Endothelial glycocalyx dysfunction in sepsis-associated acute kidney injury

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Sepsis is the leading cause of acute kidney injury (sAKI), associated with high morbidity and mortality. The renal microcirculation is profoundly disturbed in sAKI, leading to kidney damage. The endothelial glycocalyx (eGLX) is a key determinant of vascular health and is shed in vascular diseases including sepsis and AKI. We have shown for the first time that glomerular and peritubular eGLX is damaged in a mouse model of sAKI. This was associated with a corresponding increase in SDC4 mRNA expression, suggesting a compensatory response to renal eGLX shedding. Renal eGLX damage was also associated with an increase in plasma SDC4 levels, alluding to systemic SDC4 shedding. Human conditionally immortalised glomerular endothelial cell (ciGEnC) data confirm LPS-induced reduction in SDC4 cell surface expression and a corresponding feedback increase in SDC4 mRNA expression.
In this project, we sought to determine the relevance of eGLX loss in sAKI in humans. We, therefore, stimulated ciGEnC with sAKI plasma and proinflammatory cytokine TNFα, known to be increased in sAKI. Moreover, establish whether eGLX protection attenuates eGLX damage. To this end, EXT1, the gene responsible for eGLX heparan sulphate synthesis, overexpressing ciGEnC was used.

Stimulation of ciGEnC with TNFα significantly ($p=0.0011$, $n=3$) increased SDC4 mRNA expression. Treatment of ciGEnC with sAKI plasma upregulated SDC4 mRNA expression, although non-significantly with the given n number ($p= 0.0797$, $n=3$), when compared to control serum. Overexpression of heparan sulphate significantly ($p=0.0021$, $n=3$) attenuated TNFα-mediated increase in SDC4 mRNA expression and non-significantly ($p=0.4809$, $n=3$) reduced sAKI plasma-mediated SDC4 increase.

More work needs to be done to confirm these results. This will involve increasing the n number of sAKI samples to determine statistical significance. Also, repeating the experiment in lentivirus control cells to confirm the response is due to heparan sulphate overexpression rather than the lentivirus itself.

EGLX protection or restoration offers a potential novel therapeutic target in sAKI.

Feedback from Raina Ramnath

I would like to thank BMVBS for this studentship which has enabled Verity Shah to learn different lab techniques e.g. tissue culture, RNA extraction, cDNA conversion, and qPCR. Verity has also developed independent critical thinking skills, skills in analysing data and interpretation of the results, and further developed her oral and written communication skills. I have led and managed this project, helping me to further develop my teaching, leadership, and communication skills. In addition to progressing the work on this project, this studentship has improved my ability to give effective feedback to increase confidence, enhance skills and improve performance in the students.

Feedback from Verity Shah

After completing my first year of university without any lab experience, it was incredible to get involved in the practical applications of the techniques I had heard about during my studies. Additionally, for the first time it helped me get a better perspective on what research in academia has to offer and what I could do post my undergrad in medical research and further study. It was rewarding learning all the techniques from Raina and her being confident enough in me to allow some independence in carrying out RNA extraction and tissue culture work and feel like my work was contributing to a bigger piece of research that one day could be beneficial to others. It has also made my own lab skills better and more particular, looking forward to the next opportunity I have to get back into the lab.