Dynamic Effects of Transforming Growth Factor Beta-1 (TGFβ1) in Primary Human Knee Fibroblasts

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INTRODUCTION: Arthrofibrosis is a debilitating complication after total knee arthroplasties (TKAs) wherein excess scar tissue deposition restricts knee range of motion. The inflammatory cytokine transforming growth factor beta-1 (TGFβ1) is commonly used to induce the fibrotic phenotype (e.g., collagen deposition and ACTA2 expression) in tissue models of fibrosis. While a concentration of 10 ng/mL of TGFβ1 is typically used to induce the fibrotic phenotype in cell culture models, relevant TGFβ1 parameters such as dosing and timing remain largely undefined in primary knee cell models of arthrofibrosis. The purpose of the present study was to define the dosing regimen of TGFβ1 necessary to induce key fibrotic features, including extracellular matrix deposition (ECM) and ACTA2 expression, in primary knee fibroblasts.

METHODS: Patients were consented according to an approved Institutional Review Board protocol. Primary knee fibroblasts were derived from patients undergoing primary TKA (PTKA) or revision total knee arthroplasty for arthrofibrosis (RTKA-A) via collagenase digestion. The resulting fibroblasts, which were selected by plastic adherence, were maintained in Advanced MEM media supplemented with 5% human platelet lysate, heparin, GlutaMAX™, and antibiotic/antimycotic agents. At confluence (Day 0), PTKA and/or RTKA-A fibroblasts were treated with increasing TGFβ1concentrations and timing intervals in the presence of ascorbic acid (50 ng/mL) in growth medium. Three days later (Day 3), collagen deposition was assessed by picrosirius red staining (PSR). Similarly, on day 3, RNA was harvested and mRNA levels for ACTA2 were assessed relative to GAPDH (housekeeping gene) by RT-qPCR analysis. For statistical significance: *a, b, c, d* correspond to *, **, ***, and ****, respectively, relative to vehicle (-); similarly, *w, x, y, z* correspond to *, **, ***, ****, respectively, relative to the standard TGFβ1 treatment (10 ng/mL) of 72 hours. Significance is noted as follows: (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$).

RESULTS: Continuous three day TGFβ1 treatment at various concentrations ranging from 0.625 ng/mL to 40 ng/mL, which included the standard dose of 10 ng/mL TGFβ1, induced similar levels of collagen deposition in RTKA-A (**Figure 1**) and PTKA (data not shown) fibroblasts. Interestingly, while 1 and 10 ng/mL TGFβ1 three day treatment resulted in similar ECM deposition in PTKA and RTKA-A fibroblasts (**Figure 2A**), ACTA2 expression was highly induced with 10 ng/mL TGFβ1, but not with 1 ng/mL TGFβ1 (**Figure 2B**). In RTKA-A (**Figure 3A**) and PTKA (data not shown) fibroblasts, a short 5-minute exposure of 10 ng/mL TGFβ1 was sufficient to maximally induce ECM deposition. However, when compared to the three-day long treatment, short treatments (5 to 30 minutes) with 1 ng/mL TGFβ1 were insufficient in inducing ECM deposition (**Figure 3A**). Interestingly, 30-minute TGFβ1 exposure at different doses resulted in a dose-dependent ECM deposition in PTKA and RTKA-A fibroblasts (**Figure 3B**).

DISCUSSION: The present study demonstrated that ECM deposition can be stimulated by a broad range TGFβ1 concentrations, but exposure time to this potent cytokine plays a critical role during for the myofibroblastic transformation of primary knee fibroblasts. Interestingly, a divergence between ECM deposition and ACTA2 expression is observed with different TGFβ1 concentrations.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study elucidated key TGFβ1 dosing and timing parameters that will inform the development and interpretation of *in vitro* models of arthrofibrosis.

IMAGES AND TABLES:

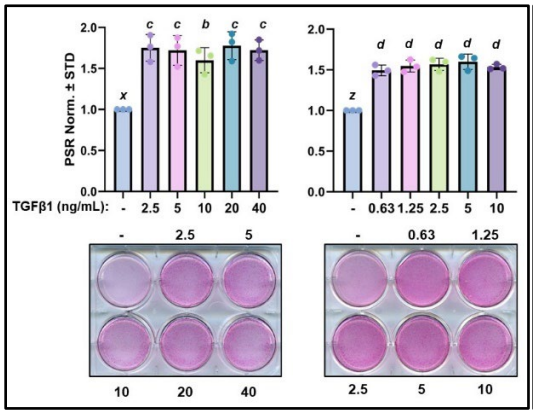

Figure 1. Similar ECM deposition rates by a broad range of TGFβ1 doses in RTKA-A fibroblasts. ECM quantification (top) and staining examples (bottom) as evaluated by PSR staining in RTKA-A cells treated for 72 hours. See methods section for interpretation of significance symbols.

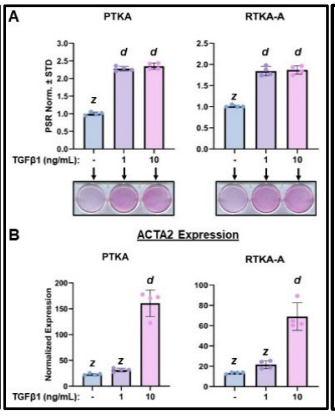

Figure 2. Divergent effects on ECM deposition and ACTA2 expression by different TGFβ1 doses. ECM quantification and examples (**A**) and ACTA2 expression (**B**) in PTKA and RTKA-A cells treated for 72 hours. See methods section for interpretation of significance symbols.

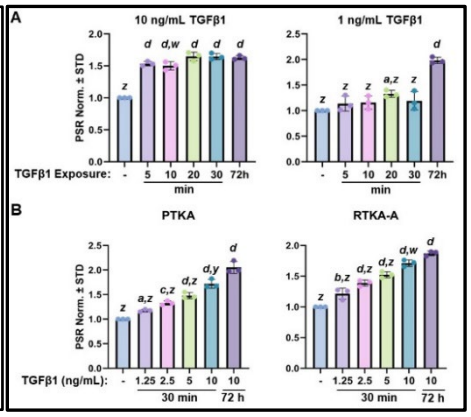

Figure 3. TGFβ1 timing and dosing in PTKA and RTKA-A fibroblasts. ECM quantification at 10 ng/mL (left) and 1 ng/mL (right) TGFβ1 with different lengths of exposure in RTKA-A fibroblast (**A**). ECM qualification of various TGFβ1 concentrations during a 30 min exposure (**B**). 10 ng/mL of TGFβ1 for 72 hours (i.e., 3 days) is used as the established standard. See methods section for interpretation of significance symbols.