12 INHIBITORY EFFECT OF MIR-29A ON THE CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Background and objectives Mesenchymal stem or stromal cells (MSC) are multipotent cells that can differentiate into different lineages, particularly osteoblasts and chondrocytes. The differentiation process of MSC is regulated by various molecules among which Sox9 and Runx2 are key transcription factors leading, respectively to chondrogenesis or osteogenesis. Recently, a new class of regulating factors, namely microRNAs (miRNAs), has been shown to be important for differentiation processes but few miRNAs have been shown

to regulate chondrogenesis. The objective of this study is therefore to identify miRNAs involved in the chondrogenic differentiation of MSC.

Materials and Methods MiRNA arrays have been done using RNA samples of MSC (day 0) and MSC-derived prechondrocytes (day 3). Analysis of miRNAs, their putative targets and of transcription factors putatively binding to their promoter regions was performed using several prediction softwares, in particular Targetscan. Pre-miRs and antagomiRs were transfected in MSC twice (day 4 and 1) using oligofectamine and chondrogenic differentiation was induced by culture of MSC in micropellets in inductive medium for 21 days. Expression of chondrocyte markers was performed by RT-qPCR.

Results Analysis of results from the miRNA arrays together with those from DNA arrays already available in our laboratory indicated that a little number of transcription factors was theoretically able to regulate the majority of the miRNAs that were modulated at day 3 of chondrogenesis. Among the transcription factors, Sox9 and YY1 can putatively bind to the promoter region of miR-29. Using real-time RT-PCR, the authors observed that the expression level of miR-29 progressively and highly decreases during the chondrogenic differentiation. Transfection of Sox9 or YY1 in the Stro-1A MSC cell line significantly reduced the expression of miR-29, while transfection of both factors totally abolished its expression. The effect of gain- and loss-of-function of miR-29 during the chondrogenic differentiation of MSCs by transfecting pre-miRs or antago-miRs confirmed the role of miR-29 during chondrogenesis.

Conclusions Our preliminary data show that, during chondrogenesis, miR-29 expression is downregulated, probably through the interaction of Sox9 and YY1 on the miR promoter region. Because miR-29 has been described to regulate different targets (DKK1, sFRP2, Kremen2 and CDK6 whose expression decreases during differentiation), future experiments will investigate whether these target genes are modulated by miR-29.