Discovery of Novel Small Molecules with Anti-TGF-β Activity that can Successfully Prevent Myofibroblast Differentiation in Post-burn Fibroblasts

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8:30 – 8:45 am

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Objective:	Upon completion of the lecture, attendees should be better prepared to: ▪ Describe how TGF-β can lead to excessive, contractile scar formation ▪ Describe the role of myofibroblasts in hypertrophic scarring ▪ Recognize that blocking excessive TGF-β activity may lead to reduced scarring

Abstract:	**Introduction:** Hypertrophic scarring and keloid formation is a well-recognized complication that can arise from burn injuries, affecting up to 90% of burn patients.[1] They not only lead to functional problems that limit range of motion, but also to chronic pain, psychological distress, and reduced quality of life.[2] While there has been some new therapies targeting such pathologic scarring recently, hypertrophic scarring and keloid formation incidence after burn injury remains high and difficult to treat. Wound healing and excessive scar formation is a complex process that involves many different pathways. One of the most significant, is transforming growth factor-β (TGF-β). TGF-β1, a cytokine that falls under a family of growth factors, participates in wound healing at various stages and promotes cell proliferation, myofibroblast differentiation/activation/proliferation, and extracellular matrix (ECM) production.[3-5] TGF-β can promote fibroblast differentiation into myofibroblast, which can lead to increased collagen, extracellular matrix (ECM), and α-smooth muscle actin (α-SMA) production;[5,6] these proteins produced by myofibroblast lead to wound contracture and excessive scar formation. It was previously shown that hypertrophic scar fibroblasts express higher levels of TGF-β1 mRNA than non-hypertrophic scar fibroblasts.[7] Furthermore, previous experiments have shown that exogenous TGF-β1 injections or TGF-β1 overexpression lead to organ fibrosis, [8,9] suggesting its crucial role in pathologic scarring and fibrosis, which can be viewed as an "over-healing" process. Hence, one can imagine that inhibition of excessive TGF-β activity may lead to prevention of hypertrophic scarring, including those after burn injuries. The goal of this study is to identify novel small molecules with anti-TGF-β activity that can effectively prevent myofibroblast formation in post-burn injuries, which may lead to the prevention of hypertrophic scarring. **Methods**: Hypertrophic burn scar fibroblasts were explanted from a scar tissue of a burn victim. Cultured fibroblasts were then treated with active, exogenous TGF-β (2.5 ng/mL) and three novel small molecules (molecule 334, 737, and 671) with anti-TGF-β activity (0.5, 1, and 1.5 uM) and incubated for 72 hours. Cells were then harvested and Western Blotting was utilized to analyze for α-SMA protein level. α-SMA is one of the most established and widely studied markers of myofibroblast, hence directly

representing level of myofibroblast activity. Furthermore, cells were examined under brightfield microscopy to look for any morphologic changes.

Results: TGF-β significantly increases αSMA expression up to 3.56 fold compared to that of vehicle cells in burn fibroblasts, indicating significantly increased myofibroblast differentiation and activity. Such changes are apparent in cellular morphology, as cells treated with TGF-β only shows prominent myofibroblastic phenotypes. Interestingly, cells treated with both TGF-β and novel small molecules show significant, dose-responsive reduction in αSMA levels. All three small molecules reduced αSMA expression down to that of vehicle with 0.5 uM treatment (relative expression of 1.04, 1.16, 0.81) (Figure 1). Furthermore, small molecule treated cells show reduced myofibroblastic phenotypic changes.

Conclusions: TGF-β can effectively push hypertrophic burn fibroblasts to differentiate into myofibroblasts, which play a major role in excessive scar development and contracture. A group of novel small molecules with anti- TGF-β activity effectively reduced myofibroblast activity; such results show that targeting TGF-β pathway may provide a novel, effective therapeutic pathway against hypertrophic scarring in burn patients.

References and Resources:

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Disclosure:

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Figure 1. Novel small molecules 334, 737, and 671 effectively reduces α-SMA production even in the presence of TGF-β.