TGF-β1 inhibits proliferation of Müller glial derived cells: potential implication in fibrosis.

Abstract Number: 2349 - B0036

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Disclosures: Md Istiaq Obaidi Tanvir: Code N (No Commercial Relationship) | Most Zarrin Tasnim: Code N (No Commercial Relationship) | Shigeo Tamiya: Code N (No Commercial Relationship)

Purpose

Müller glial (MG) cells have been implicated to contribute to fibrotic retinal conditions such as proliferative vitreoretinopathy (PVR) through transformation into myofibroblasts. TGF-β1, a key profibrotic cytokine, is implicated in driving these changes.This study investigated effects of TGF-β1 on myofibroblastic transformation using marker protein (αSMA) expression, and additionally collagen gel contraction as measure of matrix contraction, a key myofibroblast function, as read outs.

Methods

MG cells were isolated from porcine retina using a papain dissociation kit and cultured in 10%FBS-DMEM. Trypsinized cells were cultured at first passage under subconfluent (~ 50% confluent) and confluent (70% > confluent) conditions and used for experiments at second passage (p2). MG cells at p2 were plated atop collagen gels for 2 or 6 days and contraction was assessed. αSMA expression was analyzed by Western blotting and immunostaining. Cell proliferation was evaluated by EdU uptake.

Results

TGF-β1 induced myofibroblastic changes in subconfluent cultures as evidenced by increased αSMA expression. Confluent cultures had increased αSMA expression and gel contraction compared to subconfluent cultures, implicating spontaneous myofibroblastic change upon increased cell density. Interestingly higher aSMA expression in TGF-β1 treated cells did not result in enhanced gel contraction. This was due to TGF-β1-mediated inhibition of proliferation, as evidenced by reduced EdU uptake and total cell number available for contraction in both subconfluent and confluent cultures. Treatment with proliferation inhibitor aphidicolin negated the inhibitory effect of TGF-β1 on proliferation, resulting in significant increase in gel contraction with TGF-β1.

Conclusions

TGF-β1 drives myofibroblastic transformation in Müller glial cells as seen by upregulation of αSMA

expression. Sustained TGF-β1 signaling, however, may negatively impact fibrosis by suppressing proliferation of Müller glia derived cells that can contribute to the final outcome.