Prion permissive pathways: extracellular matrix genes control susceptibility to prion infection

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There are wide variations in the susceptibility of humans, animals, and cultured cell lines to infection by prions. In this issue of *The EMBO Journal*, Marbiah *et al* (2014) identified a gene regulatory network that regulates the susceptibility of cultured cells to prion infection. Surprisingly, a number of these genes impact the structure of the extracellular matrix. These results have important implications for understanding mechanisms of prion infection and also suggest new therapeutic targets.

See also: **MM Marbiah *et al*** (July 2014)

Prion diseases are transmissible neurodegenerative disorders of humans and animals characterized by dementia, motor dysfunction, and the accumulation of an abnormal isoform of the prion protein (PrP^{Sc}) in the central nervous system. PrP^{Sc} is an infectious protein that propagates itself via its ability to promote conversion of PrP^C (the normal, cellular form of the prion protein) into additional PrP^{Sc} molecules via a sequence-specific, templating mechanism (Prusiner, 1998). Examples of prion disorders include Creutzfeldt-Jakob disease and kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy in cattle.

A number of factors control susceptibility to prion diseases, most notably the endogenous gene that encodes PrP^C. Mice that lack PrP^C are completely resistant to prion infection (Büeler *et al*, 1993), and coding polymorphisms in the PrP^C gene affect disease susceptibility and incubation times in animals and humans (Westaway *et al*, 1987; Collinge *et al*, 1996). However, it is clear

from genetic studies in mice and humans that additional, non-PrP loci affect incubation times and susceptibility to infection (Lloyd *et al*, 2013). Exactly how the corresponding gene products function in the PrP^{Sc} propagation pathway remains unknown.

Prions can be propagated in cultured cell lines, as well as in laboratory animals. This is generally done by exposing cells to prion-infected brain homogenate, passaging the cells, and then assessing the presence of PrP^{Sc} via Western or cell blotting. Interestingly, only certain cell lines are susceptible to infection, while others are not. For example, N2a mouse neuroblastoma cells are easily infectible and are a commonly used model in the prion field, while CHO or HEK cells are resistant to infection (Butler *et al*, 1988). Amazingly, from a single cell line, it is possible to isolate some subclones that are highly infectible, as well as other subclones that are almost totally resistant to infection (Klohn *et al*, 2003). Importantly, these differences are not correlated with PrP expression levels and are presumably due to genetic or and/or transcriptional differences that are inherited within each subclone. Until now, there was very little insight into the molecular factors that control these variations in susceptibility. Identifying these factors is of great importance, both for understanding basic pathogenic mechanisms and for developing effective therapies. Genes and proteins that influence prion susceptibility represent potential new targets for treatment of these invariably fatal diseases.

In this paper, Marbiah *et al* (2014) employed a clever strategy to elucidate a gene regulatory network that controls prion

infectibility in cultured cells. The authors used different subclones of N2a neuroblastoma cells that are either susceptible or resistant to infection by a particular prion strain. Using transcriptional profiling, they compared three subclones that are susceptible to prion infection with three other ones that are resistant (called revertants because they were derived from susceptible N2a cells). Employing this approach, they identified a set of 95 genes that are differentially expressed in the two groups. Based on their observations that this set was enriched in genes involved in cellular differentiation and development and that the susceptible cells over-expressed genes that promoted a differentiated phenotype, the authors tested the effect of the pro-differentiation agent, retinoic acid, on prion infectibility. Treatment of resistant subclones with retinoic acid increased prion propagation up to 40-fold, rendering the cells highly susceptible to infection.

The authors then used this phenomenon as the basis for an additional filter to identify relevant genes. They first compared the transcriptional signatures of the resistant cells treated or not with retinoic acid and identified 97 genes that were over-expressed in the treated group. They then compared this list of genes with the list of 95 genes identified from their original analysis of susceptible vs. resistant subclones, yielding a small set of 18 overlapping genes that were found on both lists. They proceeded to validate this set of genes, first by quantitative, real-time PCR, and then functionally using shRNA-mediated knockdown. Strikingly, knockdown of any one of 9 genes in prion-resistant cells

caused the cells to become several-fold more susceptible to infection. These genes included fibronectin 1 (*Fn1*), integrin $\alpha 8$ (*Itga8*), chromogranin A (*Chga*), IQ motif containing GTPase-activating protein 2 (*Iqgap2*), interleukin 11 receptor alpha chain 1 (*Il11ra1*), Micalc C-terminal like (*Micalcl*), regulator of G-protein signaling 4 (*Rgs4*), 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (*Papss2*), and galactosyltransferase 2 (*Galt*). These genes thus defined a regulatory network whose upregulation suppresses prion infection.

Next, the authors carried out a series of experiments to explore the cellular roles of the corresponding gene products. Using immunostaining, they found that several of the nine proteins were associated with the extracellular matrix (ECM), including Fn1, Chga, Il11ra1, Itga8, and Micalcl. Using an improved method for visualizing extracellular PrP^{Sc}, they demonstrated that expression of some of the proteins, notably Fn1 and Chga, was negatively correlated with deposition of PrP^{Sc} in the ECM. To further document the connection between the ECM and prion resistance, the authors showed that treatment of resistant cells with the RGD

peptide, known to block Fn1-integrin interaction, caused the cells to become more susceptible to prion infection. This was accompanied by reduced secretion of the MMP2/9 metalloproteinase. To assess the role of Papss2, an enzyme critical for glycosaminoglycan (GAG) sulfation, they used Papss2 siRNA or sodium chlorate, a chemical inhibitor of glycosaminoglycan sulfation. Both agents inhibited sulfation of heparin sulfate proteoglycans and increased the prion susceptibility of resistant cells. Moreover, silencing of Fn1 or Papss2 expression caused increased deposition of PrP^C at the ECM level, an alteration that can explain why these cells are more susceptible to prion infection, because more substrate is available to be converted into PrP^{Sc}.

Overall, the results of Marbiah *et al* indicate that the ECM plays a critical role in controlling the susceptibility of cultured cells to prion infection. This conclusion is consistent with a number of lines of evidence linking PrP^{Sc} and PrP^C to sulfated GAGs, prominent components of the ECM. For example, exogenously administered, sulfated GAGs are potent inhibitors of prion propagation in cultured cells and animal

models (Caughey & Raymond, 1993). In addition, GAGs are known to bind to the N-terminal half of PrP^C, thereby enhancing its endocytosis from the cell surface (Shyng *et al*, 1995). GAGs also co-localize with PrP^{Sc} deposits in brain.

This work raises a host of interesting questions for future study. Perhaps the most pressing is exactly how upregulation of certain ECM components inhibits prion infection. One possibility is that endogenous GAGs in ECM normally bind to the N-terminal part of PrP^C, thereby inhibiting conversion to PrP^{Sc}. ECM GAGs may also bind PrP^{Sc} in the prion inoculum, impeding its access to PrP^C on the cell surface and its ability to initiate infection. In either case, downregulating GAG sulfation, or otherwise remodeling the ECM, may reverse these inhibitory processes. These mechanisms would be consistent with the effect of *Papss2* gene knockdown, which reduces GAG sulfation. Another hypothesis, suggested by the authors (Fig 1), is that PrP^C deposited in the ECM serves as substrate for the initiation of infection. If this were the case, remodeling of the ECM may allow more PrP^C to be deposited there, thereby enhancing PrP^C

Figure 1. Model for how two ECM components, the $\alpha 8$ chain of integrin (Itga8) and fibronectin 1 (Fn1), regulate susceptibility to prion infection.

In prion-susceptible cells (A), Fn1 and Itga8 are expressed at low levels, leading to a poorly developed ECM structure, and more deposition of PrP^C in the matrix that can be converted into PrP^{Sc}, thereby facilitating infection. The RGD domains of Fn1 (turquoise) are shown binding to an integrin dimer, consisting of an Itga8 α chain and a β chain (not analyzed in this paper). In prion-resistant cells (B), Fn1 and Itga8 are highly expressed, the ECM is denser, and less PrP^C is deposited, impeding generation of PrP^{Sc}. Consistent with this model, disruption of Fn1-integrin interaction by incubation of resistant cells with soluble RGD peptide rendered the cells more prion-susceptible.

formation. This scenario is consistent with the observed changes in PrP^C localization observed upon knockdown of Fn1 and Papss2. It is known that PrP^C attached to the plasma membrane via its glycolipid anchor is rapidly converted to PrP^{Sc} upon contact with exogenous prions (Goold *et al*, 2011), but how PrP^C might be released into the ECM and what role this form may play in prion propagation are open questions. How the other gene products identified in the study affect prion infection is unclear. Some of them, such as Chga, Iqgap2, Il11ra1, Micalcl, and Rgs4, are membrane or cytoplasmic proteins that are not known to be directly involved in ECM biology. These proteins could have indirect effects on the ECM, or alternatively, they may act via a completely different mechanism. Finally, it will be important to determine to what extent the current results can be extrapolated from cultured cells to tissues and organs. It seems likely that the factors that control cellular accessibility to prions in an *in vivo* setting differ from those operative in a culture dish.

The study of Marbiah *et al* (2014) has potentially important therapeutic implications. There are currently no effective treatments for prion diseases, although the utility of PrP knockdown approaches has been demonstrated in experimental animals (White *et al*, 2008). The proteins identified in this paper represent novel targets for anti-prion drugs. In this regard, compounds that cause enhanced deposition or stabilization of the ECM might be predicted to reduce or prevent prion infection. In their study, the

authors demonstrated that knockdown of ECM-related genes rendered resistant cells more susceptible to infection, but they did not determine whether over-expression of the same genes made susceptible cells resistant. This would clearly be an important first step in developing the novel therapeutic approach suggested here. Aside from their potential clinical relevance, the results presented here may also be helpful to prion biologists, by providing a way to enhance the prion susceptibility of cell types, such as primary neurons, that have been traditionally difficult to infect in culture.

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Conflict of interest

The authors declare that they have no conflict of interest.

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