Cell Research (2009) 19:1039-1040.

www.nature.com/cr

RESEARCH HIGHLIGHT

The saga of prion: to cut or not to cut

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Cell Research (2009) **19**:1039-1040. doi: 10.1038/cr.2009.102; published online 3 September 2009

Transmissible Spongiform Encephalopathies (TSE), commonly referred to as prion diseases, are a group of rare, infectious and fatal neurodegenerative diseases in mammals [1]. All prion diseases are thought to share a common pathogenic mechanism, which is based on the conversion of the normal cellular prion, PrP^{C}, into the infectious and pathogenic scrapie prion protein, PrP^{Sc} [2, 3]. The accumulation of PrP^{Sc} in the CNS is then thought to impair function, induce structural damage, and cause disease. In addition to gain of toxic function, loss of normal PrP^{C} function, a consequence of conversion to PrP^{Sc} may also contribute to pathogenesis [4].

More than 20 years after the cloning of the prion gene, *Prnp*, it is now firmly established that PrP^{C} is critical in the pathogenesis of prion diseases [2, 3]. In contrast, the normal physiologic function of PrP^{C} remains an enigma [5-7]. Human PrP^{C} is a highly conserved, relatively small (209 amino acid long), glycophos-phatidylinositol (GPI) anchored cell surface protein. A myriad of proteins have been reported to bind PrP^{C}; these proteins include cell surface proteins, secreted proteins, cytoplasmic proteins as well as nuclear proteins. In addition, PrP^{C} also binds lipids, glycosaminoglycans, nucleic acids as well as divalent cations, such as copper, zinc and iron [5-7]. As a GPI-anchored cell surface glycoprotein, PrP^{C} resides in lipid rafts, which are micro-domains on the cell surface and are important in signal transduction [8]. PrP^{C} also participates in determining apoptosis as well as regulating oxidative stress, apparently in a cell context dependent manner [5-8]. At the present time, it is difficult to conceive how a relatively small protein interacts with so many partners, mediating many cellular processes, in different cellular compartments. Despite all the important functions that have been attributed to PrP^{C}, the *Prnp*$^{-/-}$ mouse is apparently normal without an overt aberrant phenotype [9, 10].

Studies of prion biology have been limited by the lack of a large collection of anti-PrP^{C} specific monoclonal antibodies. For many years, only one PrP^{C} specific monoclonal antibody, 3F4, was available [11]. It was thought that PrP exists as three different glycoforms: a fully diglycosylated PrP^{C}, a mono-glycosylated PrP^{C} and an unglycosylated PrP^{C}. This conclusion was based on the finding that 3F4 reacts with three bands in immunoblots. More recently, it has become apparent that this view is over simplified; the synthesis, processing and transit of PrP^{C} are much more complex and not completely understood [12].

While 3F4 has been invaluable in many aspects of prion research, it has certain limitations. First, 3F4 reacts with an epitope in the central region of human PrP^{C} between amino acid residues 110 to 112; hence 3F4 does not react with some of the N-terminally truncated PrP species. Recent studies reveal that the ectodomain of PrP^{C} is subject to proteolytic cleavage by multiple proteases, such as A Disintegrin And Metalloprotease 10 (ADAM10), Tumor necrosis factor α-Converting Enzyme (TACE) and γ secretase. Furthermore, these PrP^{C} species may be important in prion biology and pathogenesis. Second, 3F4 reacts with human but not mouse PrP^{C}. In human PrP^{C} residue 112 is a methionine; in mouse, the corresponding residue is a valine. This presents a major drawback in the use of 3F4, due to widespread usage of transgenic mouse models in studying prion biology. To circumvent this problem, investigators have inserted the 3F4 epitope to mouse PrP^{C}, thus creating transgenic mouse lines or cell lines that express mouse PrP^{C} with the 3F4 epitope [9, 10]. These transgenic mouse lines and cell lines have been used extensively to study the pathogenesis of prion diseases as well as the normal functions of PrP^{C} [9, 10]. It was assumed that simply replacing a methionine with a valine should not distort the normal topology or function of PrP^{C}.

In a manuscript published in this issue of *Cell Research*, Haigh *et al.* reported findings that have implications for some of the issues discussed above: the proteolytic cleavage of PrP; the binding of copper to PrP^{C}, signal transduction and the importance of 3F4 epitope in prion biology [13]. Haight *et al.* found that PrP^{C} related signal transduction, more specifically MAP kinase signaling, is influenced by cop-

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per, membrane integrity and proteolytic cleavage, suggesting a link between the proteolytic cleavage of PrP and signaling [13]. This conclusion is based on comparing the processing and signaling of mouse PrP^C, human PrP^C and a mouse PrP^C containing the 3F4 epitope, in a rabbit cell line that lacks endogenous PrP^C. These investigators found that differences in the PrP^C primary sequence between mouse and human, especially that around the N1/C1 (3F4 epitope) cleavage site, influence the basal levels of proteolysis as well as extra-cellular signal-regulating ERK1/2 phosphorylation. While the results described in this manuscript provide new insights into the processing and signaling of PrP, it also opens another Pandora box in the saga of prion biology.

One can envision that differences in the primary sequence of PrP from different species may alter their conformation, which in turn modulates their susceptibility to proteolytic cleavage. On the other hand, the underlying mechanism by which changing one single amino acid from methionine to valine in PrP^C modulates signal transduction is less obvious. Since all three forms of PrP^C are GPI anchored on the cell surface, one possibility is that mouse PrP^C and human PrP^C interact with different proteins on the cell surface. Furthermore, the biochemical events that link proteolytic cleavage of PrP^C to signal transduction also remain to be determined. Do these results imply that cleavage of PrP^C exposes a "hidden" epitope, which then allows PrP^C to interact with another molecule to mediate signal transduction? Does the cleaved-product, the N-terminal fragment, have any biological activity? Other important points that require additional investigation are whether these *in vitro* generated membrane integrity changes occur *in vivo*, under what kind of conditions membrane perturbation occurs, and the relevance of these findings to normal PrP^C function and prion pathogenesis *in vivo*.

A more intriguing finding in this manuscript is the demonstration that the presence of the 3F4 epitope alters the proteolytic cleavage of the PrP^C. The investigators suggest that introduction of the 3F4 epitope into mouse *Prnp* may have significant biological consequences. As discussed above, over the years many different transgenic mouse lines as well as cell lines have been established and studied using 3F4-taged mouse *Prnp* [9, 10]. These investigators caution researchers using these constructs, they may wish to consider the affect this epitope may have on the function of PrP^C. Interestingly, a common polymorphism in human *PRNP*, also involving methionine and valine at residue 129, is important in the pathogenesis of human prion diseases [14]. While it is unlikely that this new data will drastically alter the interpretation of earlier results, like every thing else in life, the devil is in the details.

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