VISUALISATION OF THE ROLE OF β-CATENIN/FOXO IN KIDNEY INJURY

Padmashree Rao, Hong Yu, Yiping Wang, Guoping Zheng, David Harris
University of Sydney at Westmead Institute for Medical Research
176 Hawkesbury Road, Westmead, NSW-2145 Australia
E-mail: padmasrikaranth@gmail.com

Aim: To visually examine the role of β-catenin/Foxo and β-catenin/TCF in kidney injury healing.

Background: Transforming growth factor β (TGF-β) is known to promote healing after tissue injury, but also drives a maladaptive fibrotic response that leads to fibrosis and organ failure. β-catenin/TCF is central to TGF-β's profibrotic signalling pathways. β-catenin also binds to Foxo in competition with TCF and leads to cell survival. We propose that targeting TGF-β signalling by using an inhibitor (ICG-001) of β-catenin/TCF will promote β-catenin/Foxo which results in epithelial healing rather than mesenchymal cells.
Several microscope techniques were applied to visually examine the role of β-catenin/Foxo and β-catenin/TCF both in *in vitro* and *in vivo* healing model. Wound closure in murine kidney cell line investigated with live cell imaging while kidney injury healing investigated with slide scanner and PLA of β-catenin/Foxo and β-catenin/TCF interactions with deconvolution microscopy.

Methods: *In vitro* scratch assay was used as a model of healing in murine kidney cell line (C1.1) and treated with TGF-β1 (3ng/ml) with or without β-catenin/TCF inhibitor ICG-001 (5μM). CRISPR/Cas9 was used to knockout Foxo1 or TCF1. Wound closure was examined using Zeiss microscope and measured as the percentage area of wound closure at 48 hours (%). *In vivo* kidney injury healing was evaluated in murine unilateral ischemia reperfusion injury (UIR) by Gomori trichrome staining using the slide scanner Nanozomer and measured as percentage area of positive staining (%). β-catenin/Foxo and β-catenin/TCF interactions were examined by proximity ligation assay (PLA) and detected by Deltavision microscope. Epithelial (E-cadherin) or mesenchymal (α-SMA) healing was examined by immunofluorescence staining by Deltavison microscope and measured as percentage area of positive staining (%).

Results: TGF-β1 and ICG-001 treatment in C1.1 cells and UIR caused increased β-catenin/Foxo interaction as demonstrated by PLA. The combined treatment of rhTGF-β and ICG-001 inhibited rhTGF-β-induced α-SMA expression and showed dominant E-cadherin expression to a greater extent than seen with TGF-β alone; α-SMA, 4.9±1.2% versus 40.4±2.6%, $P<0.01$ *in vitro*, and 17.6±1.4% versus 42.1±2.8%, $P<0.01$ *in vivo*; E-cadherin, 29.1±3.6% versus 2.4±2.6%, $P<0.05$ *in vitro*, and 21.5±4.7% versus 10.5±1.8%, $P<0.05$ *in vivo* which is detected through Deltavision images. Foxo1 KO in C1.1 cells showed significant reduction in closure of the wound gap compared to WT C1.1 cells (75.0±2.9% versus 95.0±2.7%; $P<0.05$). Foxo1 KO in C1.1 cells slowed wound closure under combined treatment compared to that of WT C1.1 cells (70.0±2.9% versus 98.3±1.7%, $P<0.01$) which could be explained by absence of β-catenin/Foxo in Foxo1 KO C1.1 cells via Zeiss microscopy live cell imaging. In UIR mice, combined treatment with rhTGF-β and ICG-001 significantly attenuated kidney fibrosis compared with rhTGF-β alone by microscopy imaging.

Conclusions: These microscopy results indicate that β-catenin/Foxo may serve as a therapeutic target to prevent pathological fibrotic healing and fibrosis in the treatment of kidney diseases.