Distinct role of Snail and Twist in guiding regenerative potential of human adult stem cells

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INTRODUCTION: The epithelial-mesenchymal transition (EMT) plays a critical role in embryonic development, cancer metastasis, and injury healing [1]. Typical characteristics of EMT include a downregulation of epithelial markers and upregulation of mesenchymal markers. Given the potential role of EMT in mesenchymal tissue regeneration [2], we hypothesize that overexpression of Snail1 and Twist1, two important EMT transcription factors, have a distinct influence on mesenchymal differentiation of human adult stem cells.
METHODS: Human adult synovium-derived stem cells (SDSCs) were transduced with lentiviruses (multiplicity of infection=2) carrying Twist1 (pRRLsin-cPPT-SFFV-TWIST-wpre) (TWIST1) and Snail1 (pRRLsin-cPPT-SFFV-Snail-wpre) (SNAIL1), respectively, with lentivirus carrying green fluorescence protein (GFP) and non-transduction (CTR) as controls. Expanded cells were measured for proliferation capacity using population doubling time. Expanded cells were assessed using flow cytometry for MSC surface markers, including CD73, CD90, CD105, CD146, and SSEA4, and using TaqMan real-time qPCR (RT-qPCR) for stemness gene expression, including SOX2, NANOG, NES, POU5F1, KLF4, MYC, BMI1, and NOV. For chondrogenic capacity, expanded cells were incubated in a pellet culture system supplemented with chondrogenic induction medium for 21 days. The evaluation included RT-qPCR for expression of SNAIL1 and TWIST1 as well as chondrogenic related genes (SOX9, ACAN, PRG4, COL1A1, COL2A1, and COL10A1) and Alcian blue staining (Ab) for sulfated GAG and immunohistochemical staining (IHC) for ALPL, and types I, II, and X collagen. For adipogenic capacity, expanded cells were incubated in adipogenic induction medium for 21 days. The evaluation included RT-qPCR for expression of SNAIL1 and TWIST1 as well as adipogenic related genes (CEBPA, PPARG, and LPL) and Oil Red O (ORO) staining for lipid droplets and quantification. For osteogenic capacity, expanded cells were incubated in osteogenic induction medium for 21 days. The evaluation included RT-qPCR for expression of SNAIL1 and TWIST1 as well as osteogenic related genes (ALPL, SPARC, and COL1A1) and Alizarin Red S (ARS) staining for calcium deposition and quantification. Mann-Whitney U test was used for pairwise comparison. Different letters indicate a statistically significant difference compared to the groups within the same culture condition ($p < 0.05$).
RESULTS: The successful transduction of lentivirus was validated using immunofluorescence staining and Western blot to target SNAIL1 and TWIST1. SNAIL1 and TWIST1 transduction promoted proliferation rate, assessed by population doubling time (2.65 day for TWIST1 and 2.87 day for SNAIL1 versus 6.61 day for GFP and 3.85 day for CTR). SNAIL1 and TWIST1 transduction yielded SDSCs with increased expression of SOX2, NES, and MYC but decreased expression of POU5F1, KLF4, and NOV. Forced expression of SNAIL1 also increased expression of CD73, CD90, and CD146 but decreased expression of CD105 and SSEA4. Compared to the control group, SNAIL1 transduction yielded larger SDSC pellets with intensive staining of Alcian blue for sulfated GAG and immunostaining for types I and II collagen while TWIST1 overexpression diminished both pellet size and staining (**Fig. 1A**). Interestingly, SNAIL1 overexpression significantly increased expression of chondrogenic markers (SOX9, ACAN, COL1A1, and COL2A1) and a hypertrophic marker (COL10A1) but decreased expression of PRG4 while TWIST1 overexpression significantly decreased expression of chondrogenic markers (SOX9, ACAN, COL2A1, and COL10A1) (**Fig. 1B**). TWIST1 overexpression decreased ORO staining while SNAIL1 overexpression significantly increased ORO staining indicative of producing more lipid droplets (**Fig. 1C**). Both transcription factors benefited osteogenic differentiation, particularly for SNAIL1 transduced SDSCs (**Fig. 1D**).
DISCUSSION: A previous study showed that overexpression of either TWIST1 or SNAIL1 in breast epithelial cells exhibited multi-lineage differentiation potential similar to mesenchymal stem cells [3]. We found that TWIST1 and SNAIL1 responded differently in guiding the regenerative potential of adult stem cells. Compared to dynamic expression of TWIST1 in mesenchymal differentiation, SNAIL1 strongly supports chondrogenic/adipogenic/osteogenic differentiation of mesenchymal stem cells.
SIGNIFICANCE/CLINICAL RELEVANCE: Modulation of EMT signals might be a new approach in promoting stem cell-based tissue engineering and regeneration.
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Figure 1