

TGF- β 1 stimulates migration/proliferation in immortalised Fallopian tube epithelial cells

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Introduction:

Tubal ectopic pregnancy (tEP) occurs when an embryo implants in the Fallopian tube instead of the uterus. tEP occurs in approximately 1-2 in every 100 pregnancies, and is a major health burden which can lead to morbidity and, in rare cases, mortality. There is evidence to suggest that epithelial-to-mesenchymal transition (EMT) is involved in intrauterine embryo implantation, however, the role of EMT in tEP is unknown. We have unpublished data showing that TGF- β 1, which can induce EMT, increases blastocyst attachment in an *in vitro* model of tEP. Here, we set out to test the hypothesis that exposure to TGF- β 1 promotes EMT in Fallopian tube epithelial cells, resulting in increased proliferation and/or migration. The experiments used a wound healing scratch assay to test the effect of TGF- β 1 and of its inhibitor on cultures of an immortalised human Fallopian tube epithelial cell line (OE-E6/E7).

OE-E6/E7 cells (sourced from KF Lee, Hong Kong) were seeded in 6-well cell culture plates at 3×10^5 cells/ml per well and cultured to 100% confluency in DMEM/F-12 (ThermoFisher) supplemented with 5% charcoal-stripped serum (ThermoFisher) and 2mM non-essential amino acids (ThermoFisher). 24 hrs prior to the scratch wound assays, the culture medium was replaced with either the control (serum-free DMEM/F-12; ThermoFisher), 20ng/ml TGF- β 1 (Invitrogen), or 10 μ M TGF- β 1 inhibitor (SB431542; Stemcell technologies) in serum-free DMEM/F-12 in duplicate wells. Before scratch wounds were performed, medium was replaced with fresh DMEM/F-12 medium. Using a p200 pipette tip, a uniform scratch wound was created in the middle of each cell monolayer, using a spare culture plate lid as a guide to create an even scratch line. Scratch wounds were then imaged at 0, 4, 8, 12, and 24 hr time points. The scratch wound width was analysed using the ImageJ Wound Healing Size Tool Plugin.

After 24 hours, there was a significant increase in the percentage of wound gap closure in OE-E6/E7 cells exposed to TGF- β 1 compared to the control ($p < 0.01$). Likewise, there was a significant decrease in percentage gap closure after exposure to the TGF- β 1 inhibitor at that same timepoint, compared to either controls or to cells exposed to TGF- β 1 ($p < 0.01$ and $p < 0.001$ respectively). There was a significant reduction in the rate of migration (μ m/hr) in cells exposed to TGF- β 1 inhibitor compared to cells exposed to TGF- β 1 ($p < 0.05$). However, no difference was observed in the rate of migration of cells after exposure to TGF- β 1 compared to the control.

In conclusion, TGF- β 1 increases wound gap closure, while its inhibitor decreases it, in immortalised Fallopian tube epithelial cells. TGF- β 1 inhibition also reduced the rate of migration compared to cells exposed to TGF- β 1. Together, the results suggest that the TGF- β 1 pathway could be involved in the proliferative and/or migratory capacity of Fallopian tube epithelial cells, potentially affecting the likelihood of embryo attachment at that site.