

THE DEADLIEST AGENT PRIONS

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ABSTRACT

Prions are bits of misfolded proteins and form the basis of protein confirmation inheritance. These are deadly infectious agent with a core molecular feature and are main cause of group of fatal transmissible neurodegenerative disorders “prion disease”. Prion diseases are generally fatal and transmitted to individual by genetic, sporadic or acquired means. Till now there is no pharmacological treatment is available for prion diseases as serious damages to the brain occur before clinical symptoms manifest. So an ideal therapeutic strategy must include: reduce the formation of neurotoxic aggregates and also block the brain destruction pathways. Recent progress in applied biological research using animal models has open many therapeutic opportunities to treat prion diseases in human. Drug therapy, lentivirally mediated RNA interference (RNAi) against native prion protein (PrP) and stem cell transplantation procedure are therapeutic intervention that results in neuronal rescue, prevents symptoms and increases survival of individual.

Keywords: *Prion, Neurodegenerative Disorders, Lentivirally Mediated RNA Interference (RNAi), Stem Cell Transplantation, Neuronal Rescue*

INTRODUCTION

Prions are bits of misfolded protein that have the ability to convert other folded proteins to misfolded conformations. Prions form the basis of protein confirmation based inheritance (Gupta, 2002). Prions is otherwise known as “scrapie agent,” as after infection and a prolonged incubation period, the scrapie agent causes a degenerative disease of the central nervous system in sheep and goats (Prusiner, 1982). In the beginning it was suspected that this unknown disease is spreading by virus. But later it was found that this is not caused by any pathogens such as bacteria, virus, viroids, retrovirus and virusoids or satellite viruses having nucleic acid components as the severity of the disease spread by this deadliest agent was also found to unaffected by UV rays or some ionizing radiation and other sterilization procedure. But there was loss of infectivity on treatments involving modification or hydrolysis of proteins. Sir Stanley Prusiner won Nobel Prize (1997) for demonstrating protein of which prions were made, he called it “prion protein” (PrP for short). This paper tries to outline a unique isoform of the prion protein (the pathogenic scrapie inducing- form) that can transmit disease from cell to cell or host to host by associating and/or transforming, normal prion protein into misfolded isoform and induce cytopathologic condition in neuronal circuit.

Structure and Function of Prion Protein

Prion Protein is a 253 amino acids (aa) protein. Prion Protein may exist in two forms: a normal cellular prion protein designated as PrPC and a pathogenic misfolded conformer designated as PrPSc (Muhammad and Saqib, 2011). Both PrPC and PrPSc conformers are encoded from the same sequence of the 16 kb single copy PRNP (prion protein) gene that is positioned on the short (p) arm of human chromosome 20 (20p13). PrPSc are proteinaceous simplest infectious agent that can fold in multiple ways structurally into different confirmation. The transmissible agent of prion disease consists of abnormal β -sheet rich state (PrP(Sc) or prion) which is capable of replicating itself according to the template-assisted mechanism (only protein hypothesis). The disease-associated isoform of the prion protein gains several

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properties including ability to transmit infection, limited protease resistance, and increased ability to fibrillize and form amyloid, these observations on both etiology and biochemical nature of the agent resulted in the prion hypothesis. This postulates that the folding pattern of a newly recruited polypeptide chain accurately reproduces similar PrP(Sc) template (Ano *et al.*, 2009). Once the animal get infected after long silent stage the disease emerged with clinical symptoms with significant transformation in neuropathological properties and biochemical features of the proteinase K-resistant PrP material (PrPres) before authentic PrP(Sc) evolved is schematically represented in Figure 1. PrPSc is a protein that “teaches” the other prion proteins how to fold up into a disease state as it can escape physiologic response of cell death by overcoming host immunological barrier⁵. Thus, they slowly accumulate. Depending upon incubation time, lesions profile etc. the prion strain spread prion disease between the species to species and /or within host to host. High amount of prions deposit result neurological and neuroendocrinal disorders. The most drastic properties of prions is it can trigger other nearby PrPC to change into prion and prions never change back into PrPC.

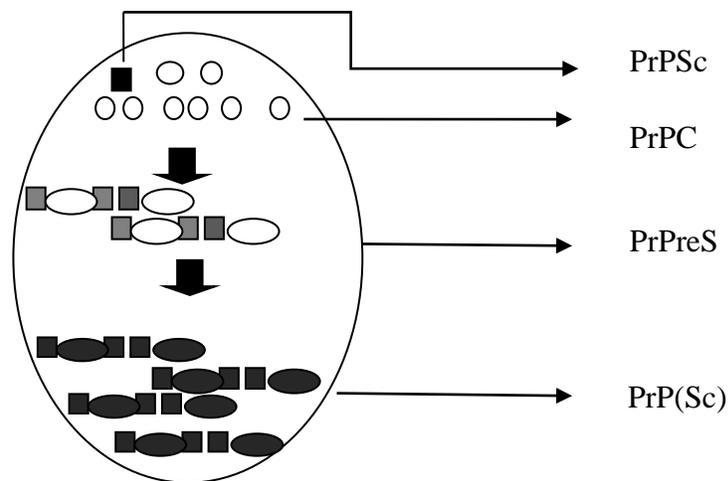


Figure 1: Misfolding of PrPC

However, the mechanism of conversion of PrPC to PrPSc is not yet completely understood. If the central of prion diseases is conversion of PrPC to PrPSc then what role played by PrPC in normal physiological condition? Possible functions of PrPC comprise roles in neurogenesis and differentiation of neural stem cells, neuritogenesis, involvement and interaction with signal transduction pathways, synaptogenesis, neuronal survival via anti- or pro-apoptotic functions, copper binding, redox homeostasis, long-term renewal of hemopoietic stem cells, activation and development of T cells, differentiation and modulation of phagocytosis of leukocytes, and altering leukocyte recruitment to sites of inflammation. A wide range of proteins may act as putative PrP interactors. It's up-regulation may occur in inflammatory conditions and may provide an increased substrate for PrPC-PrPSc conversion. Based on structural similarity, it has been proposed that PrPC might function as a member of the Bcl-2 family of proteins. Although PrPC knockout mice are healthy, the brain of these mice were found to have reduced levels of cell defense enzymes activity, such as catalase, and increased levels of oxidative stress markers. PrPC polypeptide is synthesized in the endoplasmic reticulum (ER), processed in the Golgi apparatus, and then carried in its mature form to the cell surface where most of it is found in lipid rafts of the plasma membrane. The

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pathways of conversion of PrPC to PrPSc are described detailed by Kovacs and Budka (2008). One of the hypotheses proposes generation of PrPSc from PrPC would occur after the arrival of PrPC at the cell surface. Pathogenetic events of prion diseases are yet unidentified event might occur by external prions, spontaneous conversion, or awakening of silent prions initiate conformational change of PrPC. One particle of PrPSc can cause other PrPC to convert into PrPSc. The conformational discrepancies (PrPC is predominantly rich in alpha helical contents, while PrPSc is predominantly rich in beta sheet contents render prion propagation in the brain results in the pathogenesis of prion diseases. The abnormal PrPSc isoform differs from the normal PrPC isoform in secondary and tertiary structure, but not in primary amino acids sequence. Sixteen different variants of prion disease have been reported so far: nine in humans and seven in animals (Makarava *et al.*, 2011). The Possible events may occur to convert PrPC to PrPSc that ultimately lead to prion diseases is shown in Figure 2.

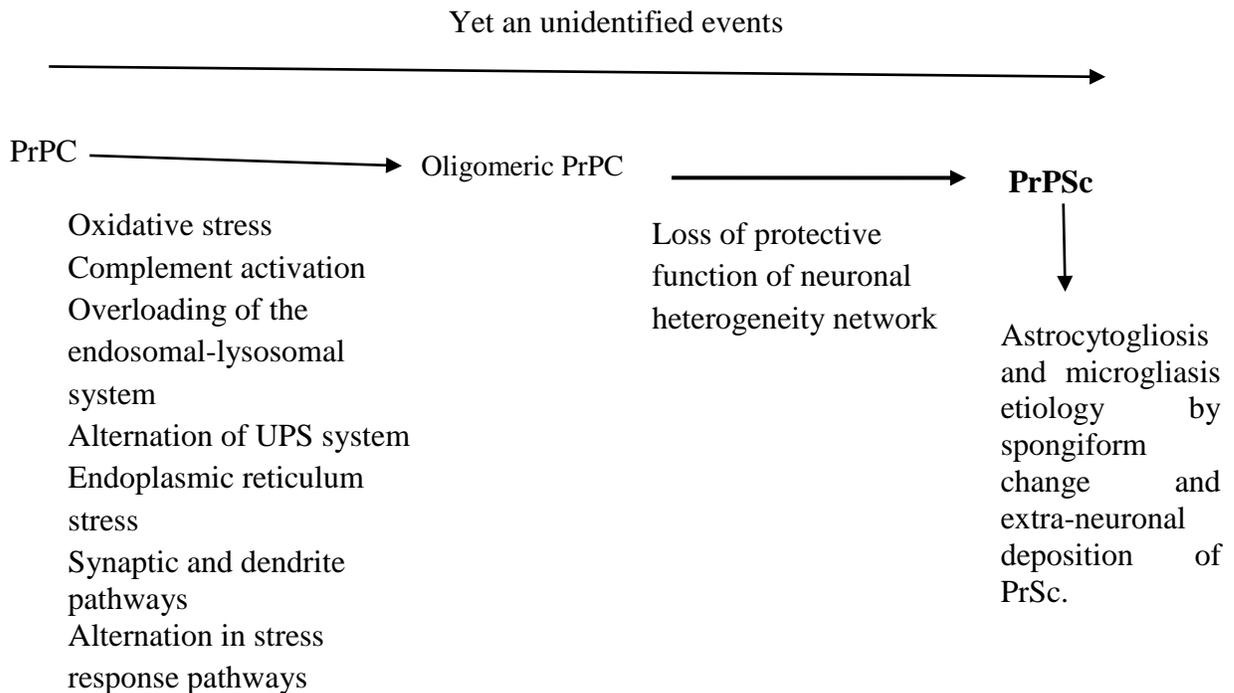


Figure 2: Events Favor Progression of Prion Diseases

Etiology and Treatment of Prion Diseases

All known transmissible prion diseases, collectively called transmissible spongiform encephalopathy (TSE). They are untreatable and often fatal. Prions cause neurodegenerative diseases by aggregating extracellularly within the central nervous system to form plaques known as amyloid, which disrupt the normal tissue architecture. This disruption is characterized by "holes" in the tissue with resultant spongy architecture due to the vacuole formation in the neurons. PrPSc may also accumulate in astrocytes and microglia. However, the diseases spreading cascade in CNS is not yet fully explored. It may assume that infectitious prion propagate in the nervous system following the sequential routes: axonal transport, passive translocation in perineural lymphatics and then spread in neural interspaces, sequential infection

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of schwann cells and a domino-like conversion of PrPC into PrPSc along neural cell membranes (Kovacs *et al.*, 2005). In the diseased human brain, PrPSc is deposited in diffuse/synaptic, patchy/ perivacuolar, perineuronal, and plaque-like patterns (Kovacs *et al.*, 2002). While the incubation period for prion diseases is relatively long (5 to 20 years), once symptoms appear the disease progresses it spread in exponential manner, leading to brain damage and death. List of prion diseases affecting mammalian species including human are given in Table 1.

Table 1: Diseases Caused by Prion in Mammals

Affected Animals	Diseases
Sheep, goat	Scrapie
Cattle	Bovine spongiform encephalopathy(BSE), mad cow disease and chronic wasting disease (CWD)
Mink	Transmissible mink encephalopathy(TME)
Deer (white-tailed deer, mule deer, moose)	Chronic wasting disease (CWD)
Human	Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), Gertsman-Straussler-Scheinker syndrome (GSS), kuru and variably protease-sensitive prionopathy (VPSPr).
Cat	Feline spongiform encephalopathy (FSE)
Antelope (nyala, oryx, greater kudu)	Exoticungulate encephalopathy (EUE)

Clinical Symptoms

Clinical symptoms include rapidly progressive dementia, cerebellar dysfunction including muscle incoordination, ataxia (balance and coordination dysfunction) and visual, speech and gait abnormalities. During the disease course, symptoms of pyramidal and extrapyramidal dysfunction with reflexes, tremors, spasticity and rigidity, and behavioral changes with agitation, confusion and depression may also be observed (Belay, 2009). At the end of the disease course, most of patients go into a state of akinetic mutism (they become unresponsive to exterior stimuli).

Transmission of Prion Diseases

Prion pathogenesis can be broken down into spatially and temporally distinct phases: (i) infection and peripheral replication; (ii) migration from the periphery to the CNS (neuroinvasion), and (iii) neurodegeneration (Aguzzi, 2006). Prion diseases may transmitted to individual by genetic (fault in the gene that codes for the prion protein), sporadic (somatic mutation in the gene which encodes the prion protein and these cells then produce a faulty prion protein which will tend to form the rogue form spontaneously) and acquired [rogue prion proteins have inadvertently been introduced into the individual as a result of accidental inoculation during medical intervention procedures like blood transfusion (termed Iatrogenic CJD (ICJD)) or by exposure to food products contaminated with BSE (variant CJD (vCJD) or human BSE)]. The routes of infection in naturally acquired prion diseases comprise uptake of prions *via* the alimentary tract or through scarification of gums (eg, in scrapie), skin, and conjunctiva (Kovacs and Budka, 2008). The prion disease is zoonotic in nature. Although presence of disease due to scarpie agent was from mid of 18th century and detection and diagnosis of diseases due to

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prion was from 1982 but complete etiology are still to achieve. The transmissions of prion diseases within and between the species remain a paradigm. One fascinating aspect of prion diseases is the very distinct transmissibility of the different disorders. It has two route of transmission. From the survey conducted by Ryou (2007) the obtained epidemiological data suggest that BSE and CJD appear to have limited or no direct transmission from one individual to another and on the other, scrapie and CWD demonstrate facile transmission between animals; resulting in endemic infections within susceptible populations. The molecular mechanisms underpinning these distinct transmissibility traits are largely unknown. However, there have been considerable recent advances in describing the routes of excretion of the prion agent within animals incubating scrapie or CWD, as well as studies describing the presence of prions within environmental samples. The prion diseases are transmitted *via*, biological materials such as skin, saliva, milk, urine, feces, septum, placenta and skin, further this transmission facilitated by environmental risk factors or vectors or reservoirs like water, food (meat and milk products) and cosmetics. As approximately 15% of prion diseases have a autosomal dominant genetic etiology The late diagnosis and rare (1 in million) occurrence of prion diseases in human make the treatment procedure more complicated.

Diagnosis of Prion Diseases

There is no absolute clinical diagnostic test as definitive diagnosis is neuropathological. So prion diseases are usually diagnosed clinically and confirmed by post-mortem histopathological examination of brain tissue. The only reliable molecular marker for prion diseases is PrP(Sc), the pathological conformer of the prion protein that accumulates in the central nervous system and, to a lesser extent, in lymphoreticular tissues. However, MRI, electroencephalogram, cerebrospinal fluid protein tests, genetic tests and tonsil biopsy are other clinical diagnosis test. The development of a noninvasive diagnostic test (e.g., on blood) would be very helpful in terms of easier, earlier clinical diagnosis, as well as having other applications (such as blood donor screening or population infection prevalence surveys). The accessible diagnostic sample for prion diseases is different species is given in Table 2.

Table: 2 Accessible Diagnostic Specimens for Prion Diseases

Collected sources	Specialty of source	Species
Cerebral spinal fluid	Best diagnostic CSF test for human sCJD,	Humans, Hamsters, Sheep
Nasal fluids	Sources of prion shedding.	Hamsters, Deer
Blood plasma	Basis for blood test for prions Easier diagnosis? Screening the blood supply?	Humans, Hamsters

Presently as described by Kübler *et al.*, (2003) the most widely used diagnostic tests are to discriminate between PrPC and PrPSc basing on the relative protease resistance of PrPSc in brain samples, in combination with immunological (anti-PrP-antibodymediated) detection of the proteinase K-resistant part of PrPSc (PrP27–30). Currently, 5 test kits such as Prionics-Check Western (Western blot, Prionics AG), Platelia test (ELISA, BioRad), Prionics-Check LIA (ELISA, Prionics AG), Enfer test (ELISA, Enfer), Prionics-Check LIA (ELISA, Prionics AG) and CDI (Inpro) use the detection of protease-resistant PrP as

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an assay principle and have been positively evaluated by the European Union in 1999 and 2003. The diagnostic tests available for different prion diseases are given in Table 3.

Table: 3 Available Diagnostic Tests of Prion Diseases.

Prion diseases		Diagnostic tests	References
Sporadic diseases (sCJD)	Creutzfeldt-Jakob	1. The EEG 2. The CSF 14-3-3 estimation 3. The MR scan	Glatzel, <i>et al.</i> (2003)
Variant disease (vCJD)	Creutzfeldt-Jakob	1. The MRI 2. Tonsil biopsy 3. CSF 14-3-3 analysis	Zeidler <i>et al.</i> (2000), Hill <i>et al.</i> (1999), Jackson <i>et al.</i> (2014)
Genetic prion diseases		Genetic analysis	Uflacker <i>et al.</i> (2014)
Laterogenic CJD		Diagnosis essentially rests on the history of a relevant known risk factor such as treatment with cadaveric derived human growth hormone or the use of human dura mater graft in surgery.	Mastrianni and Roos, (2000)

Tests in Development

Some diagnostic tools has been developed for earlier detection taking sensitivity into consideration. They are RT-QUiC and Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion and new blood test for variant CJD (McGuire *et al.*, 2012; Peden *et al.*, 2012; Panegyres and Armari, 2013). Long incubation period, varied disease patterns and least availability of diagnostic tool before first appearance of symptom of prion diseases make these infectious agents deadliest.

Therapeutics

Various therapeutic approaches have been suggested, but there is no current effective treatments are available from any candidate therapies. Importantly, the diagnosis of prion disease in humans remains difficult and often leaves a short therapeutic window after the appearance of the first clinical signs. Readers can refer the reviews on therapy for prion by Panegyres and Armari, (2013) and Gilch *et al.*, (2001) where current therapeutic interventions and modern treatment methodology for human prion diseases are extensively described. As serious damages to the brain generally occur before clinical symptoms manifest. An ideal therapeutic strategy must restrict formation of toxic aggregates and block brain destruction pathways. Among variety of therapeutic strategies have been proposed with most directed at preventing prion conversion, one approach is to reduce PrPC expression or trafficking to the plasma membrane, reducing its availability for prion conversion (Tilly *et al.*, 2003). Alternatively, chemical chaperones which stabilize PrPC structure or compounds prevent interaction of PrPC with PrPSc could be used to prevent further protein misfolding. Readers are suggested to refer review by Goold *et al.*, (2015) for degradation pathway of prion and their therapeutic interventions. Among all these therapeutic approaches administration of drugs, transplantation of stem cells and vector mediated gene transfer (RNAi) are currently recognized strategies against prion diseases in animal model (mice). So these can be used as complementary therapy to human prion diseases as mice are most popular models due to genetically equivalent construction with human (Tripathy, 2015).

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Drugs Therapy

The compounds possessing anti-prion activity are described in Table 4.

Table: 4 Medicine and Vaccines Showing Anti-Prion Properties.

Drugs and vaccines	References
Pentosan polysulfate (PPS)	Trevitt and Collinge, (2006)
Phosphorothiorate oligonucleotide	Caramelli <i>et al.</i> , (2006), Kong, (2006), Sim and Caughey, (2009)
Porphyrin and phtalocyanin	Priola <i>et al.</i> , (2010)
β -sheet-breaker peptides,	Soto <i>et al.</i> , (2000)
Amphotericin B and MS 8209 (polyene antibiotic)	Caramelli <i>et al.</i> , (2006), Kong, (2006), Sim and Caughey, (2009)
Copper chelators and copper	Caramelli <i>et al.</i> , (2006), Kong, (2006), Sim and Caughey, (2009)
Anti-PrP antibodies, PrP- dimmers, dominant-negative PrP mutants	White <i>et al.</i> , (2003)
DNA vaccines, oral vaccination with PrP-expressing Salmonella typhymurium	Caramelli <i>et al.</i> , (2006), Kong, (2006), Sim and Caughey, (2009)

The administration of above prescribed drugs effectively reduce the PrPC level or interfere with the PrPC-to-PrPSc conversion process. However, these molecules are only maximally effective when administered at or near the time of prion inoculation, before the infectious agent can reach the central nervous system. The efficacy of these compounds at early stages of the disease suggests that they might best be used for post-exposure prophylactic or preclinical treatments. In a study conducted by Doh-ura *et al.*, (2004) to evaluate efficacy of chemicals after direct infusion of pentosan polysulfate (PPS), amphotericin B and antimalarial drugs such as quinacrine in an infected transgenic mice transmissible spongiform encephalopathies intracerebrally infected with 263K scrapie agent. It was seen that PPS has most dramatic prolongation of the incubation period than other two chemicals. However, its effectiveness *in vivo* has been restricted to administration either before or soon after peripheral infection. Thus, treatment with PPS has been thought of as being preventive only for those individuals with accidental inoculation in the periphery. This study also inferred intraventricular PPS is in fact quite effective in prolonging the life spans of infected animals, even after abnormal PrP has already accumulated in the brain. However, administration a high dose of PPS (1–110 mg/kg/day of PPS over 18 days) in humans carry the risk of urinary infections, haemorrhage, seizure, and death. These findings indicate that intraventricular PPS infusion might be useful for the treatment of transmissible spongiform encephalopathies in humans, providing that the therapeutic dosage is carefully evaluated (Farquhar *et al.*, 1999). In addition, quinacrine and chlorpromazine have been described as efficient inhibitors of PrPSc formation in neuroblastoma cells (Doh-Ura *et al.*, 2000; Korth *et al.*, 2000). As these medications had already been approved for the treatment of malaria and various physiological disorders and are known to pass blood brain barrier, they were administered as a “compassionate treatment” to patients suffering from sporadic CJD or vCJD in human. However, several workers have been reported no antiprion activity

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was observed in patients with prion diseases administered with quinacrine (Barret *et al.*, 2003, Haïk *et al.*, 2003 and 2004) and the technique developed by Wood, (2011) has made detection and evaluation procedure of prion diseases easier so that remedial therapy could employed early.

Stem Cells Transplantation

Stem cell graft technology has already been employed to analyze the pathophysiology of prion disorders. In a study conducted by Aguzzi *et al.*, (1998) grafts of fetal neural stem cells derived from PrP knock-out mouse embryos when transplanted into the hippocampus of scrapie infected mice. It was seen that there was rescue from hippocampal neuronal loss and damage in animals suffering from prion diseases. However, it was not clear whether the neuronal increase was due to the replacement of damaged cells or an activation of the production of trophic factors in response to the transplantation thereby protecting the endogenous neurons. Although this approach has demonstrated that prion diseases can be treated with stem cells like other physiological disorders such as trauma, cardiac damage, cancer and diabetes. The transplanted cells can be derived from a variety of different sources such as embryonic stem (ES) cells, fetal neural stem cells, and bone marrow (BMC) or mesenchymal stem cells (MSCs).

Effective Gene Therapy for Prion Diseases

The main aim of this therapeutic approach is to inhibit formation of diseases prion protein either by using viral vector system and /or targeting PrPC/ PrPSc by Inhibiting PrnP gene expression using RNAs interference. Viral vectors constitute ideal tools to deliver genetic material into cells. Till date, five viral vector systems are available: retrovirus, adenovirus, adeno-associated virus (AAV), herpes simplex virus and lentivirus. Among all these viral mediated gene transfer approaches lentivirus and AAV therefore appear the most pertinent and have already been employed in the context of gene therapeutic approaches for brain disorders. The lentiviruses are a family of retroviruses that can integrate into the genomes of not both dividing cells and non dividing cells (such as neurons) to achieve stable, long-term expression of shRNAs. This feature makes lentivectors one of the favorite delivery systems for exogenous genes, especially in the central nervous system (Muhammad and Saqib, 2011, Pfeifer *et al.*, 2006). Lentivector-mediated RNAi has been shown to be feasible for treatment. In these si RNA is integrated into lentiviral system and helpful in silencing host PrPC expression as depicted in Figure 3.

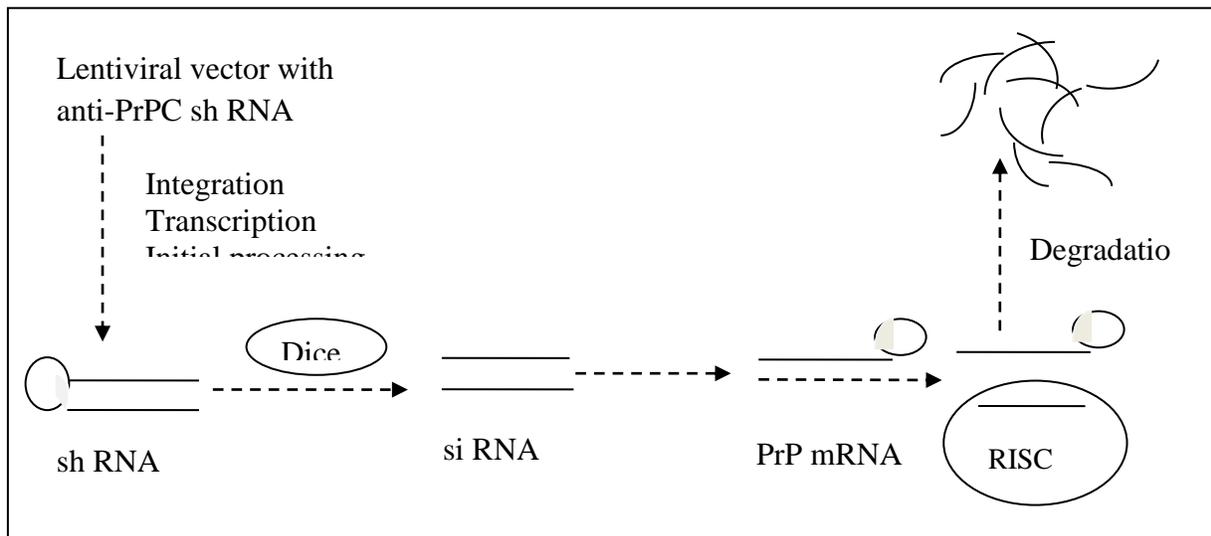


Figure 3: Lentiviral Vector Mediated RNAi

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Conclusion

The prion predicament is enigmatic as ever as the precise physicochemical nature of the agent is unknown where the process of prion replication is essentially served as black box. The precise mechanisms underlying genesis of the transmissible protein states is also required to understand. The origin of various strains of prions makes the treatment procedure more complicated. So further research is required to develop proper diagnostic tools that will help to innovate novel therapeutic strategies to treat prion diseases.

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