“Matrix metalloprotease mediated prion protein proteolysis: Investigating normal processing and links to prion disease”

CJDSGN Memorial Award in Memory of Michael Luscombe (project support 2018-2021)
CJDSGN Memorial Grant in memory of Frank Burton (salary support 2019-2021)
Awards extended for 3 months (Jan 2021- Mar 2021) due to maternity leave (Oct 2019 – Sept 2020), and Covid restrictions/delays (Oct 2020 – Dec 2020)

With overwhelming gratitude to the Luscombe and Burton families for their support of me and my research, I’m providing this final report summarising key findings of the project. As I also indicate below, these interesting results and achievements have unsurprisingly led to many further research questions and possible future directions for this research.

FINAL REPORT

The cellular prion protein, PrPC, acts as a substrate for generation of prion disease associated prion protein PrPSc, and is an absolute requirement for prion disease pathogenesis. My research is primarily focused on PrPC endoproteolytic processing, the process by which PrPC is “cleaved” (cut) at one of several sites along its length, producing several different fragments. It is known that depending on the cell type, a large proportion of the PrPC expressed can be cleaved, and there is evidence that the fragments produced may have different biological/cellular functions. The dominant cleavage event in most cells is known as “alpha-cleavage”, however the protease (i.e. the “molecular scissors”) responsible for alpha-cleavage has been debated in the prion research community and is still not confirmed. My previous preliminary studies indicated that members of the matrix metalloprotease (MMP) family of proteases are capable of cleaving PrPC in a test-tube based system, and produce fragments which appear to correspond to those produced by alpha-cleavage of PrPC in living cells. Based on these observations, I developed the hypothesis that “The MMP family of proteases are key regulators of PrPC endoproteolysis, especially at the alpha-cleavage site”. Furthermore, as PrPC acts as a substrate for PrPSc generation and PrPC cleavage can affect the PrPC-PrPSc conversion process, I hypothesized that “MMP mediated PrPC cleavage has direct influences on prion disease pathogenesis”. This project primarily involved investigating this hypothesis by determining whether MMPs were capable of cleavage of mammalian PrPC, in cultured cells, and whether this was relevant in a cellular prion infection setting.

Aim 1. To confirm the role of MMPs in PrPC alpha-cleavage and identify the specific MMP/s and pathway involved:

KEY OUTCOMES / CONCLUSIONS:

- Essential foundation work; including generation of stocks of mouse and human MMPs (2, 7 & 9) cDNA containing plasmids and successful expression of several MMP proteins in mammalian cells.
- Transient (temporary, approximately 72h) and long term (stable) mouse MMPs overexpression in MoRK13 cells causes only subtle changes to PrPC alpha-cleavage determined by measuring levels of the C1 cleavage fragment. This was a surprising finding given the high levels of recombinant PrP cleavage by these MMPs in vitro.

Despite this, MMPs involvement in PrPC alpha-cleavage cannot be entirely ruled out at this stage.

- Long term (stable) mouse MMP overexpression in MoRK13 cells causes an apparent decrease in cell associated PrPC, which is most apparent with MMP9, and to a lesser degree, MMP2. This does not appear to be due to enhanced PrPC cleavage at the cell surface, as a concurrent increase of PrPC in conditioned media was not observed, suggesting the MMPs may be acting directly on PrPC, degrading it, which is an interesting novel finding.
- Similar to the mouse PrPC/mouse MMPs result, stable over-expression of Human MMPs in HuRK13s (expressing Human PrPC) resulted in decreased cell associated PrPC, again most apparent with MMP9. Also interestingly, preliminary results indicate this effect is not seen in MoRK13 cells expressing a chimera of Mouse and human PrPC (3F4-MoPrP), with over-expression either mouse or human MMPs.

FUTURE DIRECTIONS:

- Confirm the observations on PrPC above involving mouse PrPC and MMPs in cells expressing human PrPC and MMPs, as well as the 3F4-MoPrP. Including further work to determine the reason behind reduced cellular PrPC expression, for example assessing the localisation of the PrPC and MMPs co-expressed in these cells to establish if/where they co-localise, or determining whether extracellular PrPC degradation by the MMPs is occurring.
- Confirmation of observations utilizing human and mouse derived cell lines that express endogenous PrPC (i.e. instead of RK13 (rabbit) cells over-expressing other mammalian PrPC).
- Further determination of MMPs involvement of PrPC cleavage through experiments involving pharmacological and/or biological modifiers of MMP activity, including for example using protease inhibitors to treat cells, co-over-expression of more than one MMP or siRNA knockdown of endogenous MMPs.

Aim 2. To define the influence of MMPs in prion propagation and susceptibility:
KEY OUTCOMES / CONCLUSIONS:

- Exposure of prion infected cells to extracellular MMPs produced by non-infected cells for 72h through media exchange (transwells) did not significantly reduce prion propagation (as determined by detection of PrPres levels, the biochemical equivalent of the disease associated PrPSc prion protein, produced by the infected cells).
- Similarly, transient (72h) expression of the murine MMPs directly in prion infected MoRK13 cells showed no significant difference in total PrPres levels (by cell blotting), indicating that in these conditions there is very little effect on prion propagation, although preliminary results looking at PrP profiles (by western blotting) in these cells did show there to be some MMP-dependant and strain-dependant differences in PrP fragments compared to PrP in control cells.
- Stable over-expression of mouse MMPs into prion infected MoRK13 cells, after approximately 1 month, showed striking prion strain dependent and MMP dependent differences in PrPres levels present: In M1000 infected cells, over-expression of MMP2 massively increased PrPres propagation, whereas MMPs 7 & 9 decreased the amount of PrPres to undetectable levels. 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. Interestingly, the observed effect of MMP9 on PrPC expression in uninfected cells (i.e. possible PrPC degradation), might provide an explanation and mechanism behind this reduction of prion propagation.

- Whereas there was no difference in PrPres levels produced by MMP2 expressing MoRK13 cells after exposure to prions, a significant increase in PrPres propagation was seen in MoRK13 cells stably over-expressing mouse MMP7 which were then exposed to M1000 prions, with a similar trend observed for MU02 prions, possibly suggesting expression of MMP7 increases susceptibility to prion infection.

FUTURE DIRECTIONS:

- To use western blot analysis to check for changes in PrPres profiles (i.e. glycoform ratio & cleavage fragments, including C3), in addition to total PrPres levels, in prion infected cells transiently and stably expressing MMPs.
- Investigation of whether the observed significant reductions in PrPres levels in some MMP expressing prion infected cells equates to "curing" of the cells (in that they also contain reduced prion infectivity) by using these cells as a source of prions for further infections.
- Confirmatory experiments assessing prion propagation and susceptibility after MMP modifications through pharmacological or biological means (similar to Aim 1 future directions, for example including treatment with protease inhibitors or siRNA knockdown of endogenous MMPs).