**INTRODUCTION:** Fibrocartilage is a specialized tissue sharing characteristics of fibrous tissue and, to various degree, cartilage. In humans, fibrocartilage is found knee meniscus, ligament/tendon insertion to bone, the intervertebra disc (IVD), and the temporomandibular joint (TMJ). All fibrocartilage tissues are intrinsically recalcitrant to regeneration. We recently demonstrated that fibroblasts can be derived from human mesenchymal stem/stromal cells (Lee et al. JCI, 2010). In the present study, we take an additional step to investigate whether fibrochondrocytes can be derived from human mesenchymal stem cells (MSCs), with a motivation to generate potential sources of therapeutic cells for the regeneration of fibrocartilage tissues.

Cells of fibrocartilage are not well understood but are frequently referred to as fibrochondrocytes. Although MSCs are the presumably source of fibrochondrocytes in development, there is little evidence that fibrochondrocytes derive from postnatal MSCs. In comparison with known and recently demonstrated pathways of osteogenic, chondrogenic, and adipogenic differentiation, little is known about the induction cues and signaling pathways for fibrochondrogenic differentiation. **The hypothesis of the present study is that a reproducible and reliable protocol can be delineated to derive fibrochondrocyte-like cells from human bone marrow derived MSCs.** Sequential or combined treatment of connective tissue growth factor (CTGF) and transforming growth factor β3 (TGFβ3) was tested in monolayer (2D) or 3D pellet culture of hMSCs. We show that sequential or combined CTGF and TGFβ3 treatment yielded cells characteristic of fibrochondrocytes by a multitude of assays.

**METHODS:** **Cell isolation and differentiation:** MSCs were isolated from human bone marrow and culture-expanded per our previous method (refs). P2 or 3 hMSCs (100,000 cells/well) were plated in 6-well culture plates, or 3D pellets were formed by centrifuging 2M hMSCs in 15 mL conical tubes. Monolayered (2D) cells or 3D cell pellets were treated by 1) 100 ng/mL CTGF for 2 wks followed by 10 ng/mL TGFβ3 (C-T), 2) 10 ng/mL TGFβ3 for 2 wks followed by 100 ng/mL CTGF for 2 wks (T-C), 3) mixture of 100 ng/mL CTGF and 10 ng/mL TGFβ3 for 4 wks, or 4) CTGF for 4 wks (C4), TGFβ3 for 4 wks (T4) and growth medium as controls. Fibroblastic induction supplement (FBS) (50 μg/mL ascorbic acids) and chondrogenic induction supplements (CIS) (1% ITS+1 solution; 50 μg/mL sodium pyruvate, 50 μg/mL L-asorbic Acid 2-phosphate, 40 μg/mL L-proline, 0.1 μM dexamethasone) were included in the CTGF and TGFβ3 treatment, respectively. **Analysis of fibrochondrogenic differentiation:** Upon 4 wks treatments, harvested samples (monolayers and pellet sections) were stained with Alcian blue (AB) and Picrosirious red (PR). GAGs and COL were quantitatively assayed (Biocolor, UK) and normalized to expression of proCOL-I+/proCOL-II+ in positive control. Sequential release of CTGF and TGFβ3 from microspheres: CTGF and TGFβ3 were encapsulated in 50:50 PLGA μ-spheres (μS) and 75:25 PLGA μ-spheres, respectively. Two different PLGA ratios were applied to provide sequential release of the two growth factors. Both PLGA μ-spheres (1 mg/mL containing CTGF and TGFβ3) were then embedded in 3D fibrin gel loaded with MSCs (10M cells/mL) and cultured for 4 wks in vitro.

**RESULTS:** After 4 wks, C-T and C+T groups generated notable fibrocartilaginous matrix, positively stained by AB and PR both in 2D and 3D (Fig. 1H, J, N, P) (2D data not shown). Interestingly, T-C group showed abundant COL deposition (Fig. 1G) but limited PG deposition (Fig. 1J). Positive controls, C4 and T4, showed only COL or PG, respectively (Fig. 1G, L). Quantitatively, GAGs amount in C-T was significantly higher than T-C, C+T and control (Fig. 2A). C+T group showed significantly more GAGs than T-C and control (Fig. 2A). COL amount was significantly higher in T-C group than C-T, C+T and control (Fig. 2A). In addition, COL and C+T showed significant increases in COL as compared to control (Fig. 2B). Immunofluorescence demonstrated that C-T and C+T treatments induced differentiation of hMSCs into proCOL-I+/proCOL-II+ fibrochondrocyte-like cells both in 2D (Fig. 3) and 3D (data not shown). Cells treated with T-C showed strong expression of proCOL-I but limited expression of proCOL-II+ (Fig. 3). Quantitatively, the number of proCOL-I+/proCOL-II+ in C-T (76.9±23%) was significantly higher than in control, T-C, and C+T groups (p<0.05).

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**REFERENCES**