Toward a Novel Treatment for Corneal Scarring: Establishing the Roles of TRPV1 and TGF-B1 in the Fibroblast-to-Myofiblast Differentiation

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Corneal scarring is the leading cause of blindness worldwide. Since current therapies are expensive and functionally inefficient, the scarring process (known as the fibroblast-to-myofibroblast differentiation) in the human corneal fibroblast (HCF) was explored through an interaction of TGF-B1 signal transduction and TRPV1 channel activation. Immunostaining was used to visualize a-smooth muscle actin (a-SMA). Western blots were used to view protein and phosphoprotein persistence. Small interfering RNA (siRNA) transfection, capsaicin (CAP), and capsazepine (CPZ) acted as TRPV1 expression regulators. Flow cytometry with localized reactive oxidative species (ROS) reporting dye HyPer-nuc and its cytosolic and ER equivalents was used to analyze intracellular ROS. Calcium ion (Ca2+) influx was measured by loading HCFs with Fura-2AM. CPZ diminished myofibroblast persistence by 75%. TGF-B1 rapidly yielded increased Ca2+ influx, phosphorylation of SMAD2 and p-38 over 90 minutes, ROS generation over 16 hours, and a 13-fold increase in a-SMA- and interleukin 6 (IL-6) dependent scar tissue formation. By inhibiting the ROS generation rate and TRPV1, the scar tissue phenotype was diminished. The results show that corneal scarring is controlled by a positive feedback loop in which p-SMAD2-induced ROS activates TRPV1, TRPV1 causes activation of p38, p38 enhances the activation of SMAD2, and SMAD2 promotes the fibrotic mechanism. The inhibitors studied have significantly higher rates of conjugation to new, more efficient drug delivery systems like gold nanorods compared to current candidates. Thus, the resultant model has significant applications in improving drug delivery, streamlining ocular targeted inhibition, and preventing over 300 million cases of blindness worldwide.

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