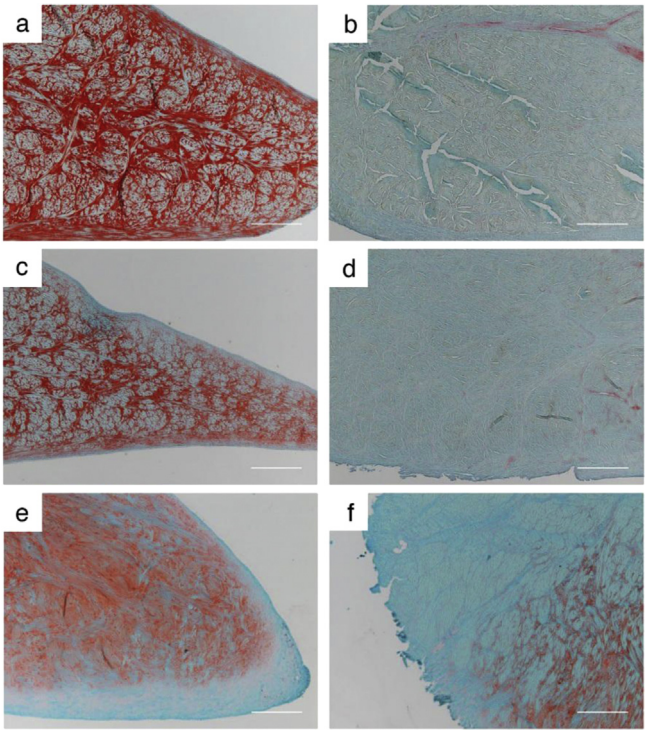

Fig.2, GAG was stained by Picrosirius Red in the central portion.

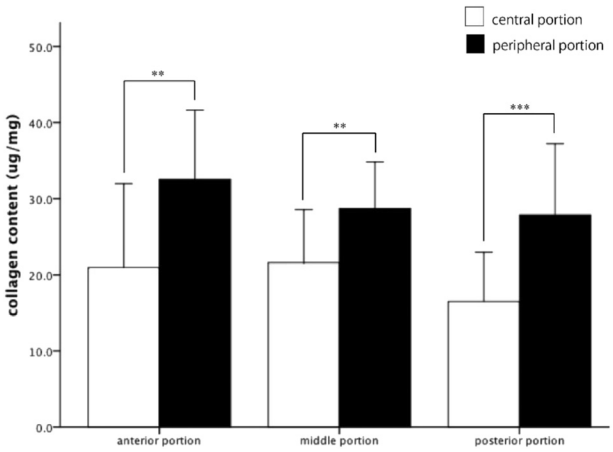

Fig.3, In hydroxyproline assay, collagen content in the peripheral portion was more than in the central portion.

miRNA

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

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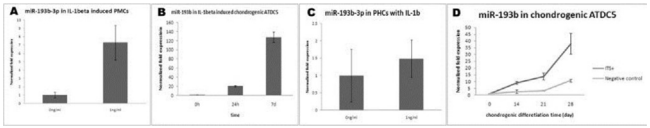
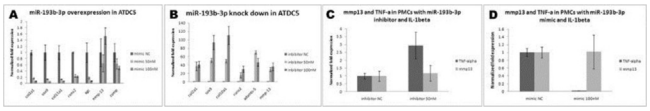
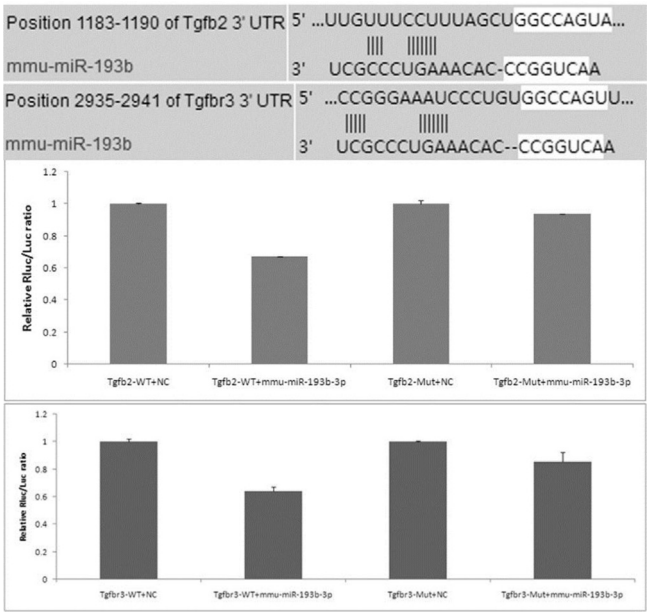
Purpose: To investigate the biological effect of mmu-miR-193b-3p on chondrogenic differentiation.
Methods: Chondrocyte-like ATDC5 cell line was stimulated with ITS+ premix to form cartilage nodules. Total RNA was isolated and reverse transcripted into cDNA. The 3'-UTR of predicted target genes, TGFBR3, 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.







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

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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.

Results: Chips data analysis demonstrated not only that 15 miRNAs varied significantly during MSC proliferation and preconditionning but also that 17 MSC miRNA varied significantly during chondrogenic 3D differentiation. By quantitative PCR, we confirmed that miR-29b expression level was significantly down-regulated by PAD supplement at passage 3 stage during hMCS monolayer expansion. On one hand, during 3D differentiation we confirmed by qPCR miR-29b down-regulation until Day 14. On the other hand, miR-140 expression was strongly significantly increased throughout the chondrogenic differentiation. MSC chondrogenic differentiation was confirmed at the gene (COLA21, ACAN, SOX9) and tissue levels: type II collagen was present in extracellular matrix (immunohistochemistry) as proteoglycans (Alcian blue staining).
Conclusions: As miR-29b is known for its anti-proliferative properties under certain conditions, its inhibition/repression may favour cell proliferation and matrix synthesis. Besides, miR-140 is one of a very limited number of noncoding microRNAs (miRNAs) mainly expressed in cartilage. Our results confirm that miR-140 increases during MSC driven chondrogenesis. Finally, miR29 repression and mir140 induction likely play a role in MSC-driven cartilage engineering and may have value as biomarkers and/or therapeutic target.

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METHODS FOR THE IDENTIFICATION OF MICRORNA-455 TARGETS

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Purpose: MicroRNAs (miRNAs) have emerged as a new class of gene expression regulators that are important in both normal cartilage physiology and pathology. We have previously identified several microRNAs that are regulated in osteoarthritis, including microRNA-455, a key Sox9-responsive microRNA. MiRNAs are 20-24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. MicroRNAs are known to simultaneously target many transcripts, possibly to fine tune complex signalling pathways. A single miRNA can regulate multiple mRNAs via binding to sequences in the 3'UTR either promoting mRNA decay or repressing translation. mRNA profiling can be used to identify some miRNA targets, but fails to identify targets that change only at the protein level and to distinguish direct from indirect targets. We have over-expressed or inhibited miR-455-3p and performed whole genome arrays to identify genes that are regulated at the mRNA level. By combining the analysis of whole genome array data and the analysis of the promoter sequences of regulated genes, the purpose of this study was to identify both targets that are regulated at the mRNA or only at the protein level.
Methods: Primary chondrocytes were transfected with either 50nM miR-455-3p mimic or inhibitor or non-targeting controls. Cells were incubated for 48 hours and harvested for mRNA using Trizol (Invitrogen). Illumina whole genome micro-array analysis was performed to identify genes regulated at the mRNA level. We further analysed the 3'UTR sequence of regulated genes for mir-455-3p binding sites using the software package R. We also used pathway analysis to identify groups of functionally related genes. To identify genes that change only at the protein level, the promoter sequences (2000nt downstream of the transcriptional start site) of all regulated genes were downloaded from Ensembl (www.Ensembl.org) and analysed for all 6mer binding motifs (AAAAAA, AAAAAT, etc 4^6 = 4096 motif sequences) using the software package R. To analyse putative targets of miR-455-3p the 3' UTRs of potential targets were subcloned downstream of a luciferase gene for experimental validation.
Results: Following the over-expression or inhibition of miR-455-3p, we have generated mRNA expression profiles that have identified a number of genes regulated at the mRNA level or protein level. Genes where the mRNA was both decreased by over-expression of miR-455-3p and increased by its inhibition, and contain a miR-455-3p-binding site, include members of the WNT signalling pathway including DKK1, beta-catenin, and GSK. Following analysis of the promoter sequences of regulated mRNA, two motifs were identified, CACGTG and CAGGTG which are binding sites for the transcription factor TCF3, itself implicated in WNT signalling. Luciferase assays confirmed TCF3 as a direct target of miR-455-3p.
Conclusions: We have combined different bioinformatic analyses of mRNA datasets to identify direct targets of miR-455-3p which change at the mRNA level or only at the protein level. Pathway analysis has also revealed that miR-455 may regulate multiple points in the WNT signalling pathway.

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miRNA EXPRESSION PROFILE IN MESENCHYMAL STEM CELLS FROM OA PATIENTS DURING OSTEOGENIC DIFFERENTIATION

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Purpose: Osteoarthritis (OA) is a common and disabling musculoskeletal disorder. The most evident feature of OA is articular cartilage loss however changes in bone also occur, including increased volume and reduced mineralization. Homeostasis of bone tissue largely relies on the ability of self-renewal and differentiation of precursor mesenchymal stem cells (MSCs) driven by the activity of signalling pathways such as the Wnt, MAPK, TGF-β and Hedgehog. These set of cellular processes interact and integrate the signals in a highly regulated manner in which miRNAs can play a critical role. miRNAs act silencing the expression of different genes at the translational level and therefore can interfere through the activation or inhibition of essential processes involved in cell fate commitment.
The main objective of this work was to identify comparatively the miRNA expression profile occurring during MSCs osteogenic differentiation in vitro in MSCs isolated from OA patients and healthy subjects to decipher their possible relevance in OA pathology.
Methods: MSCs isolated from the bone marrow of 5 OA (OA-MSCs) and 3 healthy individuals (H-MSCs) with similar ages were isolated, characterised and further induced to osteogenic differentiation at the sixth passage. mRNA was collected at different time points: 0, 10 and 21 days. The miRNA pool was isolated and the expression profile of 754 well-characterized miRNA sequences from the Sanger miRBase v14 were obtained using the TaqMan® OpenArray® Human miRNA Panel, according to the manufacturer instructions. Raw data was analyzed using generalized lineal models. The potential role of the differentially expressed miRNAs was assessed using the predicted targets provided in the mirTar database.
Results: Expression profiling during osteogenic differentiation revealed at t=0 the differential expression of 3 miRNAs: miR-320; miR-616 and miR-99b (p=0.002) in undifferentiated OA-MSCs compared with H-MSCs. miR-320 and miR-99b, initially downregulated In OA-MSCs, showed a progressive increase at t=10 and t=21 during differentiation process. Conversely, expression levels of miR-616, initially upregulated in OA-MSCs, progressively decrease during differentiation process. Although no significant differences were found, the opposite trend was observed for H-MSCs. The functional pathway analysis for the differentially expressed miRNAs showed that several of their predicted or validated target genes were involved in signalling pathways essentials for the maintenance of bone homeostasis, these include several proteins of the Wnt, MAPK, TGFβ and Hedgehog signalling pathways.
Conclusions: Bone homeostasis alterations are one of the features characterising OA pathology. Here we have described that miR-320; miR-616 and hsa-miR-99b likely involved in the modulation of crucial signalling pathways for MSCs osteogenic commitment. To our knowledge none of them have been described previously in this biological context. Although further validation of results is required to define their particular or jointly contribution, these preliminary results suggest their role in OA pathophysiology. A more complete understanding of these differential expressed miRNA regulatory functions is likely to shed light on the molecular mechanism dysregulated in OA an thus could be useful for the development of novel therapeutic approaches.

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MIR-381-3P PARTICIPATES IN CHONDROGENESIS AND CARTILAGE DEGRADATION BY ALLEVIATING COLLAGEN 2 EXPRESSION AND ENHANCING MMP13 EXPRESSION

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Purpose: To investigate the biological effect of mmu-miR-381-3p on chondrogenic differentiation and interleukin-1beta (IL-1b) induced arthritis model.
Methods: Chondrocyte-like ATDC5 cell line was stimulated with ITS+ premix to form cartilage nodules. Total RNA was isolated and reverse