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THE HUMAN ENTHESIS CONTAINS POPULATIONS OF MESENCHYMAL STEM CELLS WITH DISTINCT FUNCTIONAL CHARACTERISTICS

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Background: Ankylosing spondylitis (AS) is associated with enthesal inflammation and new bone formation. Resident populations of lymphocytes have been identified at the enthesis that on stimulation with IL-23 produce pro-inflammatory cytokines including IL-17A and IL-22 (1) which drive inflammation and may also influence osteogenesis. Surprisingly, enthesis resident mesenchymal stem cells (MSCs) have not been phenotypically or functionally characterised.

Objectives: To determine if human enthesis including enthesal soft tissue and peri-enthesal bone harbours a population of MSCs. Furthermore, to investigate the effect of spondyloarthritis associated pro-inflammatory cytokines on MSC osteogenesis.

Methods: Samples from healthy the spinous process and interspinous ligament (male = 5, female = 10, median age = 19) were divided into enthesal soft tissue (EST) and peri-enthesal bone (PEB) and enzymatically digested (1). MSCs content was assessed using a CFU-F assay. Flow cytometry was used to examine expression of MSCs specific markers in plastic adherent cultures. Following osteogenic, chondrogenic and adipogenic inductions, osteogenesis was qualitatively by alkaline phosphatase and alizarin red staining and quantitatively by measurement of calcium accumulation. Chondrogenesis and adipogenesis were assessed using glycosaminoglycan assay and oil red o staining respectively. Osteogenic cultures were also supplemented with IL-17A (50ng/ml), IL-22 (10ng/ml) or TNF- α (1ng/ml).

Results: As a proportion of total cellularity EST developed approximately 5 fold more colonies than matched PEB ($p < 0.0001$). Cultured cells were overwhelmingly positive for expression of MSC markers CD73, CD90, CD105 (PEB median 98.66% range: 95.17-98.96%, EST median 98.42% range: 87.69-98.7%) and negative for CD14, CD19, CD45 and HLA-DR (PEB median 0.34% range: 0-2.91%, EST median 1.1% range: 0-2.53%) however some CD34 expression was noted particularly in EST cultures (median 3.74% range: 0-29%). Both populations were capable of tri-lineage differentiation, although PEB MSCs had greater osteogenic ($p < 0.025$) and lower adipogenic ($p < 0.05$) potential than matched EST MSCs. Addition of IL-22 ($p < 0.01$) or IL-17A ($p < 0.005$) caused a significant decrease in the calcium deposited in PEB MSCs but not in EST MSCs.

Conclusion: Both the EST and PEB contain cells that meet the ISCT criteria defining MSCs. However, MSCs from these sources are functionally distinct in terms of their differentiation potential and response to inflammatory cytokines. The cytokines tested had a negative influence on osteogenesis in the conditions tested.

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MIRNA-150 AS A NOVEL REGULATOR OF POST-TRAUMATIC OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a chronic joint disease causes irreversible damage to the articular cartilage resulting in loss of joint function and subchondral bone remodeling. There is no cure for OA and currently the available treatment like pain management, and joint replacement surgery is used to treat this disease. MiRNAs are small non-coding RNAs consist of 21-29 nucleotides which regulate gene expression by targeting the 3' untranslated region (UTR) leading to translation inhibition. In recent years, many studies showed that microRNAs could be a target for many diseases including OA. Our preliminary data on osteoactivin showed that the GPNMB acts as a protective protein in osteoarthritis by reducing catabolic gene expression. Our lab group results have been shown that miRNA-150 targets the 3'UTR of osteoactivin in osteoblast cells.

Objectives: There is no study regarding the role of miRNA 150 on cartilage homeostasis and because the miRNA 150 targets osteoactivin in lung and bone cells, we hypothesized that miRNA-150 targets osteoactivin in chondrocytes and protect cartilage ECM degradation.

Methods: RNAs were isolated from primary chondrocytes of miRNA-150 knockout pups, then was used in qPCR to measure gene expression. Western blot used to detect MMP-3, MMP-9, MMP-13, IL-6, collagen type-II and aggrecan. In addition, histological analysis was used to test the role of miRNA-150 vivo through evaluating proteoglycan degradation by toluidine blue staining and immunohistochemistry after 12 weeks from DMM surgery. PCR transcription factors and signaling pathway finder array were used to elucidate the altered genes and transcriptions factors compared to the wild type in primary chondrocytes culture.

Results: Primary chondrocytes from 150 KO mice showed high mRNA expression levels of GPNMB compared to the wild type mice. Medial tibia plateau (MTP) and medial femur condyle (MFC) have been graded by OARS scoring system after 12 weeks of DMM surgery. Histological results showed that the 150 KO mice have less cartilage damaged compared to the wild type. To determine whether epigenetic modifiers (miRNAs) regulating GPNMB/Osteoactivin expression can modulate the cartilage protective effects of GPNMB/Osteoactivin, we found that miRNA 150 targets GPNMB/Osteoactivin in chondrocytes. Next, we examined the effects of miRNA 150 deficiency on the OA progression in vivo and found that miRNA 150 KO are more protected against post-traumatic OA compared to WT mice. Furthermore, we assessed the expression of GPNMB/Osteoactivin in miRNA 150 KO chondrocytes and showed a significant increase compared to WT cells.

Conclusion: These results showed that miRNA is negative regulator of cartilage homeostasis and blocking miRNA might have therapeutic potential for the treatment of age-related osteoarthritis and post-traumatic induced osteoarthritis.

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