Progress Report – September 2017-12-21

Name of Grant Recipient: **University of Sydney**

Project Title: ***Treating pancreatic cancer with FXYD3 peptide analogues***

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1. Summarise the aim of your research

"FXYD" refers to proteins named after the one-letter code for amino acids the proteins share. They associate closely with the membrane sodium-potassium pump that pumps sodium out of cells and potassium in. The pump is present in all mammalian cells and its function is critical for their survival, including for heart cells.

While the association of FXYD proteins with the sodium-potassium pump is established and a role for them in regulation of pump activity implicated the mechanism by which they regulate pump activity has been poorly defined.

In our research on defining how FXYD proteins modify sodium-potassium pump activity in heart cells we developed a recombinant derivative of one of the FXYD proteins, FXYD3, in which we had mutated an amino acid we suspected is critical for function. FXYD proteins are highly soluble in the lipid milieu of cell membranes and exposing heart cells to the recombinant protein displaced the native FXYD protein and eliminated its function, making the recombinant FXYD3 a useful tool.

In some cancers, including those of the pancreas and breast, FXYD3 is present in much greater abundance (i.e. "overexpressed") than in non-cancer cells. Using our FXYD3-derived tool we found that native FXYD proteins protect sodium-potassium pump function against oxidative stress. Based on the full-length FXYD3 mutant protein we

developed a much shorter derivative (FXYD3-pep) that retains the properties of the full-length version but is much easier and cheaper to make.

Our aims are to determine to what extent FXYD3-pep can amplify effects of chemotherapy and explore the mechanism by which such amplification occurs.

2. **What have the outcomes been to date?**

Exposing pancreatic cancer cells that overexpress FXYD3 to FXYD3-pep amplifies the cytotoxicity of the chemotherapeutic drug doxorubicin many-fold while FXYD3-pep does not amplify effects in cancer cells that do not overexpress FXYD3 (only ~75% of pancreatic cancers overexpress FXYD3). Results are similar for breast cancer cells. Surprisingly, the membrane sodium-potassium pump does not seem to be the primary target for FXYD3-pep that we have found to distribute inside of pancreatic cancer cells rather than at the cell membrane where functional sodium-potassium pumps are located.

Exposure of pancreatic cancer cells to FXYD3-pep almost eliminates expression of a protein, glutathione-S-transferase , that is implicated in treatment-resistance and the exposure amplifies a doxorubicin-induced up-regulation of a protein called p53 that is important for programmed cell death.

FXYD3-pep when given to mice by injection is distributed to tissues and combined with doxorubicin it has no apparent ill effects on the mice after 6 weeks treatment.

3. **What are the next steps?**

FXYD3 by itself has little effect on cancer cells alone but is highly effective in amplifying effects of treatment. Determining how FXYD3 works will be important for what kind of treatments are best combined with it, and we will continue to explore mechanistic details. We will also express human pancreas cancers in mice to determine how much FXYD3-pep amplifies effect of standard treatments. Properties of the peptide will be further refined.

4. **What has it meant to receive funding from the Avner Pancreatic Cancer Foundation?**

Funding from the Foundation has allowed us to explore a promising, multidisciplinary approach to treatment never previously considered.