Scientists develop new tests that identify lethal prion strains quickly and accurately

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One of the new in vitro tests, called the Standard Scrapie Cell Assay, measures prion infectivity levels in a highly accurate and extremely rapid way, producing results in less than two weeks. The second test, called the Cell Panel Assay, allows researchers to quickly distinguish between several prion strains in various cells lines. Using the new assays, the scientists were able to show that four different cell lines exhibited widely different responses to four different strains of the infectious protein particles.

The research is being published in an advanced online edition of the *Proceedings of the National Academy of Sciences* the week of December 3, 2007.

“These new assays vastly accelerate the measurement of prion infectivity and the determination of those cell lines that are able to sustain high infection rates of some prion strains,” said Sukhvir P. Mahal, an author of the study who is a senior staff scientist in the laboratory of Charles Weissmann, chair of the Scripps Florida Department of Infectology. “The current test, which takes anywhere from 150 to 250 days and involves large numbers of laboratory mice, is slow, imprecise, and expensive. Our new assays will replace the current mouse brain-bioassays.”

The current method of measurement and identification involves injecting a prion-containing sample into the brains of mice and then waiting to see how long it takes for the animals to succumb to disease;

the higher the prion level, the less time it takes for them to become lethally infected.

In contrast, the new Standard Scrapie Cell Assay is based on prion-susceptible cell lines. In the test, cells are exposed to prions and then the infected cells are identified and counted using automated imaging equipment.

A Unique Pathogen

Prions (the name stands for proteinaceous infectious particles) are unique infectious pathogens associated with some 15 different diseases, including Bovine Spongiform Encephalopathy ("mad cow") and its rare human form, variant Creutzfeldt-Jacob disease. Infectious prions, which are thought to consist mainly of an abnormally structured or misfolded protein, have the ability to reproduce, despite the fact that they contain no nucleic acid genome as do viruses or bacteria.

Mammalian cells normally produce what is known as cellular prion protein; during infection, the abnormal protein converts production of normal host prion protein to its infectious form. The full details of this process are still not understood.

Prions develop in distinct strains, initially characterized by incubation time and the pattern of brain damage that develops during infection. It is currently thought that strain-specific properties of prions are determined by the three-dimensional structure of the misfolded protein, although the amino acid sequence remains the same. During infection with a single type of prion, several different prion strains can be propagated indefinitely in a single host.

"Some cell lines can be persistently infected by prions and show preference for certain strains," Mahal said. "One intriguing finding of

our new study is that a cell line's ability to replicate a particular prion strain is a trait that varies significantly among the members of the cell population-even sibling cell lines may show different relative susceptibilities to various prion strains."

This suggests that the capacity of a cell line to replicate a particular prion strain is controlled epigenetically without any changes to the DNA sequence, she said.

Another fascinating question raised by the study is how cells come to distinguish between prion strains; that is, between the various proteins that differ only in the way they are folded. The exact nature of that recognition process is now the target of a new Scripps Research study using the Cell Panel Assay.

Source: Scripps Research Institute