Nanomechanics of human adipose-derived stem cells: small GTPases impact chondrogenic differentiation.

Abstract:
Human adipose-derived stem cells (ASCs) show gene expression of chondrogenic markers after three-dimensional cultivation. However, hypertrophy and osteogenic transdifferentiation are still limiting clinical applications. The aim of this study was to investigate the impact of small GTPases (Rac1 and RhoA) on transforming growth factor (TGF)-β1-mediated chondrogenic differentiation of ASCs and compare it with BMP-2-induced hypertrophy, by assessing effects on intracellular and extracellular matrix. In a novel experimental approach we characterized differentiation of living stem cells by single-cell elasticity measurements using atomic force microscopy. Results were matched with single-cell size measurements (diameter and volume) and quantitative real time-polymerase chain reaction for osteogenic and hypertrophic (alkaline phosphatase [ALP], collagen type X) as well as chondrogenic (collagen type II) gene expression. Intracellular F-actin expression was visualized by phalloidin staining of alginate-embedded ASCs. Statistical analysis was performed using analysis of variance (ANOVA) and two-sided t-test. Nontreated two-dimensional cultured ASCs (2D ASC) showed a significantly lower deformability than chondrocytes (Young’s modulus: 294.4 vs. 225.1 Pa; ANOVA; p<0.001). Standard chondrogenic stimulation
decreased stem cell elasticity to chondrocyte values (221.7 Pa). All other chondrogenic differentiated ASCs presented intermediate elasticity (BMP-2 stimulation: 269.1 Pa; Rac1 inhibition: 279.8 Pa; RhoA inhibition: 257.8 Pa; p<0.05 compared to 2D ASC). F-actin fluorescence was visually decreased in Rac1-inhibited cells and increased in BMP-2-stimulated cells. Cell volume of 2D ASCs (6382.3 fL; p<0.001) was significantly higher than in all stimulated samples (BMP-2: 3076.7 fL; RhoA inhibition: 3126.0 fL). Volume of stem cells after standard chondrogenic stimulation (2590.0 fL) was not significantly different from chondrocyte volume (2244.9 fL). Rac1-Inhibitor reduced stem cell volume significantly below chondrocyte volume (1781.1 fL). Regarding mRNA expression, Rac1-Inhibitor reduced late hypertrophic transdifferentiation (collagen type X), while collagen type II production slightly increased (p<0.05). RhoA-Inhibitor increased osteogenesis (ALP) and slightly decreased collagen type II production (p<0.05). Biologically relevant nanomechanical parameters contribute to the evaluation of stem cell differentiation, in view of increased deformability of stem cells after chondrogenic stimulation. Regarding gene expression, Rac1 inhibition reduced hypertrophic chondrogenic differentiation and RhoA inhibition increased osteogenic transdifferentiation. Thus, the control of small GTPases is promising for cartilage tissue engineering purposes of stem cells.

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