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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 (PrPSc) in the central nervous system. PrPSc is an infectious protein that propagates itself via its ability to promote conversion of PrPC (the normal, cellular form of the prion protein) into additional PrPSc 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. Mice that lack PrP are completely resistant to prion infection ( Büeler *et al*, 1993), and coding polymorphisms in the PrP 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 PrPSc 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, passing the cells, and then assessing the presence of PrPSc 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 α8 (Itga8), chromogranin A (Chga), IQ motif containing GTPase-activating protein 2 (Iqgap2), interleukin 11 receptor alpha 1 chain (Il11ra1), integrin a8, Mical C-terminal like (Micalcl), regulator of G-protein signaling 4 (Rgs4), 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (Papss2), and galactosyltransferase (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 matrix (ECM), including Fn1, chromogranin A (Chga), interleukin 11 receptor alpha 1 chain (Il11ra1), integrin a8, and Mical C-terminal like (Micalcl). This work raised 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 PrPSc, thereby inhibiting conversion to PrPSc. ECM GAGs may also bind PrPSc in the prion inoculum, impeding its access to PrPSc 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 PrPSc deposited in the ECM serves as substrate for the initiation of infection. If this were the case, remodeling of the ECM may allow more PrPSc to be deposited there, thereby enhancing PrPSc conversion into PrPSc, 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 PrPSc is deposited, impeding generation of PrPSc.

Figure 1. Model for how two ECM components, the α8 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 PrPSc in the matrix that can be converted into PrPSc, 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 PrPSc is deposited, impeding generation of PrPSc.

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\textsuperscript{C} localization observed upon knockdown of Fn1 and Papp2. It is known that PrP\textsuperscript{C} attached to the plasma membrane via its glycolipid anchor is rapidly converted to PrP\textsuperscript{Sc} upon contact with exogenous prions (Goold \textit{et al}, 2011), but how PrP\textsuperscript{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, Mical1, 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 \textit{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 \textit{et al}, 2008). The proteins identified in this paper represent novels 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.

References

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