Effects of late passage small umbilical cord-derived fast proliferating cells on tenocytes from degenerative rotator cuff tears in interleukin 1β-induced tendinopathic conditions

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INTRODUCTION: Tendinopathy, a degenerative tendon disease, manifests through pain, swelling, and tenocyte dysfunction as a result of chronic inflammation. Mesenchymal stem cells (MSCs) are recognized as a promising therapeutic tool in regenerative medicine due to their anti-inflammatory effects on various musculoskeletal diseases. However, for these cells to be clinically viable, extensive culturing is required to expand the cell population, a process which may compromise their anti-inflammatory capabilities. In previous research, we established a unique set of MSCs, dubbed "small, umbilical cord-derived fast proliferating cells" (smumf cells), derived from the umbilical cord using a novel minimal cube explant isolation method. These smumf cells retained their superior MSC characteristics even after undergoing long-term culture. Thus, the purpose of present study was to assess the anti-inflammatory effects of smumf cells at a late passage stage (P10) on tenocytes derived from degenerative rotator cuff tears under IL-1β-induced tendinopathic conditions.

METHODS: Conditioned medium (CM) and bioactive materials secreted from IL-1β treated smumf cells were analyzed using multiplex assays. Using co-culture with IL-1β treated smumf cells, proliferation of tenocytes was evaluated. Expression and synthesis of molecules with respect to inflammation, tendon matrix turnover, apoptosis, and upstream signal pathway of MAPK and NF-κB of tenocytes were evaluated with qPCR and Western blotting.

RESULTS: Among substances secreted by smumf cells, the levels of HGF (18.2-fold), G-CSF (2.4-fold), PDGFR-β (1.7-fold), IGBP-3 (1.6-fold) and FGF-7 (1.5-fold) were found to be the highest. Co-culture of smumf cells under normal culture conditions without IL-1β significantly increased the proliferation of tenocytes by 6.1%, 13.5%, and 43.3% at 1, 3, and 7 days, respectively. On the other hand, in an inflammatory environment in the presence of IL-1β, co-culture of smumf cells did not affect tenocytes proliferation, but when IL-1β was removed, tenocytes proliferation increased 1.2-fold at 7 days. After co-culture with IL-1β, *Scx, Mkx*, and *Col1a1* expression was not changed in tenocytes, while the gene expression of *Egr-1, Egr-2, Collagen type 1/3* ratio, and *bFGF* were found to be significantly up-regulated, which were 1.3-, 1.4-, 2.1- and 1.8-folds higher than IL-1β treated tenocytes. smumf CM reduced the protein expression of COX-2 and IL-6 induced by IL-1β by 28.3% and 23.3%, respectively, and enhanced IL-10 by 1.4-fold. CM also decreased the protein expression of MMP-1, -3, and -9 by 31.3%, 77.9%, and 56.3%, respectively, and the BAX/BCL2 ratio by 44.9% compared to IL-1β-treated tenocytes. NF-κB and MAPK signaling pathways were activated by IL-1β, and CM reduced the levels of p-IκBα and p-p65 by 24.2 % and 55.5%, respectively, and p-JNK and p-p38 by 38.5% and 65.1%, respectively.

DISCUSSION: The most important findings of this study were: 1) The smumf cells of P10, which are not different from the early passage cells, secrete a lot of various growth factors at P10, especially, 1,938 pg/ml bFGF and 2,043 pg/ml HGF in normal conditions, and 2.2-fold and 1.9-fold more bFGF and HGF in inflammatory conditions, respectively; 2) Tenocytes co-cultured with smumfs cells increased ten proliferation by 1.5-fold, and showed no difference in the inflammatory environment, but increased by 1.2-fold when inflammation was removed; 3) Co-culture with smumf cells in an inflammatory environment increased the expression of *Egr-1* (1.3-fold), *Egr-2* (1.4-fold), *Collagen type 1/3 ratio* (2.1-fold) and *bFGF* (1.8-fold) of tenocytes compared to IL-1β-induced tenocytes; 4) smumf CM reduced the expression of pro-inflammatory related proteins IL-6 and COX-2 induced by IL-1β and enhanced the anti-inflammatory related protein IL-10. smumf CM also reduced the protein expression of MMP-1, -3, and -9 and decreased the anti-apoptosis-related BAX/BCL2 ratio compared to IL-1β-treated tenocytes; 5) NF-κB and MAPK signaling pathways were activated by IL-1β, and these pathways were attenuated in tenocytes by smumf cells CM. Taken together, these findings demonstrated that smumf cells at a late passage stage (P10) have anti-inflammatory effects on tenocytes derived from degenerative rotator cuff tears under IL-1β-induced tendinopathic conditions.

CLINICAL RELEVANCE: smumf cells at late passage which mimic stem cell therapy in real-world clinical practice exhibit anti-inflammatory on tenocytes from human degenerative rotator cuff tears in tendinopathic conditions. Therefore, smumf cells have good potential in tendon repair and regeneration.

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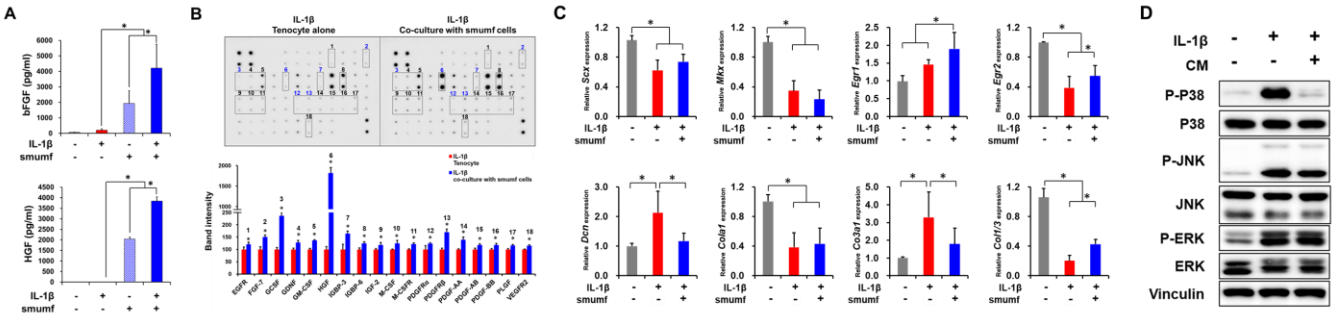

Figure. A, B. Bioactive factors secretion in co-cultured smumf cells under inflammatory conditions C. Effects of smumf cells co-culture on expression of tenogenic marker D. Effects of smumf CM on IL-1β-induced MAPK signaling pathways. The quantitative results are means ± SD of three independent experiments. The significances of differences were determined using the independent t-test and one-way analysis of variance with Tukey's test. * $P < 0.05$