The Malignant Protein Puzzle

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Editor’s Note: When most people hear the words malignant and brain, cancer immediately comes to mind. But our authors argue that proteins can be malignant too, and can spread harmfully through the brain in neurodegenerative diseases that include Alzheimer's, Parkinson's, CTE, and ALS. Studying how proteins such as PrP, amyloid beta, tau, and others aggregate and spread, and kill brain cells, represents a crucial new frontier in neuroscience.
On the Australian island of Tasmania around 20 years ago, a disfiguring, fatal cancer of the face was reported to be rapidly spreading among Tasmanian devils. The disease, known as devil facial-tumor disease, happens to be an extraordinary instance of infectious cancer. It is caused not by a virus but by the direct transfer of cancer cells from one devil to another, possibly through biting.\(^1\) And it is not unique to devils; other examples of unusual infectious cancers have been described in species such as dogs\(^2\) and clams.\(^3\)

These curious cases reveal that some cancer cells can “infect” receptive hosts, but they by no means indicate that all malignancies should be treated as infectious diseases. The great majority of cancers arise within the body of the host when normal cells transform and proliferate uncontrollably. Infectious cancers do, however, highlight the impartial resourcefulness of biology in both health and disease.

We now believe that many of the neurodegenerative diseases that increasingly plague modern humans bear an intriguing similarity to cancer, except that the disease agents that proliferate in these brain disorders are not transformed cells, but rather transformed proteins that have folded into the wrong shape. Such “malignant” proteins are key players in such devastating diseases as Alzheimer’s, Parkinson’s, Huntington’s, frontotemporal dementia, chronic traumatic encephalopathy (CTE), amyotrophic lateral sclerosis (ALS), and Creutzfeldt-Jakob disease (CJD). Most of these maladies are thought to be non-contagious under ordinary circumstances, but CJD and its variants have been transmitted to humans by tainted meat, cannibalism, and tissue transplants, and research suggests that other disease-linked malignant proteins can in some circumstances transmit their properties from one organism to another.

As in the case of cancerous cells, though, these rogue proteins almost always emerge and propagate within the affected host. Once the misfolded proteins gain a foothold in the nervous system, they effectively compel normal versions of the same protein to adopt the same malformed state. In this faulty configuration, the proteins stick to one another and, in a molecular chain-reaction, structurally corrupt like molecules that are generated in the course of normal cellular metabolism. In many instances, the final products of this process are clumps of the protein called
amyloid (Figure 1). Central to this process is what we call seeded protein aggregation (seeding for short), a surprisingly common disease mechanism that first came to light with the discovery that protein seeds called prions can act as infectious agents.

![Image of amyloid formation](Image courtesy of Lary Walker/Mathias Jucker)

**Figure 1.** The steps leading from a single protein molecule to clumps of amyloid. The misfolded proteins act as seeds that accelerate the crystallization-like disease process. The seeds can vary in size, and very small assemblies called oligomers can be particularly toxic to cells. In prion diseases, the seeds can sometimes be transferred from one organism to another (“infection”).

The Prevailing Prion

The improbable tale of infectious proteins began in the 1730s, when reports of a slowly progressing and ultimately fatal disease of sheep first appeared in the European scientific literature. British farmers called the disease scrapie because affected sheep were seen to scrape the wool from their skin by compulsively rubbing against farmyard objects. The farmers suspected even then that scrapie was contagious, but it wasn’t until the 1930s that transmission of the disease was demonstrated experimentally by Jean Cuillé and Paul-Louis Chelle in France.\(^4,5\) To establish infectivity, Cuillé and Chelle injected homogenized nervous tissue from scrapie-affected donor sheep into healthy host sheep. Infectious illnesses usually emerge within days or weeks, but earlier experiments had failed to demonstrate transmission of scrapie in this timeframe. Cuillé and Chelle,
however, were patient; the sheep that they injected with scrapie-tainted tissue finally succumbed to the disease more than a year later.

Thus began a long and prickly debate about the nature of the scrapie agent: What kind of infectious pathogen causes disease only after months or years of incubation? Furthermore, infections usually announce their presence with inflammation and fever, yet scrapie showed no such signs. The term “slow virus” was adopted by many, but evidence gradually mounted that the culprit was not a virus at all, but rather, just possibly, an infectious protein.

Interest in the problem intensified in the 1960s when D. Carleton Gajdusek and his colleagues made the Nobel Prize-winning discovery that, like scrapie, two rare human neurodegenerative diseases, kuru and Creutzfeldt-Jakob disease, are transmissible with very long incubation periods. By then it was becoming clear that the agent of infection was strange indeed. Radiobiological experiments performed by Tikvah Alper strongly suggested that the agent did not require nucleic acids to replicate, and the mathematician John Griffith described, prophetically, how a protein-only agent might multiply using the host’s genetic machinery to generate more protein. In 1982, Stanley Prusiner crystallized the protein-only concept (and enraged its opponents) by naming the scrapie agent a “proteinaceous infectious particle,” or “prion.” In subsequent years, Prusiner’s group, along with a growing cadre of allies, amassed persuasive experimental support for the prion concept, for which Prusiner was awarded the Nobel Prize in 1997. Although echoes of the old debate about the causative agent still sometimes find their way into print, the prion paradigm has prevailed, and today is evolving into a far-reaching new concept of disease.

Assembling Into Amyloid
The prions of CJD and scrapie are submicroscopic assemblies of a natural mammalian protein known as prion protein, or PrP. Prions consist of misfolded versions of PrP that can seed the formation of similar assemblies by a process resembling the seeded crystallization seen in some chemical reactions (Figure 1). In this sense, PrP prions can be viewed as malignant proteins that multiply and spread within the nervous system, eventually causing neurological dysfunction and death. In humans, PrP prions trigger progressive, fatal neurodegenerative disorders that include CJD, kuru, Gerstmann-Sträussler-Scheinker disease, and fatal insomnias. In nonhuman species, the
PrP prion diseases include scrapie, bovine spongiform encephalopathy (“mad cow disease”), and chronic wasting disease (CWD) of North American deer, elk, and moose.\textsuperscript{16,17}

Before the discovery of prions, these diseases often were called spongiform encephalopathies due to the appearance of sponge-like vacuoles in the brain (Figure 2). Despite being caused by just one type of protein—PrP—different prion diseases display remarkably different clinical and pathological signs, and these differences appear to be related to distinct molecular features of the prions.\textsuperscript{18} The exact mechanisms by which nervous tissues are damaged in prion diseases (and other neurodegenerative disorders) remain incompletely understood—and that poses a pressing challenge for future research.

The enigma of infectious proteins was deepened with the discovery that PrP prion diseases also can be caused by mutations in the gene that encodes PrP.\textsuperscript{9,12} These hereditary forms of prion disease, ironically, helped to establish the protein-only hypothesis of the infectious agent, as they confirmed the importance of normally generated PrP in the creation of new prions.

In other words, hereditary (and presumably also spontaneous) PrP prion disease begins when prions are formed by the misfolding of PrP that is generated by cells inside the body. Infectious prion disease, in contrast, results when external prions invade the body of the host. But in all cases, once the process begins, the abnormal prion protein accumulates in the nervous system and triggers the impairment and death of neurons. As abnormal PrP aggregates build up in the brain, they sometimes clump into distinctive masses of amyloid.\textsuperscript{18}

This attribute of misfolded PrP—its ability to assemble into amyloid—furnishes telling clues to the nature of the prion. Surprisingly, amyloid-forming proteins also characterize more common age-related neurodegenerative diseases, such as Alzheimer’s disease. While amyloid is obvious under
the light microscope (see Figure 1), the amyloid itself may only be the tip of the iceberg; the aberrant proteins often form assemblies that are not amyloid in the strict sense of the word, and in many instances, much smaller clusters of misfolded protein molecules called oligomers have been found to be quite toxic to cells.

**Other Plaques and Tangles**

Intriguing similarities, such as the presence of amyloid and relentless decline of brain function, suggested to Gajdusek, Prusiner, and others that the prion diseases might yield insights into the cause of Alzheimer’s, Parkinson’s, and other human neurodegenerative disorders. Virtually all of these maladies involve the appearance of characteristic protein deposits in the brain. For example, in Alzheimer’s disease (the most frequent cause of dementia), a protein called amyloid beta (Aβ) aggregates to create the “senile plaque” formations seen in the gray matter of all Alzheimer’s brains (Figure 3), as well as cerebral amyloid angiopathy, a buildup of amyloid in the walls of brain blood vessels. Another protein called tau also can adopt the amyloid structure, forming neurofibrillary tangles in Alzheimer’s (Figure 3). While both plaques and tangles are necessary to drive Alzheimer’s disease, the prime mover in the degenerative cascade appears to be Aβ. In Parkinson’s disease, yet another protein known as α-synuclein assembles into intracellular amyloid clumps called Lewy bodies. The list of diseases and their misshapen proteins continues to grow. In each disease, the flawed proteins are associated with distinctive signs and symptoms. But are they, like PrP prion disease, transmissible?

In the 1960s, Gajdusek’s group began a massive study to address this very question. Specifically, they wanted to know if non-PrP neurodegenerative diseases such as Alzheimer’s are transmissible to nonhuman primates? The outcome was essentially negative. In Great Britain, however, a team
led by Rosalind Ridley and Harry Baker reported in the early 1990s that Aβ plaques and cerebral amyloid angiopathy are increased in the brains of marmosets several years after injection of Alzheimer brain homogenates into the brain. The actual agent that precipitated these amyloid deposits, however, remained uncertain.

These researchers logically used nonhuman primates to assess the potential transmissibility of Alzheimer’s disease, since close evolutionary relatives are most likely to manifest the same type of disease. Such experiments, however, were hampered by issues of time and cost. Normal laboratory mice and rats were not suitable for these experiments because the chain of amino acids that makes up rodent Aβ differs from that in humans and monkeys; for that and perhaps other reasons, rats and mice do not naturally develop amyloid deposits in the brain as they grow old. In the mid-1990s, however, genetically engineered mouse models were introduced that make human-sequence Aβ. These “transgenic” mice generate amyloid plaques within a matter of months, and thus were widely adopted as the first practical animal models for studying Alzheimer-like Aβ aggregation in the brain.

Testing a Hypothesis

With this important new tool in hand, the two of us set out to test the hypothesis that Aβ-amyloid can be induced to form in the brains of transgenic mice by a mechanism similar to the infectivity of PrP prions. In our earliest studies, we homogenized brain tissue from Alzheimer patients, spun it briefly in a centrifuge to remove larger debris, and injected a small amount (usually one to four millionths of a liter, or microliters) of the clear extract into the brains of transgenic mice expressing human-sequence Aβ. After an incubation period of several months, the mice began to develop Aβ plaques and cerebral amyloid angiopathy in the injected region, similar in many ways to the Aβ amyloid pathology seen in Alzheimer’s. Subsequent experiments in our labs and others have shown that the seeding agent is indeed aggregated Aβ.

The mice did not develop full-blown Alzheimer’s disease, which, to the best of our current knowledge, occurs only in humans. Research has shown, however, that at the molecular level, Aβ seeds resemble PrP prions in virtually every way: they consist solely of a particular protein; the seeds vary in size; they resist destruction by high temperature or formaldehyde; they can spread
within the brain and to the brain from elsewhere in the body; and different seed structures have different biological properties (variants that are referred to as strains).11,14

More recently, numerous elegant studies have found that proteins involved in other neurodegenerative diseases also have prion-like properties. These proteins include tau (which forms neurofibrillary tangles in Alzheimer’s disease, CTE, and many other disorders), α-synuclein (which forms Lewy bodies in Parkinson’s disease, Lewy body dementia, and multiple system atrophy), huntingtin (which forms inclusion bodies in Huntington’s disease), and several proteins with prion-like properties that accumulate in such disorders as ALS and frontotemporal dementia.11,12,22-25

**Are Neurodegenerative Proteopathies Infectious?**

A growing awareness of the similarities between PrP prions and other protein seeds has revived speculation that Alzheimer’s and other neurodegenerative diseases might be infectious. This question gained recent prominence with a report from a team led by Sebastian Brandner and John Collinge in Great Britain showing that at least one facet of Alzheimer’s disease—Aβ-amyloid formation—appeared to be induced in patients who were treated as children with human growth hormone in order to correct short stature.26 It was discovered in the mid-1980s that some of the growth hormone used for treatment, which had been isolated from large batches of human pituitary glands collected at autopsy, was contaminated with PrP prions. As a result, some recipients died of Creutzfeldt-Jakob disease many years after their growth hormone treatments had ceased. Brandner, Collinge, and co-workers were able to examine the brains of eight of them who were 36- to 51-years-old at the time of death. In addition to PrP prion pathology, four of the patients also had substantial Aβ accumulation in plaques and cerebral blood vessels, and two others had sparse Aβ deposits.

The appearance of Aβ plaques and vascular amyloid in people at such a young age is quite unusual. The findings strongly suggest that some batches of growth hormone were contaminated with Aβ seeds in pituitary glands that were inadvertently collected from Alzheimer patients. Remarkably, none of the eight subjects had evidence of neurofibrillary tangles, the other defining brain abnormality in Alzheimer’s disease. Because all of them had died of prion disease, we cannot know
whether they eventually would have developed Alzheimer’s. If so, the incubation period would likely be at least as long as that of prion disease.

This presumed transmission of Aβ-amyloidosis to humans occurred under extraordinary circumstances—repeated, long-term injections of a hormone derived from pooled human pituitary glands. By sheer good luck, recombinant growth hormone (produced by genetically modified bacteria) became available in 1985, just at the time when the cadaver-derived hormone was confirmed to be contaminated with PrP prions. The recipients were quickly switched to this safer version of the agent. Strangely (or perhaps not), a black market continued to flourish for cadaver-derived growth hormone, sustained in large part by body-builders and other athletes; the cadaver-derived hormone is indistinguishable from that produced by the recipient, and thus is difficult to detect in doping tests.²⁷

Fortunately, most of the patients treated with growth hormone prior to 1985 have not developed prion disease. It will be important to follow them in the coming years to determine whether they are at higher risk of Alzheimer’s disease and other neurodegenerative disorders involving protein seeds. Interestingly, a team of Swiss and Austrian researchers recently reported a similar induction of Aβ deposition in CJD patients many years after they had received transplants of PrP prion-contaminated dura mater that had been harvested from human cadavers.²⁸ These studies by no means indicate that Alzheimer’s disease can be transmitted from person-to-person under everyday circumstances; rather, they do provide the first evidence that the aggregation of a protein other than PrP might be induced in the human brain by exogenous seeds. Just as cancer cells can occasionally transmit disease from one animal to another, the same appears to be true—under exceptional circumstances—for some pathogenic protein seeds.

Promise and Pitfalls of the Prion Paradigm

In Alzheimer’s and other non-PrP neurodegenerative diseases, malignant protein seeds arise from normally generated proteins inside the body, just as malignant cells stem from normal cells in cancer. Nature also has exploited the prion mechanism for beneficial ends; proteins that form prion-like aggregates handle functions ranging from information transfer in yeast²⁹ to the storage of peptides³⁰ and the consolidation of memory³¹ in mammals. In light of these discoveries, we have
argued that the term “prion” should be re-defined as a “proteinaceous nucleating particle” to stress the molecular process of seeded protein aggregation (nucleation) that is common to all of these phenomena. By removing the disquieting word “infectious,” the new definition accommodates the many instances in which such proteins are not infectious by any customary definition of the term.

Recent research has brought into clearer focus the devastating role of malignant proteins in diverse diseases. Infectivity is undoubtedly an important characteristic of PrP prions, particularly in some nonhuman species. Chronic wasting disease, for example, is rapidly spreading among members of the deer family in western North America. In humans, though, most cases of PrP prion disease do not result from infection. Because prions achieved notoriety largely due to their infectivity, this peculiar feature has colored our view of all cases of PrP prion disease, whether they are caused by infection or not. In light of the history of the prion concept, one has to wonder how we would view cancer if the first malignancy discovered had been devil facial-tumor disease, and only later did we learn that most cancerous cells actually develop within the body of the affected organism. Would we now consider all cancers to be potentially infectious? And at what cost to the patients and those who care for them? Our perception of disease, and the language we use to define it, must continually adapt to new information. By highlighting the molecular properties of malignant proteins, the evolving prion concept will help to guide future experimental strategies for defeating a multitude of intractable conditions.

**Bios**

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