodules. Total RNA was isolated and reverse transcribed into cDNA. The 3'-UTR of predicted target genes, TGFBR3, were cloned into luciferase reporter plasmids. The mmu-miR-193b-3p mimic/inhibitor, and luciferase reporter plasmids were transfected into cells with lipofectamine 2000. Alcian blue were used to stain the cartilage nodules.

Results: The miR-193b expression was elevated in chondrogenic ATDC5. The miR-193b suppressed the expression of several chondrogenic markers in chondrogenic ATDC5 in a dose dependent manner,

including col2a1, sox9, col10a1, col11a1, runx2, aggrecan, and comp. The mouse TGFBR3 were predicted as the potential target gene of mmu-miR-193b-3p.

The luminescence decreased more than 30% in 3T3 cells cotransfected with TGFBR3 3'-UTR reporter plasmids and miR-193b-3p mimic, while the mutation of predicted seed sequences of TGFBR3 3'-UTR partially restored the luminescence.

Conclusions: The miR-193b may inhibit chondrogenesis of ATDC5 via targeting TGFBR3.



555

BOTH miRNA-29B DOWNREGULATION AND miRNA-140 OVEREXPRESSION DRIVE RESPECTIVELY MSC PROLIFERATION AND CHONDROGENIC DIFFERENTIATION IN COLLAGEN SCAFFOLD

V. Salone, C. Henrionnet, C. Branlant, P. Gillet, A. Pinzano. UMR 7365 CNRS-Université de Lorraine, IMoPA, Vandoeuvre Les Nancy, France

Purpose: MicroRNAs (miRNAs) play an important role in the regulation of chondrogenesis of human bone mesenchymal stem cells (hBMSC), however their respective expression during 2D expansion and 3D chondrogenic differentiation in collagen scaffold still remains poorly known. In this study, miRNA profile expressions during hBMSC chondrogenic differentiation was explored as putative biomarkers of chondrogenesis.

Methods: Mesenchymal stem cells issues from human bone marrow (hip replacement) were amplified and pre-conditioned by a specific medium (PAD) in the last passage (P3). Cells were then seeded in collagen sponges (Day 0) and cultured 28 days in a chondrogenic medium containing TGF-β1. The expression of miRNA was analyzed by hybridization on DNA microarrays then confirmed by qRT-PCR. The expression of these miRNA was compared with the extracellular matrix synthesis, and more particularly type II collagen and proteoglycan synthesis/content with qRT-PCR and histology respectively.