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Expressions of pathological markers in PRP based chondrogenic differentiation of human adipose derived stem cells

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Background & Aim: Optimization of the differentiation medium through using autologous factors such as PRP is of great consideration, but due to the complex, variable and undefined composition of PRP on one hand and lack of control over the absolute regulatory mechanisms in *in vitro* conditions or disrupted and different mechanisms in diseased tissue microenvironments in *in vivo* conditions on the other hand, it is complicated and rather unpredictable to get the desired effects of PRP making it inevitable to monitor the possible pathologic or undesired differentiation pathways and therapeutic effects of PRP. Therefore, in this study the probable potential of PRP on inducing calcification, inflammation and angiogenesis in chondrogenically differentiated cells was investigated.

Methods: The expressions of chondrogenic, inflammatory, osteogenic and angiogenic markers from TGF or PRP-treated cells during chondrogenic differentiation of human adipose-derived stem cells (ADSCs) was evaluated. Expressions of Collagen II (Col II), Aggrecan, Sox9 and Runx2 were quantified using q-RT PCR. Expression of Col II and X was investigated by immunocytochemistry as well. Glycosaminoglycans (GAGs) production was also determined by GAG assay. Possible angiogenic/inflammatory potential was determined by quantitatively measuring the secreted VEGF, TNF and phosphorylated VEGFR2 via ELISA. In addition, the calcification of the construct was monitored by measuring ALP activity and calcium deposition.

Results: Our data showed that PRP positively induced chondrogenesis; meanwhile the secretion of angiogenic and inflammatory markers was decreased. VEGFR2 phosphorylation and ALP activity had a decreasing trend but tissue mineralization was enhanced upon treating with PRP.

Conclusions: Although reduction in inflammatory/angiogenic potential of the chondrogenically differentiated constructs highlights the superior effectiveness of PRP in comparison to TGF for chondrogenic differentiation, yet further improvement of the PRP-based chondrogenic differentiation media is required to inhibit the production of angiogenic/inflammatory markers, calcification and the release of synthesized GAG out of the construct.

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