INTERIM REPORT

# *"Matrix metalloprotease mediated prion protein proteolysis: Investigating normal processing and links to prion disease"*

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The cellular prion protein, PrPC, is expressed in most cells and tissues, and has been linked to several cellular processes and diseases, most commonly to prion diseases. In the context of prion disease, PrPC acts as a substrate for generation of disease associated PrPSc, and is an absolute requirement for prion disease pathogenesis. My work over the last few years has focused on PrPC endoproteolytic processing, which is where PrPC is "cleaved" (cut) at one of several sites along its length by proteases, producing fragments of various lengths and features. We know that depending on the cell type, a large proportion of the PrPC expressed is cleaved, and there is evidence that the fragments produced may have different functions. Importantly, as PrPC acts as a substrate for PrPSc generation, PrPC cleavage can affect this process.

The most prevalent cleavage event in most cells is alpha-cleavage, however the protease responsible is still not confirmed. My preliminary studies using recombinant proteins (proteins made in bacteria) indicated that members of the matrix metalloprotease (MMP) family of proteases are capable of cleaving PrPC, and produce fragments which appear to correspond to those produced by alpha-cleavage. Hence we developed the hypothesis that "The MMP family of proteases are key regulators of PrPC endoproteolysis, especially at the alpha-cleavage site, which has direct influences on prion disease pathogenesis". The next step in investigating this hypothesis was to determine whether MMPs were capable of cleavage of mammalian PrPC, in cultured cells, and whether this was relevant in a prion infection setting.

To that end, here is a summary of key achievements and findings, and future work required:

1. Generation of stocks of mouse and human MMPs 2,7 & 9 cDNA containing plasmids for protein expression in mammalian cells.

2. Transient transfection of mo-MMPs 2,7 & 9 into MoRK13 cells, to determine any effect on PrPC expression levels or profiles. After 72hr overexpressing the MMPs, I observed approximately only a 10% increase in alpha-cleavage with each of the proteases.
    - *TO DO: Repeat this experiment with hu-MMPs transiently expressed in HuRK13s, and both mo- and hu-MMPs into 3F4-RK13s (which express mouse PrPC which has been modified to contain the "human" 3F4 epitope which is near the alpha-cleavage site)*

- *TO DO: Repeat experiments adding pharmacological or biological modifiers of MMP activity*

3. Stable (long term) over-expression of mo-MMPs into MoRK13 cells and hu-MMPs into HuRK13 cells, as the 72h transient expression had minimal effect on PrPC. The primary observation I found was a massive decrease in cell associated PrPC levels.
    - *TO DO: Effects on alpha-cleavage have not yet been established – cell lysates and media were collected, but further analyses of these is required (eg PNGaseF digest, epitope mapping, western blotting)*
    - *TO DO: Determine the reason behind the reduced cellular PrPC with MMP over-expression – ie are MMPs altering PrPC cleavage, PrPC shedding from the cell, or are they degrading PrPC? Check localization of PrPC and MMPs in the cells.*
    - *TO DO: Treatment of the stably transfected cells with pharmacological or biological modifiers of MMP activities*

4. Transient transfection of mo-MMPs 2,7 & 9 into M1000 or MU02 prion infected MoRK13 cells, to determine any effect on PrPres propagation. After 72hr overexpressing the MMPs, I observed no significant difference in absolute PrPres levels by cell blotting.
    - *TO DO: check for changes in PrPres profile, including C3 fragment, in cells transiently expressing MMPs*

5. Stable over-expression of mo-MMPs into M1000 or MU02 prion infected MoRK13 cells, to determine any effect on PrPres propagation, as only 72hr over-expressing the MMPs elicited none. After four passages (approximately 1 month), I observed striking differences in PrPres levels present which were both MMP and prion strain dependent. In M1000 infected cells, over-expression of MMP2 massively increased PrPres propagation, determined by by cell blotting, whereas MMPs 7 & 9 decreased the amount of PrPres to the limit of detection. In MU02 infected cells, MMP2 over-expression had no effect on PrPres levels, MMP7 over-expression increased PrPres propagation, and MMP9 over-expression again reduced PrPres levels to the limit of detection.
    - *TO DO: determine whether the cells with reduced PrPres also contain less infectivity by using them as a source of prions for further infection*

6. Utilizing stably MMP-transfected MoRK13 cells for susceptibility studies – ie infect cells with M1000 or MU02 prion trains and compare to untransfected MoRK13 cells. After allowing the cells to develop prion infection and produce PrPres, I observed no difference in PrPres levels produced by MMP2 over-expressing cells, however there was a significant increase in PrPres production in MMP7 over-expressing cells exposed to M1000 prions, with a similar trend seen with MU02 exposed cells, possibly suggesting increased prion susceptibility.
    - *TO DO: Repeat this experiment with MMP9 as for unknown reasons this didn't work on the first attempt at transfection unlike the other two MMPs*
    - *TO DO: Repeat experiments adding pharmacological modifiers of MMP activity*