RNAi: a novel strategy for the treatment of prion diseases

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Commentary

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Prion diseases

Prion diseases, or transmissible spongiform encephalopathies, have 3 etiologies: genetic (due to pathogenic mutations of the gene encoding the prion protein [PrP]), acquired (as a result of exogenous prion infection), and sporadic (of unknown causes) (1). Prion disease is caused by prions, in which a self-replicating, infectious protease-resistant form of PrP (termed PrP^{Sc}) is the only essential component identified to date. PrP^{Sc} multiplies through conversion of the normal cellular PrP (PrP^C) (Figure 1) (1). Host PrP^C is essential for prion propagation, since no prion replication occurs in mice devoid of PrP^C (2). Host PrP^C expression is also essential for prion pathogenesis. When normal neural tissues were grafted to the brains of mice devoid of PrP^C, only the grafted tissues developed neurodegeneration after prion infection, but the PrP^C-free brain tissues of the host were virtually unaffected despite the presence of large amounts of PrP^{Sc} (3). These and other reports establish the central role of host PrP^C in both prion replication and prion pathogenesis.

Development of treatments for prion disease: current status

Prion disease is invariably fatal, and no therapy is available. Because serious damage to the brain has already occurred before clinical symptoms manifest, prion disease treatments have aimed not to cure the disease but to slow disease progression (4). There have been significant efforts to develop treatments for prion disease with limited success since the 1960s, as summarized in the recent reviews by Trevitt and Collinge (5) and Caughey et al. (6). Given the essential role of PrP in prion disease, 2 basic therapeutic strategies have been adopted: reduction of PrP^C levels and prevention of the conversion from PrP^C to PrP^{Sc}. Direct targeting of PrP^{Sc} is not viable due to the lack of understanding about the mechanism of PrP^{Sc} clearance in vivo and the risk of exacerbating the disease (5). Reducing the levels of PrP^C expression is known to attenuate prion replication and prolong survival time, and reagents that interfere with the conversion process also suppress prion replication. A large number of methods/reagents have been tested in animal models, cultured cells, or cell-free in vitro conversion systems (refs. 5, 6, and references therein). These include hundreds of compounds, e.g., polycationic, polyanionic, polyphenolic, tetrapyrrolic, tetracyclic, tricyclic, and related compounds (including some antimalarials); phenothiazines, steroids, antihistamines, polyene antibiotics, RNA aptamers, dominant-negative proteins (certain PrP mutants, PrP-dimers), copper chelators and copper, inhibitors of signal transduction, β-sheet–breaker peptides, anti-PrP antibodies; as well as immuno-modulation and reagents that cause temporary depletion of follicular dendritic cells. Some of these compounds, such as pentosan polysulphate (PPS), which has been in use for the treatment of osteoarthritis in dogs, appear to be promising (Figure 1). Extended intraventricular infusion of PPS has been shown to significantly increase survival time of prion-infected mice even when administered at the onset of clinical symptoms, but earlier treatment gave better results (7). Some other strategies also have potential (Figure 1). For example, a recent report showed that active DNA vaccination against PrP^C dramatically delayed disease onset in a transgenic mouse model, but it was followed by rapid disease progression (8).

RNAi

RNA interference (RNAi) is a highly conserved, sequence-specific posttranscriptional gene-silencing mechanism, whereby siRNA targets homologous mRNA for degradation (9). siRNAs are generated from endogenous or exogenous double-stranded RNAs (dsRNAs) by the dsRNA endonuclease Dicer; subsequently, siRNA activates the RNA-induced silencing complex (RISC) to degrade the target mRNAs (9) (Figure 1). Rapid advancement in the understanding of RNAi in general, and mammalian RNAi in particular (10), makes it feasible to use RNAi to develop therapeutics for a variety of human diseases. The huge potential of RNAi in research and therapeutics has been reflected by the awarding of the 2006 Nobel Prize in Medicine to Craig Mello of University of Massachusetts Medical School and Andrew Fire of Stanford University School of Medicine for their contributions to the discovery of RNAi.

There are major challenges in developing therapeutic approaches that utilize RNAi. Since RNAi requires expression of dsRNAs
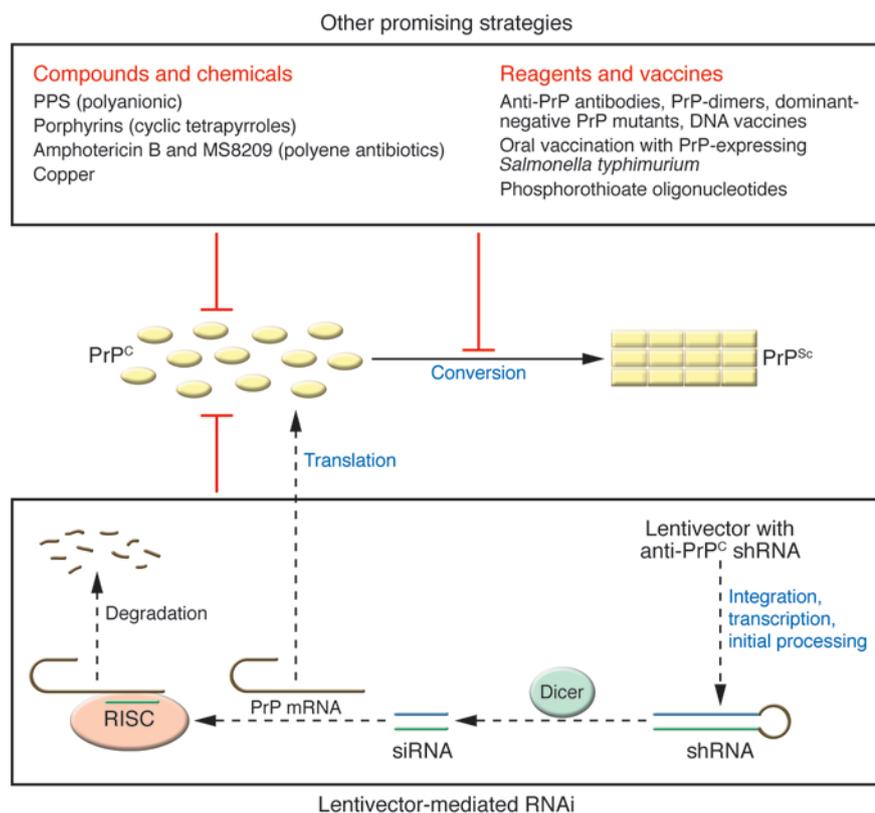
Figure 1
RNAi and other strategies for prion disease treatment. Monomeric PrP^C (yellow ovals) converts into multimeric PrP^{Sc} (yellow rectangles) in the process of prion replication and prion pathogenesis. Reagents or strategies that effectively reduce the PrP^C level or interfere with the PrP^C-to-PrP^{Sc} conversion process have shown therapeutic potential for prion disease. In this issue of the *JCI*, Pfeifer et al. (20) show that anti-PrP^C shRNA carried on a lentivector is transfected into neuronal or ES cells, integrated into chromosomal DNA, and transcribed; anti-PrP^C shRNA is released into the cytoplasm, where it is processed by endonuclease Dicer into siRNA. This in turn activates the RNA-induced silencing complex (RISC) to degrade PrP mRNAs, leading to reduced expression of PrP^C and consequently diminished PrP^{Sc} accumulation and significantly improved survival time after prion infection. Other promising reagents and strategies are listed in the top box. Only the most effective and least toxic compounds or chemicals that have demonstrated therapeutic effects in animals after intracerebral prion inoculation are included; a few other reagents and vaccines that have potential but need further research are also listed (5–6).

including short hairpin RNAs (shRNAs), these dsRNAs could potentially activate the innate immune system, inhibit nontarget genes, or saturate the RNAi machinery (11–12). The biggest factor that hinders therapeutic application of RNAi is the need for a safe and efficient delivery system for dsRNAs. The lentiviruses are a family of retroviruses that can integrate into the genomes of not only dividing cells but also nondividing cells (such as neurons) to achieve stable, long-term expression of shR-NAs. This feature makes lentivectors one of the favorite delivery systems for exogenous genes, especially in the central nervous system (13). Lentivector-mediated RNAi has been shown to be feasible for treatment of

Alzheimer disease and amyotrophic lateral sclerosis (ALS) in mouse models (14–15), and it is even being used for genome-scale loss-of-function screening (16). However, lentivectors have their own drawbacks: they require high-titer virus stocks; the consequences in the event that recipients are infected with related retroviruses are unknown; and their effectiveness may be reduced due to silencing of many integrated lentivector copies (17).

Development of RNAi-based therapeutics for prion disease
RNAi has demonstrable therapeutic potential in animal models of several neurodegenerative diseases, including Alzheimer disease

(14), ALS (15), spinocerebellar ataxia (18), and Huntington disease (19). The effectiveness of RNAi is particularly impressive in the ALS model, where it was reported to double the latency period and prolong the lifespan by approximately 80% after intramuscular injections of shRNAs (15).

In this issue of the *JCI*, Pfeifer et al. (20) report in vitro and in vivo data that raise hope for an RNAi-based therapy for prion disease. They showed that lentivector-mediated anti-PrP^C shRNA expression effectively suppressed prion replication in a murine neuroblastoma cell line persistently infected with a mouse prion strain. More significantly, they also created chimeric mice using embryonic stem cells transfected with a lentivector carrying an anti-PrP^C shRNA and showed that the survival time after prion inoculation was markedly lengthened. In the chimeric mice, only cells derived from the lentivector-transfected embryonic stem cells expressed the anti-PrP^C shRNA, and the degree of chimerism was positively correlated with the increase in incubation time in general, implying that the degree of PrP^C knock down is directly related to the improvement in survival time. These results indicate that lentivector-mediated expression of properly designed anti-PrP^C shRNAs in neuronal cells has significant therapeutic effects in vitro and in vivo. It is also encouraging that no apparent abnormality was noted in the chimeric mice, suggesting that lentivector-mediated RNAi is well tolerated in at least the mouse model system. These data indicate that RNAi has therapeutic potential for prion disease, providing a novel venue for the search of an effective prion disease treatment. It may also be possible to utilize RNAi to prevent or delay the occurrence of prion disease in subjects carrying pathogenic mutations in the human PrP gene, *PRNP*.

Much more research is needed before RNAi can be harnessed to treat prion disease. Since Pfeifer et al. (20) demonstrated the therapeutic effect of the shRNAs only in chimeric mice where PrP expression was suppressed from the embryonic stage in shRNA-expressing cells, future studies need to establish that shRNAs carried in a lentiviral vector can still prolong the lifespan of affected animals when administered into adult mice at least at presymptomatic stages of prion disease or, better yet, at early symptomatic stages of prion disease. It is noteworthy that only a moderate reduction in PrP^C expression was observed in the brain area of adult mice where the lentivector was

injected (see Figure 1, G and H, in ref. 20); even in the most highly chimeric mouse (approximately 95% chimeric), the increase in survival time to 231 days was modest when compared with that of 167 ± 1.2 days for the control mice. These observations point to the need for a vast improvement of the lentivector to achieve more efficient transfection of neurons and much higher levels of shRNA expression in those cells that are transfected. A safe and effective protocol also needs to be developed to allow brain-wide lentivector transfection. Combined therapeutic strategies, such as application of both RNAi and PPS or other effective compounds (such as porphyrins), may hold real promise for the effective therapy of prion disease in the near future.

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Search for the preadipocyte progenitor cell

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An increase in adipocyte number is a major contributor to the increase in adipose tissue mass that is characteristic of obesity. The identity and regulation of the adipocyte precursor cell (or preadipocyte) and the preadipocyte precursor cell (or progenitor cell) have been intensely studied for many years. In this issue of the *JCI*, Crossno et al. report that progenitor cells originating from outside the adipose tissue, in particular the bone marrow, can contribute to an increase in adipocyte number (see the related article beginning on page 3220). Their study in mice reveals that treatment with the thiazolidinedione rosiglitazone or exposure to a high-fat diet promotes the trafficking of circulating bone marrow–derived progenitor cells into adipose tissue, where they become multilocular adipocytes. This adds a new and unexpected dimension to this research arena.

Historical perspective

Early investigations noted that the full range of cells common to red bone marrow, including monocytes, reticular cells, lymphocytes, normoblasts, erythroblasts, and basophils, were observed in developing adi-pose tissue (1). In particular, a wide range of cell phenotypes common to red bone marrow, including endothelial cell precursors, pericytes, macrophages, immature macrophages, fibroblast-like perivascular reticular cells, and perivascular mesenchymal cells, were among those implicated as the adipocyte precursor cell (1). Adipose tissue and bone marrow–derived mesenchymal cells have a similar protein expression phenotype based on cell-surface markers (2, 3). Furthermore, adipogenic differentiation of both adipose tissue and bone marrow–derived mesenchymal cells are similarly characterized by increases in the expression of key adipocyte markers such as fatty acid–binding protein, lipoprotein lipase, PPARγ, and CCAAT/enhancer-binding protein α (C/EBPα) (4, 5). Bone marrow–derived adipocytes also secrete the adipocyte-specific factors leptin and adiponectin, indicating functional similarity between bone marrow–derived and adipose tissue–derived adipocytes (4). Interestingly, clusters of red bone marrow cell types have been observed in developing adipose tissue (6). The number of these cell types was associated temporally and spatially with the development of large clusters of multilocular (comprising many compartments) adipocytes representative of an immature stage of adipocyte development in white adipose tissue. These observations would seem to counter the long-held belief that new adipocytes arise solely from resident preadipocyte progenitors and hint that progenitor cells from tissues outside adipose depots could contribute to the formation of new adipocytes. In support of the latter concept, in this issue