Cell density alters growth factor response in cultured Müller glia cells.

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Purpose
Müller glia (MG) cells have been implicated to play key roles in the formation and contraction of retinal scars such as epiretinal membranes involved in retinal folding and detachment. The detailed mechanism involved in the progression of this common fibrotic condition is still unclear. In this study, the effect of a key profibrotic cytokine TGF-β1 (Transforming Growth Factor-β1) and/or IGF-1 (Insulin-like Growth Factor-I) on MG cells on collagen gel contraction was examined.

Methods
MG cells were isolated from retina of freshly enucleated porcine eyes using a papain dissociation kit and cultured for 8–9 days in 10%FBS-DMEM (passage 0 [p0]). Trypsinized p0 cells were cultured for either 5-6 days (Set1, non-confluent) or 10-11 days (Set2, confluent) as p1 and then used for experiments at p2. Collagen gel contraction assay was used to assess the effect of growth factors on cellular ECM contraction, and western blot analyses utilized to examine cell phenotype marker expression. Briefly, cells were plated on top of solidified gels in 24 well plates and cultured for 6 days in four different conditions: 2.5% FBS (used as control), 2.5% FBS with 10ng/ml TGFbeta1, 2.5% FBS with 100ng/ml IGF-1, 2.5% FBS with TGFbeta1 and IGF-1. Then, gels were released from plates and photographed after 4 hours and 24 hours.

Results
Collagen gel contraction by p2 MG cells were affected by cell culture status at p1. p2 cells from confluent MG cells at p1 (set 2), achieved by extended culture period, showed higher contraction in 2.5% FBS than p2 cells from subconfluent p1 cells. Gel contraction was further enhanced by IGF stimulation for post-confluent p2 cells (Set2). Interestingly, the effect of IGF1 was reversed for (Set1) p2 MG cells.

Conclusions
Culture conditions, and resulting cell densities, play key roles in fibrotic changes of MG cells. IGF-1 plays a prominent role in enhancing collagen gel contraction and myofibroblast transdifferentiation of MG cells for confluently cultured MG cells, whereas the effect of IGF-1 is reversed for non-confluently cultured MG cells. Our present data shed light on importance of cell culture condition on growth factor signaling that regulates fibrotic transformation of MG cells.