

Induction of Cellular Autophagy Impairs TGFβ1-Mediated Myofibroblast Differentiation of Primary Knee Fibroblasts

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INTRODUCTION: Arthrofibrosis, characterized by aberrant matrix deposition (i.e., scar tissue formation) and resulting knee stiffness, is a common (~5%) complication of total knee arthroplasty (TKA). The role of cellular autophagy in organ fibrosis (e.g., pulmonary and hepatic) is conflicting. To date, studies have yet to address whether autophagy plays a role in arthrofibrotic pathways. Thus, the purpose of this study was to evaluate the contribution of cellular autophagy on TGFβ1-mediated myofibroblast differentiation of primary human knee fibroblasts.

METHODS: Patients were consented according to an approved Institutional Review Board protocol. Primary knee fibroblasts were isolated from patients undergoing primary TKA (pTKA) and revision TKA for arthrofibrosis (rTKA-A) via collagenase I digestion and Advanced MEM media supplemented with 5% human platelet lysate, heparin, GlutaMAX™, and antibiotic/antimycotic. At confluence (Day 0), cells were treated with Rapamycin (mTORC1 inhibitor and autophagy inducer, 10 nM or 50 nM), Bafilomycin A1 (autophagy inhibitor, targets autophagosome-lysosome fusion, 5 nM), or Pepstatin A (5 μg/mL) and E64D (5 μg/mL) combination (autophagy inhibitors – lysosomal protease inhibitors). The next day (Day 1), myofibroblast differentiation was induced via TGFβ1 (10 ng/mL) in the presence of ascorbic acid (50 ng/mL). On Day 3, extracellular matrix (ECM) deposition was visualized and quantified by picrosirius red staining. For starvation-based autophagy induction (i.e., amino acid deprivation), confluent cells were cultured for three hours in Earle's Balanced Salt Solution. Following the nutrient deprivation regimen, cells were treated with TGFβ1 in the presence of ascorbic acid. Three days later, picrosirius red staining and RNA isolation were performed. mRNA transcript levels for ACTA2 (myofibroblast marker) were evaluated, relative to GAPDH (housekeeping gene), via RT-qPCR analysis.

RESULTS: Induction of autophagy through mTORC1 inhibition by Rapamycin (10 nM and 50 nM) reduced ECM deposition, a critical cellular process associated with myofibroblast differentiation and arthrofibrosis, in both rTKA-A (data not shown) and pTKA fibroblasts (**Figure 1**). Disruption of cellular autophagy by blocking autophagosome-lysosome fusion (Bafilomycin A1) or inhibiting lysosomal proteases (Pepstatin A and E64D) did not alter the fibrotic phenotype (**Figure 1**). As observed with Rapamycin treatment, amino acid deprivation also suppressed TGFβ1-mediated collagen deposition in both pTKA (data not shown) and rTKA-A cells (**Figure 2A**). Furthermore, starvation-induced autophagy decreased the expression of ACTA2, a key myofibroblast marker, in pTKA and rTKA-A fibroblasts (**Figure 2B**).

DISCUSSION: This study demonstrated the anti-fibrotic properties of mTORC1 inhibition, suggesting that induction of autophagy may present a mechanism to suppress ECM deposition associated with arthrofibrosis. Beyond pharmacologic intervention, our studies also reveal that amino acid deprivation-based inactivation of mTORC1 reduced the fibrotic phenotype.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study suggests that induction of autophagy in the knee joint prior to TKA may be prophylactic against the pathogenesis of arthrofibrosis.

IMAGES AND TABLES:

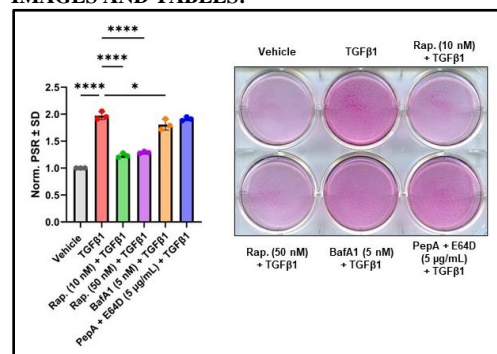


Figure 1. Rapamycin suppresses collagen deposition in pTKA fibroblasts. Fibroblasts were treated with Rapamycin (Rap.), Bafilomycin A1 (BafA1), or Pepstatin A (PepA) + E64D at confluence. ECM deposition by picrosirius red staining in pTKA cells (left = quantification, right = staining example). Significance is noted by asterisks (* = $p \leq 0.05$, **** = $p \leq 0.0001$).

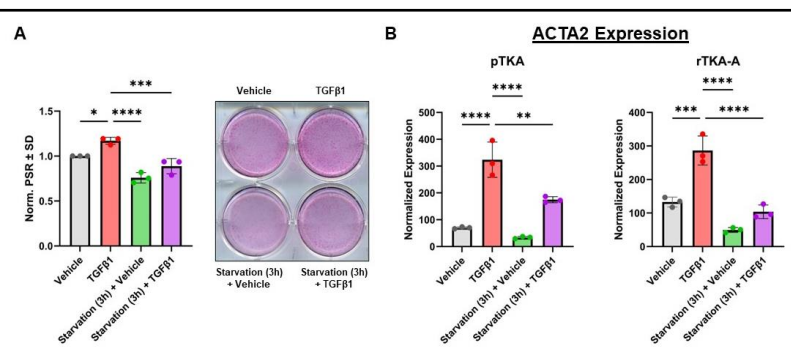


Figure 2. Starvation-induced autophagy impairs matrix deposition and ACTA2 mRNA expression in pTKA and rTKA-A fibroblasts. Collagen deposition, as shown by picrosirius red staining, in rTKA-A cells (A) (left = quantification, right = staining example). ACTA2 mRNA transcript levels, relative to GAPDH, in pTKA and rTKA-A fibroblasts (B). Significance is noted by asterisks (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$).