More tools for prion research

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Monoclonal antibodies that recognize both normal cellular prion protein (PrP^{C}) and abnormal disease-associated isoforms (PrP^{Sc}) without denaturation became commercially available on August 2 this year. These antibodies will be useful for basic research, diagnosis and possibly therapy, says London-based academic spin-out company D-Gen (http://www.d-gen.co.uk). Meanwhile, the search continues for a PrP^{Sc}-specific antibody and a blood-based test for PrP^{Sc}.

New antibodies for studying prions

Prion diseases, such as variant Creutzfeldt-Jakob disease (vCJD) in people and scrapie in sheep, are characterized by the deposition of PrP^{Sc}, an abnormal amyloid protein, in the brain. By changing the conformation of PrP^{C}, PrP^{Sc} propagates throughout the brains of infected people and animals, causing neurodegeneration and death. Over the years, many antibodies have been developed for use in basic research and diagnostics that recognize PrP^{C} alone or both PrP^{C} and PrP^{Sc}. However, PrP^{Sc}-specific antibodies have been more elusive.

The antibodies now being marketed by D-Gen were raised by immunizing mice lacking PrP^{C} with either an α-helical form of recombinant human PrP representative of PrP^{C}, or a form enriched in β-sheets representative of PrP^{Sc}, explains D-Gen consultant Jonathan Wadsworth of the Medical Research Council's Prion Unit at the Institute of Neurology, London, UK (http://www.ion.ucl.ac.uk). 'What is important about our antibodies is that they recognize both PrP^{C} and PrP^{Sc} in the native form — most other

commercially available antibodies only recognize denatured PrP^{Sc}.'

Recognizing native isoforms

This difference has several consequences. For example, some of the D-Gen antibodies can delay prion disease development in animal models [1]. Furthermore, the antibodies should make it possible to determine exactly what prion forms are present in which tissue, including blood.

'With many antibodies, biological tissues have to be treated pretty harshly for PrP^{Sc} to be recognized,' says Wadsworth. 'But with our antibodies, abnormal PrP forms can be isolated without altering their physicochemical properties.' Thus, research using the D-Gen antibodies could yield new information about the blood form of PrP^{Sc} that could lead to a diagnostic test for it.

Although the D-Gen antibodies are not unique, having them commercially available will be very useful, comments Neil Cashman, Professor of Neurology at the University of Toronto (http://www.utoronto.ca). 'The widespread availability of any antibodies against prion protein isoforms is a great boon to research on this enigmatic molecule.'

The missing antibody

Foremost among antibodies that would advance prion research is one specific for PrP^{Sc}. Last year, Cashman's team described a PrP^{Sc}-specific antibody generated by immunizing animals with a tyrosine-rich motif exposed in PrP^{Sc} but not in PrP^{C} [2]. Caprion (http://www.caprion.com), a company founded by Cashman, is using this discovery to develop new diagnostics for prion diseases.

This July, Anthony Williamson, Assistant Professor of Immunology at the Scripps Research Institute in La Jolla (http://www.scripps.edu), and co-workers described two more PrP^{Sc}-specific antibodies. Previous research highlighted two PrP^{C} regions that are important for the PrP^{C}~PrP^{Sc} interaction that precedes PrP^{C} conversion into PrP^{Sc}. Could transplantation of these regions into an antibody framework provide a PrP^{Sc}-specific reagent? The researchers reported a positive answer to this question [3]. 'We are now selecting variants of our engineered antibody with higher affinities and specificities that could form part of a diagnostic test', says Williamson.

Premortem diagnostic test

With the recent report in the *Lancet* of a second UK patient who had contracted vCJD from a blood transfusion [4], the need for a test to detect PrP^{Sc} in blood is more urgent than ever. Antibodies specific for PrP^{Sc} and D-Gen's antibodies could help in the development of such a test, although the task will not be easy because PrP^{Sc} levels in blood are likely to be very low. 'A blood-based test for human prion disease may ultimately rely on a surrogate marker that has yet to be identified through basic research,' concludes Wadsworth.

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Abundant A-to-I editing in the human transcriptome

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RNA editing by ADAR (adenosine deaminases acting on RNA) enzymes convert adenosine to inosine (A-to-I) in precursor mRNAs and plays a crucial role in vertebrate and invertebrate development. If ADAR-mediated editing is knocked out, the organism dies. Evidence of this editing has been found in inflamed tissues, malignant gliomas, epileptic mice, suicide victims experiencing chronic depression, and in individuals with amyotrophic lateral sclerosis [1], implicating the process in a number of diseases.

New targets

Previously, the glutamate and serotonin receptors were the only known ADAR substrates. Recently, however, a report from Compugen (http://www.cgen.com), based in Tel Aviv, Israel, describes identification of 12,723 A-to-I editing sites in 1637 different genes using computational methods. Among these, 26 novel substrates were validated experimentally.

'Previously, only two targets were known,' says Compugen scientist and spokesman, Eli Eisenberg. 'But there are three different enzymes that do that editing,' he explains. 'It can't be that three enzymes only have two targets. Other evidence suggests that there are many more editing sites.'

Hunting for novel substrates

Putting that theory to the test, bioinformatics specialists at the company developed a computational approach designed to overcome recognized difficulties associated with identifying unknown ADAR substrates.

ADAR substrates consist of imperfectly matched dsRNA stems that are formed when an exon containing an adenosine to be edited base pairs with a complementary region of pre-mRNA, which can be thousands of nucleotides apart. Compugen's approach entailed searching for mismatches in potential double-stranded regions. Human expressed sequence tags (ESTs) and cDNAs were aligned with the genome and organized into gene groups and partial gene groups. The algorithm they developed then honed the search for A-to-I editing sites.

In humans, they discovered that editing sites are typically found in noncoding regions of RNA, particularly among Alu repeats, transposable elements unique to primates and responsible for >10% of our genome. Two Alu regions oppositely oriented within a gene can form dsRNA. The ADAR enzyme recognizes the dsRNA stem and then changes some of the adenosines to inosines.

'The editing prefers to attack sites that are not perfectly paired… and is related to the stability of the dsRNA,' explains Eisenberg. Furthermore, it was found to occur in a variety of organs. 'A couple of years ago, this was thought to be restricted to the nervous system – all edited mRNAs before were found in the brain,' says biologist Gordon Carmichael, also at the University of Connecticut Health Center, Farmington, Connecticut, USA (http://www.uchc.edu). 'This shows us that [this RNA editing] is way more prevalent and it's in messages we didn't think it would be in.'

The Compugen team reasons that such widespread editing contributes to greater diversity of the human transcriptome than could be achieved by alternative splicing alone.

But Robert Reenan, a biologist also at the University of Connecticut Health Center, suspects that stability might not be solely responsible. 'It certainly is possible that, rather than just reflecting an effect on the stability of the RNA secondary structure, that it's a reflection of the enzyme's preference,' he says.

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