Prion Diseases: A Journey Through Therapeutic Strategies

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Abstract

Prions are unconventional infectious agents that cause lethal transmissible neurodegenerative diseases in humans and animals. They were the main cause of a number of diseases including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia and Kuru. Prions can be distinguished from other pathogens by their lack of nucleic acids. The most important process for prion propagation is the conversion from normal cellular prion protein on the cell membrane to insoluble, limited protease digestion-resistant, pathogenic scrapie prion protein. For several years, many pharmacological and biological tools have been targeting different stages of disease progression. A very few numbers of them have been upgraded to clinical trials. Despite all these treatments being tested, none has been approved as a therapeutic drug for prion diseases in general. In this review, some of these treatments will be discussed to get a basic knowledge of different possible therapies.

I. Introduction

Prion diseases, or transmissible spongiform encephalopathies, are fatal neurodegenerative diseases in the central nervous system [1] which include Creutzfeldt - Jakob disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, and Kuru.

Prions are the main infectious agents of these diseases. They behave like any other infectious pathogens, except the fact they lack the most fundamental features of any organism, in particular, genetic material (DNA, RNA). But it turns out that they are caused by the misfolding of a host-encoded prion protein (PrP). Prion Protein is a 253 amino acid AA protein. After its transport to the endoplasmic reticulum in the process of synthesizing proteins, the first 22 N-terminal AA are removed from PrP, while the last 23 C-terminal AA are cleaved off after the addition of glycosylphosphatidylinositol (GPI) anchor. GPI helps the proteins attach to the outer surface of the cell membrane.

PrP could be found in two forms: a normal cellular prion protein (PrPC) and a pathogenic misfolded conformer (PrPSc), both of which are encoded from the same sequence from base pairs 4,666,796-4,682,233. The abnormal PrPSc and the normal PrPC differ in secondary and tertiary structure, but not in primary amino acids sequence. [2] [3].

According to the seeding-nucleation model*, the preexisting or acquired PrPSc oligomers catalyze the conversion of PrPC molecules into PrPSc fibrils. This breakage provides more PrPSc templates for the conversion process [4]. This process of conversion is self-propagating, with PrP SC acting as a conformational template forcing more PrP C to convert. The conversion reaction itself is crucial to neurotoxicity in prion diseases. Nonetheless, the exact identity of the neurotoxic prion species and the mechanism of neurotoxicity are still unknown. Figure #1 illustrates the process of conversion from

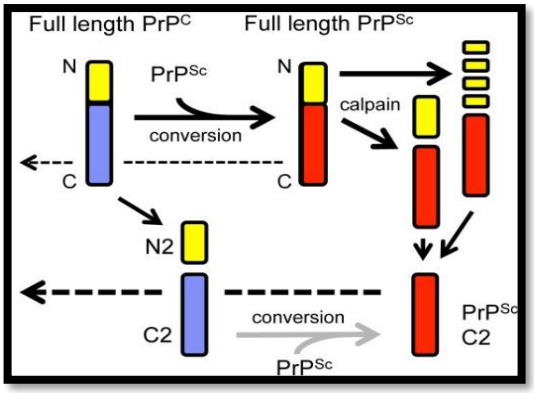

Figure #4: pathogenic signaling

the cellular protein PrPc to pathogenic misfolded conformer (PrPSc).

Prion diseases are rare in humans, but they indeed have a unique biology that had caused them to be the focus of the scientific world for several years. There is currently no effective treatment for prion diseases or clear evidence of how they are transmitted, but mostly they are transmitted through contaminated blood products [5].

II. Treatments for prion diseases: Do they exist?

A short answer is that there is no effective disease-modifying treatment for prion diseases until now. None has string preclinical evidence from experiments on animals. A good understanding of the agent's structure and mechanism is essential for discovering a decent treatment, which is improving every day.

The most frequent targets are PrPc, PrPsc, and the conversion process between PrPc to PrPsc. The main goal of targeting PrPc is to enable knocking down or completely remove substrates for prion propagation, which can be applied for all types of prion diseases.

Targeting PrPsc may be the most logical approach; however, such therapies could not influence disease progression, and they may even enhance or prolong the disease. Conversely, targeting the process of conversion from PrPc to PrPsc includes various pathways. Inhibition of PrPC trafficking to the plasma membrane, stabilization of the PrPC structure with chemical chaperones, and interference of the interaction between PrPC and PrPSc are examples of such therapies [6] [7].

Any successful therapy for prions would either prevent the formation or block the action of the neurotoxic species. Pathological changes and infectivity are associated with (PrPsc), but there is no evidence for in vivo toxicity caused by PrPsc [8] [9].

Another reason for arguing about the toxicity of PrPsc is the existence of the subclinical disease, in which high levels of PrPsc are present, but with no clinical symptoms [10]. As well as several inherited prion diseases in which PrPsc is not detected in any significant amounts, the level of PrPsc accumulation in certain brain regions has nothing to do with any clinical features [11]. But despite all of the previous, expression of PrPsc is essential for both prion propagation and pathogenesis.

III. Timing of treatment: Does it make a difference?

Before any neural loss in mouse scrapie, spongiform change and synapse loss occur [12] and before the end stage motor symptoms, some changes in the species' typical behaviors take place [13] [14] and they are associated with the early loss of presynaptic terminals in the dorsal hippocampus [15]. In other neurodegenerative disorders, such as Alzheimer's and Huntington's diseases, the loss of synapses precedes neuronal loss.

The early spongiform change had some kind of relation with cognitive and behavioral deficits and synaptic transmission in the hippocampus in prion-infected mice with PrP depletion; these mice recovered just one week after depletion has occurred [16].

Although the link between spongiosis and synaptic loss is not completely clear, it may give some information like reflecting a stage of functional impairment of synapses before they are physically lost. The recovery of early spongiform change bears with the idea of the early stage of the disease being a key for prion diseased neurons to be rescued.

IV. Using RNAi for Therapeutic Gene Knockdown

"Transgene-mediated reduction of PrPC" is an expression that opens a new door when it comes to possible therapeutic strategies regarding prion diseases in general. It has been discussed before how the prevention of PrPsc formation may be a possible cure for prion diseases; a way to do so is by disabling or "silencing" the gene responsible for the formation of PrPsc. Nevertheless, that expression does not contain any direct therapeutic possibilities in human patients. Recent developments in the field of RNA interference (RNAi) constitute a new opportunity to achieve such therapeutic gene silencing in vivo.

RNAi is a naturally occurring highly-conserved sequence-specific mechanism for post-transcriptional gene silencing in eukaryotes. It is associated with the presence of double-stranded RNA (dsRNA), which is exogenously introduced as viral RNA to the cell and endogenously encoded as microRNAs (miRNAs), which is an RNA responsible for regulating gene expression. Exogenously introduced dsRNA is recognized as a cytoplasmic ribonuclease known as Dicer. Its function is to cleave dsRNA into 21–23 nt sequences called short interfering RNAs (siRNAs) [17]. Both siRNAs and miRNAs interact with a multi-protein RNA-induced silencing complex (RISC) that unwinds the RNA duplex and destroys one of the strands [18]. This type is also known as passenger RNA.

We can call the remaining "guide" strand a template, which is used to locate cellular mRNAs containing a homologous sequence. The degree of homology between the guide strand and the mRNA determines whether RISC initiates endonucleolytic cleavage or translational arrest of the target mRNA and, consequently, silences the expression of that gene. Generally, siRNAs mediate the destruction of target mRNAs whereas miRNAs silence gene expression through translational repression due to their imperfect complementarity to the target mRNA [19] [20]. Interfering RNA sequences can be designed to enter the RNAi pathway at various points. siRNA duplexes can be synthesized for direct loading into RISC without requiring further processing [21]

Targeting of brain structures has been achieved successfully with an infusion of naked siRNA duplexes in conjunction with transfection reagents and conjugated to a peptide derived from the rabies virus glycoprotein [22] [23]. Because of the promising results being attained, current technologies mean clinical translation for the treatment of many neurodegenerative diseases. The previous process will require the continuous or repeated long-term infusion of the interfering RNA directly to the CNS.

Alternatively, stable, long-term expression of interfering RNA sequences can be achieved through the use of recombinant viral vectors (see schematic in Fig. 2).

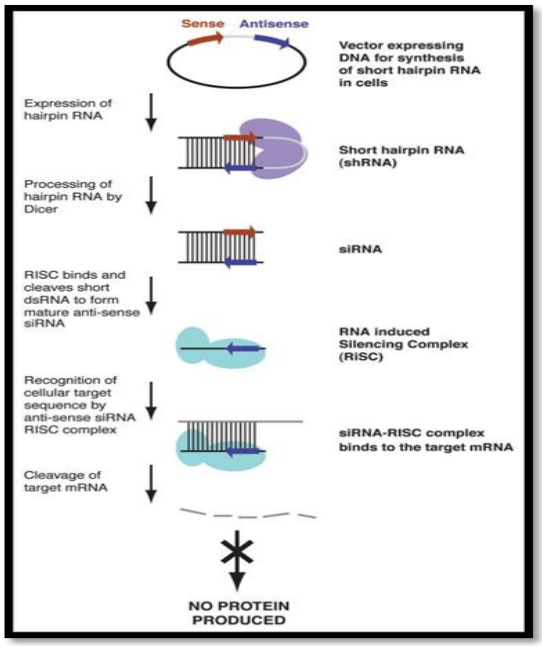

Figure #5: Schematic representing virally mediated RNAi

V. Chemotherapeutic Approach

Treatments targeting early or preclinical acquired prion diseases may find success by targeting peripheral replication and blocking neuroinvasion. However, effective therapies for symptomatic disease will most likely require a combination of approaches, such as inhibiting pathogenic PrP formation, destabilizing or

enhancing the clearance of existing pathogenic PrP, blocking neurotoxic effects of the infection, or promoting the recovery of lost functions in the central nervous system. There is a long list of chemical classes of compounds that have been screened and tested in vitro, and some even in vivo.

The majority of anti-prion compounds were examined to stop the conversion of PrPC to PrPSc. This may happen through direct binding of PrPC and/or PrPSc, causing prevention of interaction or block polymerization. Hence, a compound may redistribute PrPC to a location where conversion cannot occur. Others affect conversion by interfering with important accessory molecules or by suppressing PrPC expression altogether.

VI. Conclusion

Our vision and knowledge about Prion diseases and PrPsc especially are in constant progress each day. While increasing our understanding of their structure & formation, various methods of treatment have emerged on the scene, perhaps not all of them have proven remarkable progress in vivo, but this increased knowledge will help in the future to open other doors for various biological and pharmacological tools.

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