

# Paracrine Senescence Signals Promote Fibrotic Reprogramming in Fibroblasts

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**DISCLOSURES:** N/A

**INTRODUCTION:** Senescent cells secrete factors that can drive fibrosis and propagation of senescent cells. This secretome is often described as the senescence-associated secretory phenotype (SASP). We have previously shown that senescent dermal, connective tissue, and tendon fibroblasts are marked by an abundant SASP expression of Transforming Growth Factor beta (TGF $\beta$ ) and interferon beta (IFN $\beta$ ). TGF $\beta$  is a well-characterized SASP factor that promotes fibrotic signaling and can initiate the senescence program in neighboring cells. Further, these connective tissue senescent cells have high expression of p15<sup>INK4b</sup>, a cell cycle dependent kinase, which we and others have identified as a putative maker of cellular senescence that drives fibrosis. Additionally, another SASP factor we have identified is interferon beta (IFN $\beta$ ), known to increase protein levels of p15. Our hypothesis is that dermal fibroblasts treated with SASP factors, TGF $\beta$  and IFN $\beta$ , drive markers of fibrosis including increased p15 expression.

**METHODS:** To test our hypothesis, human dermal fibroblasts were treated with TGF $\beta$  (5 ng/mL) and IFN $\beta$  (5 ng/mL), and senescence-associated  $\beta$ -galactosidase (SABG) activity was assessed after 3 days in culture. Cell proliferation and migration were evaluated to determine functional changes (n=2). Gene expression responses to TGF $\beta$  and IFN $\beta$  were analyzed using qRT-PCR (n=3), and p15 protein expression was assessed by immunohistochemistry (IHC, n=1). To determine whether fibrotic and senescence-associated genes could be downregulated, cultures treated with TGF $\beta$  and IFN $\beta$  were exposed to senolytic drugs, including Fisetin (5  $\mu$ M) and a combination of Dasatinib (50 nM) + Quercetin (20  $\mu$ M), which selectively target and eliminate senescent cells.

**RESULTS:** A marker of cellular senescence is lysosomal accumulation, often assessed by SABG activity. To examine this, human dermal fibroblasts were treated with TGF $\beta$  for 3 days, resulting in a notable increase in SABG activity compared to untreated controls (Figure 1). To further investigate the effects of TGF $\beta$  and IFN $\beta$  on fibroblast function, we performed proliferation and migration assays. We observed that TGF $\beta$  and IFN $\beta$ , both individually and in combination, led to a significant reduction in cell proliferation (Figure 2). While migration was not substantially altered, a modest decline was noted with combined TGF $\beta$  and IFN $\beta$  treatment. We next assessed p15 protein expression by immunohistochemistry and found that p15 levels were elevated in response to TGF $\beta$  and IFN $\beta$  treatment (Figure 3). At the transcript level, qRT-PCR analysis revealed upregulation of p15 (CDKN2B), SERPINE1 (PAI-1), THBS1, and COL3A1 following treatment with TGF $\beta$  and IFN $\beta$ , supporting a shift toward a senescence-like and pro-fibrotic phenotype. Treatment of TGF $\beta$  and IFN $\beta$  stimulated fibroblasts with senolytic agents, Fisetin and the combination of Dasatinib + Quercetin (D+Q), resulted in a significant downregulation of p15 and SERPINE1, suggesting that senescence and fibrosis-associated gene expression can be partially reversed through senolytic intervention.

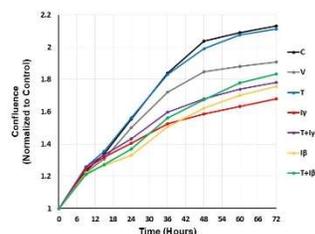
**DISCUSSION:** Senescent cells can induce changes in neighboring cells that promote both fibrosis and secondary senescence. We have previously identified TGF $\beta$  and interferons (IFNs) as key components of the SASP in connective tissue fibroblasts. In this study, we assessed lysosomal SA- $\beta$ -Gal (SABG) activity in dermal fibroblasts treated with TGF $\beta$  and observed a marked increase in SABG expression, indicative of senescence-like changes. Using immunohistochemistry (IHC), we found that p15 protein expression was elevated in fibroblasts treated with TGF $\beta$ , IFN $\beta$  and their combination. TGF $\beta$  is a well-established inducer of both fibrosis and senescence and is known to transcriptionally activate p15 in fibroblasts. Interestingly, recent findings suggest that IFN $\beta$  may increase p15 protein levels through post-transcriptional mechanisms, further supporting its role in promoting senescence-like phenotypes. The increase in p15 in neighboring (bystander) cells may reflect a cytostatic response to the paracrine secretome of senescent cells, pushing these fibroblasts into a senescence-like, non-proliferative state. Proliferation assays revealed no increase in cell division following TGF $\beta$  or IFN $\beta$  treatment. In fact, a significant decrease in proliferation was observed in cells exposed to combined TGF $\beta$  and IFN $\beta$ , suggesting that SASP components actively suppress fibroblast proliferation. In contrast, migration assessments did not show significant differences across treatment conditions. The treatment of fibroblast cultures with the senolytic agents Fisetin and Dasatinib + Quercetin (D+Q) resulted in downregulation of p15 and SERPINE1 (PAI-1), further implicating these genes in the senescence and fibrosis-associated phenotype. Taken together, our findings suggest that SASP factors, particularly TGF $\beta$  and IFN $\beta$ , drive p15 expression in fibroblasts, promoting a senescence-like, low-proliferative phenotype that may contribute to fibrosis. Ongoing work is aimed at further characterizing the fibrotic phenotype and exploring p15 as a potential therapeutic target in fibrotic disease.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The overlap between cellular senescence, wound healing, and fibrosis in connective tissues suggests that modulating p15 expression may represent a valuable therapeutic strategy. Our findings highlight that SASP from senescent cells may exert paracrine effects on neighboring fibroblasts, promoting senescence-like phenotypes that may contribute to the development and persistence of fibrotic tissue pathology.

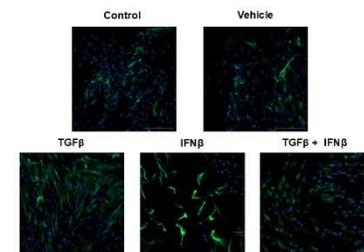
## IMAGES AND TABLES:



**Figure 1. SABG Expression is Increased in TGF $\beta$  Treated Fibroblasts.** Primary human dermal fibroblasts (ATCC PCS-201-012 (passage 3)) were treated with vehicle or TGF $\beta$  (5 ng/mL) for 6 days and were assessed for SABG expression. SABG staining is shown in blue.



**Figure 2. Proliferation Assay of Fibroblasts Treated with SASP Factors.** Human dermal fibroblasts were treated with control (C), vehicle (V), and SASP factors TGF $\beta$  (5 ng/mL, T), IFN $\beta$  (5 ng/mL, I $\beta$ ) separately and in combination with TGF $\beta$  (T+I $\beta$ ) and IFN $\beta$  (I $\beta$ ), separately and in combination with TGF $\beta$  (T+I $\beta$ ) using IncuCyte proliferation assays. IncuCyte software was used to quantify proliferation. Results illustrate the decrease in proliferation in the presence of TGF $\beta$  and IFNs. n=2.



**Figure 3. p15 Expression in Fibroblasts Treated with SASP Factors.** Human dermal fibroblasts were treated with Control (C), Vehicle (V), and SASP factors TGF $\beta$  (5 ng/mL, T), and IFN $\beta$  (5 ng/mL, I $\beta$ ) separately and in combination (T+I $\beta$ ) for 5 days. Cells were fixed and assessed for p15 expression.