Final Report: PKD Foundation of Australia

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Genes and Cellular Stress in PKD

Our team consisting of Prof. Jacqueline Phillips, Prof Julie Atkin, Ms Shabarni Gupta and Ms Vereena Bottrill were privileged to be funded by the PKD Australia to undertake a study examining how genes responsible for PKD could contribute to an increase in stress responses in kidney cells that drive cell damage and progression of kidney disease.

Specifically, damage to cells by a PKD mutation may result in impaired endoplasmic reticulum activity, altering critical cellular functions that otherwise serve to protect the cell. This will result in a state of increased oxidative stress and potential cell death. Furthermore, given that these same stress signals can be driven by the build-up of toxins in the blood that arises when kidney function declines, we also tested if the combination of PKD mutation and toxins worsens the stress response in the cells.

Aim 1: Using cell-culture, determine if expression of different PKD gene mutations for PKD1 (ADPKD) and NEK8 (ARPKD) induces common changes in mitochondrial function, ER stress and increased levels of ROS.

Aim 2: Using cell-culture, determine if exposure to a uraemic environment potentiates PKD gene driven changes in mitochondrial function, ER stress and increased levels of ROS.

Outcomes

The funding for this project has allowed us to construct a number of cell line models, and develop a pipeline of protocols and techniques for use in the study the effect of mutations in ARPKD and ADPKD on fundamental cellular processes such as ER and mitochondrial stress responses.
It has allowed us to establish baseline data on the effects of ARPKD mutations and uraemic toxins as drivers of cellular stress responses, which will be used as the foundation for ongoing studies in this area. Results so far suggest the impact of the ARPKD mutation on select markers of cellular stress on its own is negligible, however the presence of uraemic toxins does trigger a cellular stress and there is initial evidence to suggest this is potentiated by the presence of the NEK8 mutation.

Specifically, using a tissue-culture cell based system, we constructed cell line models expressing a human pathogenic mutant of NEK8, mutations of which are responsible for a recessive form of PKD called nephronophthisis 9 (NPHP9). NPHP is an autosomal recessive form of cystic kidney disease that can present anywhere between birth and adolescence in affected individuals.

We then looked at changes in expression of markers which indicate activation of cellular stress. This included protein levels of BiP (a 78-kDa glucose-regulated protein (GRP78), which functions as a chaperone for new proteins and is essential for the normal function of the endoplasmic reticulum) and mRNA expression of a splice variant of the transcription factor XBP1 (X-box binding protein 1). We studied the expression of these markers in cells with wild type NEK8 and with the mutant NEK8 and looked to see if there was any impact of a uraemic toxin on their activation.

Our results showed that while the NEK8 mutation alone did not change the stress response, the uraemic toxin significantly upregulated both BiP and XBP1 and that this was potentiated by the presence of the mutation. Our next steps in this project will be to examine other markers of the stress pathway and determine if the same effect is seen in cell lines expressing different forms of PKD mutations.

Overall, the funding for this project has allowed us to establish new cell line models, and develop a pipeline of protocols and techniques for use in the study the effect of PKD mutations on fundamental processes such as cellular stress that would not have been possible without the support of the PKD Australia. Importantly, determining how PKD leads to cell damage may provide viable therapeutic strategies to preserve kidney function and delay the progression of PKD.