TGF-β3 and Three-dimensional Culture of Human Adipose-Derived Mesenchymal Stem Cells Stimulate a Disc-like Proteoglycan and Collagen Rich Extracellular Matrix

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INTRODUCTION:

During disc degeneration, disc cell viability and gene expression fail to adequately maintain the extracellular matrix (ECM); this results in proteoglycan loss, disc dehydration and annular tears, which may progress to disc herniation. Stem cell therapy has potential application in the biologic treatment of disc degeneration. Due to ease of harvest and abundance, adipose-derived mesenchymal stem cells (AD-MSC) have shown utility in tissue engineering [1, 2]. Treatment with transforming growth factor-beta 3 (TGF-β3) has been found to be a useful tool in manipulating stem cells [3]. Here we show that human AD-MSC grow well in three dimensional (3D) culture, and respond to TGF-β3 by production of a disc-like extracellular matrix.

METHODS:

Studies using human tissue were approved by our Institutional Review Board. Adipose tissue was brought directly from surgery to the laboratory. AD-MSC were extracted by collagenase digestion, filtration, centrifugation and plating.

Isolated AD-MSC were confirmed to be stem cells by their plastic adherence, chondrogenic and osteogenic differentiation, and localization of surface markers. Chondrogenic differentiation was confirmed using micromass culture; cells were grown for 7-10 days in Chondrogenic Induction Medium (Cambrex BioScience) (Figure 1A). Osteogenic differentiation of AD-MSC used culture with the Osteogenesis Kit (Chemicon Internatl.), following which positive alizarin red staining confirmed mineralized matrix formation (Figure 1B). Positive localization of the cell surface markers CD105, CD29, CD90 and CD 44, and negative localization of CD45 and CD44 also supported the isolation of stem cells [4].

AD-MSC were directed toward a disc-like phenotype by seeding 100,000 cells into a 0.5 mm3 3D collagen scaffold and exposure to TGF-β3 for 2 weeks. Proteoglycan content was characterized qualitatively by toluidine blue staining for proteoglycans, and quantitatively by DMB measurement of sulfated proteoglycans. ECM produced within the 3D constructs was characterized using immunohistochemical localization of types I and II collagen, chondroitin sulfate, decorin and keratin sulfate.

Statistical analysis used SAS version 8.2. A p-value of less than 0.05 was considered statistically significant. Data are presented as means ± SD. Experiments were replicated with AD-MSC from 5 patient samples, and experiments were run in duplicate.

RESULTS:

When AD-MSC were exposed to TGF-beta3 in 3D culture, greater extracellular matrix was formed which contained types I and II collagen, keratin sulphate, and decorin. Figure 1C showed localization of chondroitin sulfate and collagen type II (black localization product); note greater localization in TGF-β3 treated AD-MSC vs control (non-treated) cultures. Biochemical measurement of proteoglycan production showed that proteoglycan production was significantly greater in TGF-β3 treated AD-MSC in 3D culture vs 3D untreated control cells (P < 0.05, Figure 1D).

CONCLUSIONS:

Stem cell plasticity offers potential for future biologic therapies for disc degeneration. Data presented here show that human AD-MSC can successfully be manipulated in 3D culture to express gene products important in the ECM of the disc, such as types I and II collagen, chondroitin sulfate, keratin sulfate and decorin. Further work is underway to more fully characterize the expression patterns of AD-MSC in 3D and growth factor-enhanced microenvironments which could be adapted to autologous cell therapy for disc degeneration.

REFERENCES:


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