P342

P342 -Post-translational regulation of endothelial derived CCN proteins in response to uremic serum from haemodialysis patients

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Introduction
Non-traditional risk factors play an important role in cardiovascular disease (CVD) observed in renal patients. We have previously reported neointimal hyperplasia in arteries in patients with chronic kidney disease (CKD). Vascular smooth muscle cells (VSMC) dedifferentiation is a key step in neointimal hyperplasia. Endothelial cell derived CCN2/CTGF and CCN3/Nov could play opposing roles in regulating VSMC dedifferentiation and migration modulating neointimal hyperplasia.
We aim to investigate the expression and alteration in the CCN protein axis in human endothelial cells in response to uremic serum donated by haemodialysis patients.

Methods
Following approval by Research Ethic Committee and registration of the project, blood samples were obtained from consented haemodialysis patients (n=10) and healthy control subjects (n=6). Serum was prepared and stored at -80°C. Human Umbilical Vascular Endothelial Cells (HUVEC) derived from a mixed pool of donors was obtained from Lonza and cultured in supplemented EGM™ growth medium on collagen IV coated culture ware. Cells were incubated in serum-free (sf) media, sf media containing 10% v/v patient serum (PS) and sf media with 10% v/v control subject serum (CS). After 24 h, media was removed, cells lysed and RNA extracted. RNA was subject to reverse transcriptase followed by real time QPCR. Relative gene expression was calculated by δδCt analysis. Following 72 h incubation of HUVEC in the conditions above, media was removed, centrifuged and stored. Cells were lysed and lysates prepared for PAGE Western Blotting.

Results
There was no significant difference in TGFβ1 or CCN2/CTGF expression in sf, PS or CS treated cells after 24h. Although, serum significantly reduced CCN3 expression, there was no difference between PS or CS treated cells.

There was no measurable difference in LDH between cultures with any of the protocols. Serum treatment for 72h, increased eNOS protein expression but there was no significant difference between PS and CS.

Although there was some variation in the protein expression from cells exposed to sera from different patients, there was still an overall increase in the intact 36/38Kda CTGF from cells treated with PS compared to both CS and sf ($p<0.05$). PS treatment induced significantly less CCN3 protein than CS treatment ($p<0.05$). Active and latent (lap-bound) TGFβ expression was also investigated. No measurable TGFβ was seen in media from sf treated cells. There was substantial variation in the TGFβ isolated from PS treated cells with more in the latent form, but no statistical difference was observed between media from CS and PS treated cells.

Conclusion
From our data, we conclude that serum from haemodialysis patients induces endothelial cells to alter the CCN2/CCN3 axis substantially in favour of VSMC differentiation and migration potentially driving neointimal

hyperplasia and hypertension in these patients. Our data indicates that 72 h exposure to patient sera induces a moderate dedifferentiation of the endothelial cells, but without cell necrosis.

Regulating the CCN2/CCN3 axis offers a novel attractive target for reducing vascular disease in CKD patients, but further work is required to clarify the nature of the molecular interactions.