[Frontiers in Bioscience 15, 1150-1163, June 1, 2010]

Interactions between PrP^c and other ligands with the 37-kDa/67-kDa laminin receptor

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1. ABSTRACT

The 37-kDa/67 kDa laminin receptor (LRP/LR) represents a multifunctional protein. It is a receptor for viruses such as Dengue Viruses, Alphaviruses and Adeno-associated-Viruses (AAV), as well as the cellular prion protein (PrP^c) and infectious prions (PrP^{Sc}). The 37-kDa/67-kDa LRP/LR plays furthermore fundamental roles in basic cell biological processes such as cell adhesion and cell growth and acts as a key player in metastatic cancer, affecting invasion, adhesion and apoptotic processes. This review gives fundamental insights into basic cellular processes affected by LRP/LR including signal transduction and cell cycle progression and focuses on pathophysiological implications of the interaction of prion proteins, laminin, viruses and other ligands with LRP/LR affecting the development of highly-prevalent diseases such as cancer, neurodegenerative diseases such as prion disorders and Alzheimer's Disease as well as viral infections. Molecular tools such as LRP/LR specific antibodies and siRNAs targeting LRP expression as possible alternative therapeutics for the treatment of neurodegenerative diseases, metastatic cancer and viral infections are emphasized.

2. THE 37-kDa/67-kDa LRP/LR REVEALS A MULTIFUNCTIONAL PROTEIN

The 67-kDa laminin receptor (LR) is a non-integrin cell surface receptor with high affinity binding for its corresponding ligand, laminin, an extracellular matrix glycoprotein involved in cell growth, movement, attachment and differentiation (for review: (1-4)). Laminin interacts with the 67-kDa LR via two binding domains; the first domain is known as the peptide G sequence which is located between amino acids 161-180 and the second laminin binding domain is located between amino acids 205-229 at the C-terminal of the 37-kDa LRP (5). Besides having high affinity for laminin, 67-kDa LR also acts as a receptor for other extracellular matrix molecules such as elastin and carbohydrates (1). Furthermore, 67-kDa LR is reported to mediate laminin-induced tumor cell growth, migration, invasiveness and metastasis (6, 7) . This is due to the high laminin receptor levels found in a number of cancer types which correlates with an increase in the invasiveness and metastasis of tumor cells (8, 9). The relationship between the 67 kDa high affinity receptor (LR) and the 37-kDa laminin receptor precursor (LRP), is poorly understood. Several reports suggest that 67-kDa LR

might be formed through homo/heterodimerization reactions involving 37 kDa LRP and fatty acid acylation (10, 11). The fact that recombinant LRP is monomeric and fails to interact with itself in yeast two hybrid system (12), however, strongly suggests that 67 kDa LR is not the dimer of 37 kDa LRP. It is reported that both the 37-kDa LRP and the 67-kDa LR forms of the protein are found on the cell surface and also function as receptors for cellular prion proteins (PrP) (13) and infectious prions (14). The interaction between the 37-kDa/67-kDa LRP/LR and the prion proteins (PrP) was demonstrated by using several cell culture models as well as the yeast two-hybrid system. Furthermore, data from yeast two-hybrid system demonstrated the presence of two binding domains for 37-kDa/67-kDa LRP/LR on PrP that are of importance in the PrP-37-kDa/67-kDa LRP/LR interaction. These include a direct (PrPLRPbd1, aa144-179) and an indirect (PrPLRPbd2, aa53-93) binding domains. The interaction between PrP and 37-kDa/67-kDa LRP/LR through the indirect binding domain is reported to be dependent on heparan sulfate proteoglycans (HSPGs) that function as co-factors or co-receptors for the binding of PrP^c to the 37-kDa/67-kDa LRP/LR (12). HSPGs are multifunctional macromolecules comprising of a core polypeptide covalently bound to glycosaminoglycans (GAGs) which act as initial attachment receptors for several extracellular molecules (15, 16) Additionally, HSPGs are known to be associated with Amyloid-beta (A-beta) deposits in Alzheimer's disease (AD) (17). Experimental investigations using the yeast two-hybrid system demonstrated that the direct PrP-binding domain on 37-kDa/67-kDa LRP/LR is located between amino acid residues 161 and 180 (12) (Figure 1). On the other hand, a HSPG-dependent binding site has so far not been identified on LRP/LR but it is presumed to be located between amino acid 180 and 285 (17). The 37-kDa/67-kDa LRP/LR has also been shown to function as a receptor for a number of viruses such as Dengue virus (18), Sindbis virus (19), Venezuelan equine encephalitis (20), Adeno-associated viruses serotypes 2, 3, 8 and 9 (21). Recently, 37-kDa/67-kDa LRP/LR has been demonstrated to play a role as a receptor and mediator of epigallocatechin-3-gallate (EGCG)-induced anti-cancer and anti-allergic activity in human colon cancer Caco2 cells (22). In another study, the down-regulation of 37-kDa LRP expression using siRNAs in Hep3B cells was shown to lead to the induction of apoptosis suggesting a role of 37-kDa LRP in tumor cell viability (23).

In addition to its role as a cell surface receptor, the 37-kDa LRP is also reported to be present in the cytoplasm where it exists as p40 ribosome-associated protein and is involved in the maturation of the 40S subunit of the ribosomes (24, 25). Moreover, the 37-kDa LRP is localized in the perinuclear compartment and nucleus where it interacts with PrP and histones, respectively. (24, 26, 27). The presence of the 37-kDa LRP in various parts of the cell suggests that it is a multifunctional protein that plays an important role in a variety of cellular processes including proliferation, viability and protein synthesis and could be a potential target for the treatment of various human diseases.

3. THE 37-kDa\67-kDa LRP\LR AND ITS INFLUENCE ON PRION AND ZOONOTIC DISEASES

The transmission of prions amongst individuals of different species (interspecies) is characterized by prolonged incubation and survival periods, a phenomenon termed the "species barrier" (28). Factors influencing this phenomenon are numerous and may include: variations in the prion protein (29) and laminin receptor sequences in the donor and recipient species; the strain of PrP^{Sc} employed for experimentation and the route of infection. Although intraspecies transmission efficiencies vary (high amongst captive deer suffering from Chronic Wasting Disease (CWD) and low in cattle and sheep infected with Bovine Spongiform Encephalopathy (BSE) and Scrapie respectively, (Figure 2) (30, 31), interspecies transmissibility is still lower and may fail completely. BSE is the only TSE that has been transmitted from animals to humans and thereby caused the zoonotic disease variant Creutzfeldt-Jakob disease (vCJD) (32). The consumption of infected meat is the most common mode of infection and thus further investigations to study the mode of oral prion uptake were undertaken (33). The 37-kDa/67-kDa LRP/LR is expressed on the surface of enterocytes (the major cell population of the intestinal epithelium) as well as in the apical brush border of the small intestine and Paneth cell secretory granules (34). Thus, it was hypothesized that the 37-kDa/67-kDa LRP/LR may influence the susceptibility to oral prion infection and the transmissibility of Transmissible Spongiform Encephalopathies (TSEs) across the species barrier. Recent research suggests that the transmissibility of TSEs is dependent on the strain of the infectious PrP as PrP^{BSE} (Cattle strain of the infectious prion) agents were internalized via 37-kDa/67-kDa LRP/LR receptors on human enterocytes (Caco-2/TC7 cells) whereas PrP^{Sc} (Sheep strain of the infectious prion) failed to do so (34). The mode of transmission is a further factor that must be taken into consideration. Sheep, Goats (35), minks and mice (36) are susceptible to oral infection with PrP^{BSE} in contrast to pigs which are only successfully infected upon parenteral inoculation (37). To investigate whether other prion diseases in livestock and game, such as sheep scrapie and CWD in deer, may cause zoonotic diseases further binding studies investigating the interactions between bovine, ovine, cervid and porcine prions and human enterocytes, and possibly the 37-kDa/67-kDa LRP\LR receptor, are being undertaken.

4. THE ROLE OF PRION PROTEINS AND THE 37-kDa\67-kDa LRP\LR IN SIGNAL TRANSDUCTION AND CELL CYCLE

Data from studies performed by (38) demonstrated that the activation, via phosphorylation, of Fyn (a tyrosine kinase) is PrP^c dependent. This activation is supposedly related to neuronal cell maturation, morphology and functions associated with neurotransmitters (38). Fyn is an intracellular protein and PrP^C is bound to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor thereby suggesting that intermediate proteins function in the signaling cascade. Caveolin has been identified as one

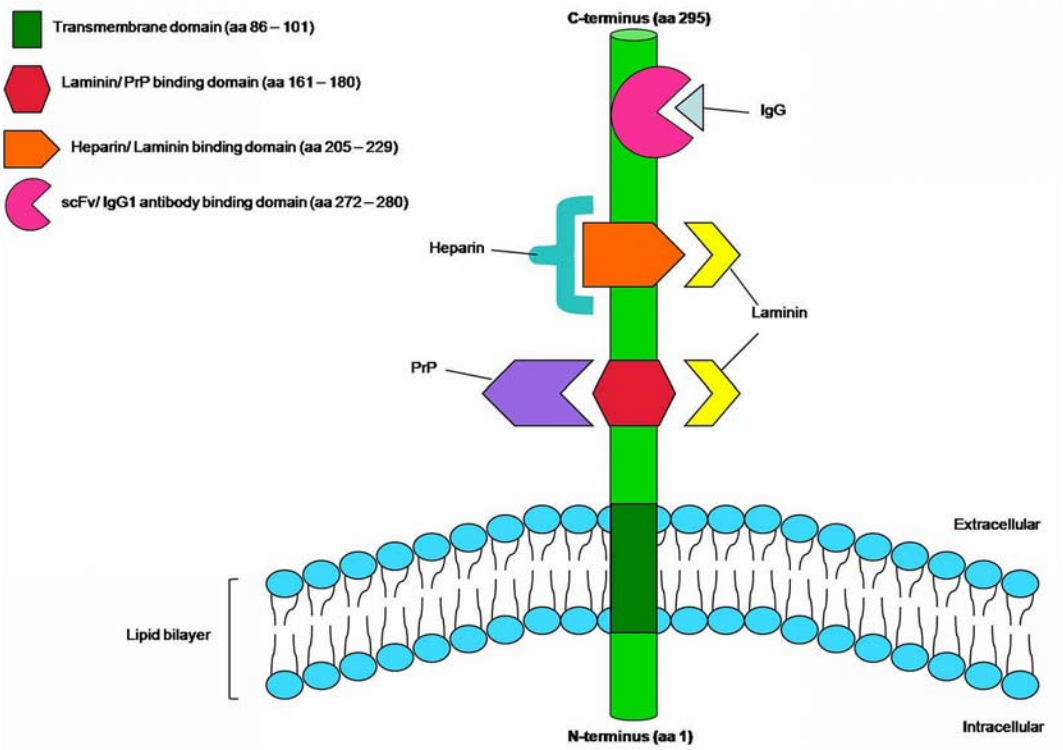

Figure 1. Schematic representation of the 37-kDa laminin receptor precursor (LRP). The 37-kDa LRP is 295 amino acids in length located on the cell surface encompassing four functional domains. A transmembrane domain located between amino acids 86-101 which docks the receptor to the cell membrane. Two laminin binding domains localized between amino 161-180 and 205-229 which also serves as attachment sites for PrP and heparin, respectively. The third binding domain is localized towards the C-terminus of the receptor between amino acids 272-280 and serves as an attachment site for IgG antibodies. Figure modified from (4).

such intermediary protein (38). The C-terminus of the GPI anchor and the dimer formation by the PrP^{c}'s hydrophobic domain confer stress-protection to the cell. The infectious prion protein (PrP^{Sc}) is suggested to induce apoptosis by promoting the phosphorylation of the mitogen-activated protein kinase (MAPK) termed Jun-N-terminal kinase (JNK) (39). However, this PrP^{Sc} activation of JNK is dependent on the presence of the GPI-anchored PrP^{c} (39).

The 37-kDa/67-kDa LRP/LR is suggested to have an influence on cell signaling. The is attributed to its involvement in the progression of the cell cycle, namely from Gap phase (G1) to synthesis phase (S phase) and the suppression of the receptor results in transient cell cycle arrest (24). It has been illustrated that the receptor may suppress the expression of cyclin dependent kinase inhibitors such as p21, tumor suppressors and Survivin (an anti-apoptotic protein) (24). Thus, the receptor may function in anti-apoptotic signaling. Furthermore, evidence showed that 37-kDa/67-kDa LRP\LR induces MMP-2 and enhances the expression of the enzyme MKP-1 which promotes the dephosphorylation of MAKs (40). The 37-kDa/67-kDa LRP\LR inhibits the granulocyte macrophage colony stimulating factor (GM-CSF) receptor (GMR) by interacting with the beta-subunit of the receptor complex (41). GM-CSF is a cytokine and functions in inducing the phosphorylation of MAPK (Erk-1 and Erk-2) and Stat5 (signal transducer and activator of transcription) and promotes tumor necrosis factor-alpha (TNF-alpha) synthesis by neutrophils (41). Therefore, since the 37-kDa/67-kDa LRP\LR inhibits GM-CSF signaling, the aforementioned processes will be hindered.

5. EMPLOYING ANTIBODIES DIRECTED AGAINST 37-kDa/67-kDa LRP/LR AS THERAPEUTIC TOOLS FOR THE TREATMENT OF PRION DISEASES

Antibodies are becoming increasingly favourable as potential therapeutic tools in the treatment of multiple human diseases. This is evident as numerous antibodies, in particular monoclonal antibodies, are being used in clinical trials and have obtained U.S. food and Drug Administration (FDA) approval for therapeutic applications (for example Herceptin™, Avastin ™, Erbitux ™ and Vectibix ™ targeting receptor tyrosine kinases for cancer therapy).

Prion diseases, fatal neurodegenerative diseases, are caused by the modification of the cellular prion protein (PrP^{c}) that results in increased beta-sheet content thereby forming the infectious prion protein (PrP^{Sc}). The 37-kDa/67-kDa laminin receptor has been identified as the receptor for both the aforementioned proteins (13, 14). Thus, antibodies directed against the receptor have been developed as

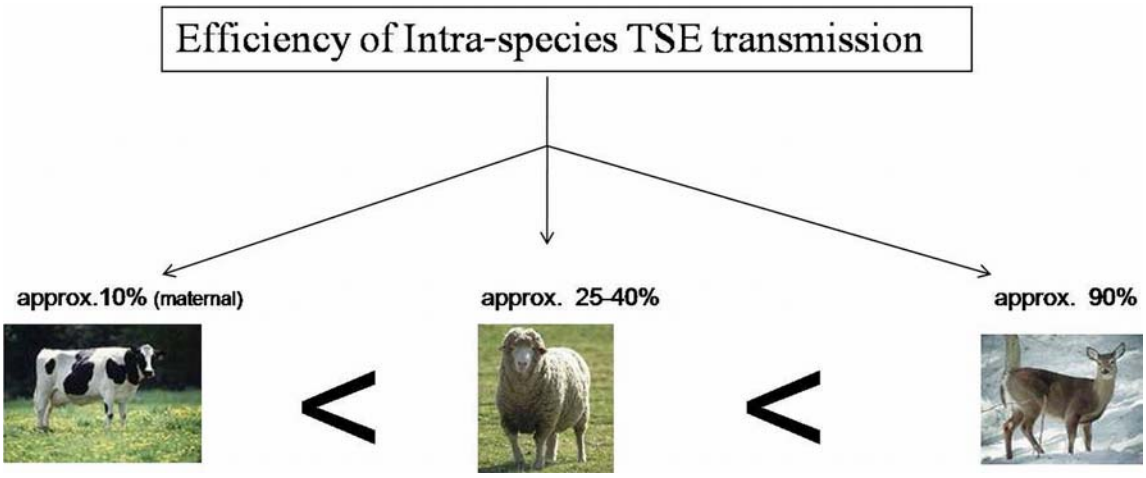

Figure 2. The efficiency of intraspecies transmision of transmissible spongiform encephalopathies (TSEs). Studies in cattle demonstrated that the maternal transmission of BSE to offspring, although at the rate of 10 %, is of statistical significance. The calculated rate of scrapie transmission 25-40% (32) amongst sheep, although greater than that amongst cattle, is still 50-65% lower than those reported in captive deer suffering from CWD (101).

potential therapeutic and prophylactic tools against prion infections and diseases (42-44). W3, a limited polyclonal antibody, represents such an antibody (Figure 3). This antibody has reduced PrP^{Sc} propagation in cell culture (45) and hampered the binding of PrP^{BSE} and PrP27-30 to enterocytes (34). Furthermore, upon passive immune-transfer of the antibodies into PrP^{Sc}- infected mice, a 66% reduction in peripheral propagation of the infectious protein and a 1.8 fold enhancement in the survival of the mice were observed (46).

Single chain anti-LRP antibodies (scFv), referred to as S18, have also been employed in this regard. The size of the antibody, a mere 30-kDa, renders it more effective in tissue penetration and enhances the probability of the antibody passing the Blood Brain barrier (BBB). Upon passive immunotransfer of these antibodies into PrP^{Sc}-infected mice, a 40% reduction of peripheral PrP^{Sc} propagation in the spleen was observed (47). However, the antibodies failed to extend incubation times or survival and this may be attributed to their instability and short half-life (approximately 12 hours). For permanent expression of the single chain antibodies, recombinant Adeno-associated-viruses encoding scFvs directed against LRP/LR were constructed and delivered to the brain of Scrapie infected mice by microinjection (48). Surprisingly, a significant reduction of peripheral PrP^{Sc} propagation was observed in the spleen, which was due to the presence of recombinant AAV in the spleen. This was the first proof for trafficking of recombinant AAVs from the brain to the spleen (48) Unfortunately, also here no significant prolongation of incubation times and survival was recorded, which might be due to the short half-life times of scFvs in blood. As a result thereof, an improved full-length antibody termed IgG1-iS18 was engineered (5). Due to the nature of full-length antibodies with regard to enhanced stability and an approximately 21-day half-life, we proposed that the antibody might be more successful in the survival prolongation of scrapie infected mice.

Epitope mapping has demonstrated that the LRP/LR antibody-binding site (scFvS18) is located at the C-terminus of the receptor (aa 272-280) (47), and not at the direct PrP/LRP-LR binding site located between aa161-180 of LRP (12). This suggests that the antibody prevents the PrP-37-kDa/67-kDa LRP/LR interaction through sterical hindrance as opposed to direct competition.

6. PENTOSAN POLYSULFATE IN THE TREATMENT OF PRION DISEASES

Pentosan polysulfates (PPS), sulfated polyanions (49) which imitate the physiological roles of heparans (50), are amongst the most widely tested and effective drugs used in the treatment of prion diseases. These compounds have been demonstrated to hinder PrP^{Sc} propagation in cell culture (51); significantly prolong incubation times and survival of infected mice (49, 52-55) as well as reduce infectious prion protein deposition and the associated neurodegenerative cerebral alterations (53). It is suggested that PPS compete with endogenous heparan sulphate proteoglycans (HSPGs) (co-receptors for the prion proteins) (12). They thereby inhibit the binding of PrP^{Sc} to 37-kDa/67-kDa LRP/LR (14) (Figure 3) by binding to the receptor's heparan binding site and thus potentially alter the physiological activities of 37-kDa/67-kDa LRP/LR (50). The combined treatment of PPS and Fe (III) meso-tetra (4-sulfonatophenyl)porphine has been shown to further enhance the survival of infected mice (56). However, the efficacy of an oral or intra-peritoneal administration of PPS in the treatment of TSEs is questionable as the compound is unstable and unable to penetrate the blood-brain barrier (54). PPS has been administered via chronic intraventricular infusion in patients diagnosed with vCJD (50, 57) with no clinical improvement. However, recent findings indicate that PPS therapy over a period of 6 months may significantly prolong the mean survival of humans but further in vivo animal experiments are required to assess the efficacy of this treatment.

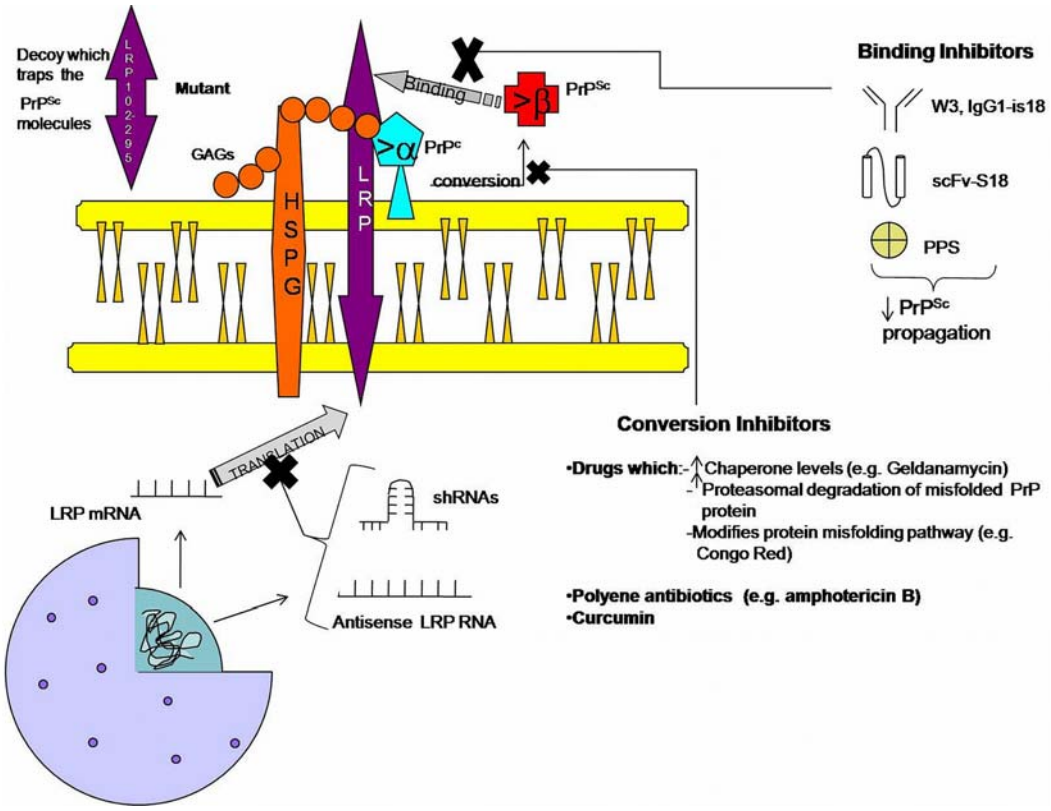

Figure 3. Therapeutic strategies for prion disorders. LRP/LR has been identfied as the receptor for both the cellular (PrP^c) and the infectious prion protein (PrP^{Sc}). Several therapeutic strategies have been developed to inhibit (i) the binding of the PrP^{Sc} to LRP/LR, (ii) conversion of PrP^c to PrP^{Sc} and (iii) the expression LRP/LR mRNA. Antisense RNA and short hairpin RNA have been employed to down-regulate LRP/LR expression and thereby inhibit PrP^{Sc} propagation in scrapie infected cells (45). Lentiviral vector delivery of siRNAs downregulating LRP expression, resulted in a prolonged pre-clinical phase in Scrapie infected mice (61). Blocking the 37-kDa/67-kDa LRP/LR receptor serves as an alternative strategy. Anti-LRP/LR antibodies (full length such as W3 and IgG-is18 and single chain scFv s18) as well as the drug pentosan polysulfate (PPS) have been used in this regard (14, 45-47). Experiments with neuroblastoma cells infected with scrapie illustrate that the LPR/LR mutant (LRP102-295::FLAG) sequesters PrP^{Sc} thus reducing PrP^{Sc} accumulation (62, 63). Curcumin (65) and Polyene antibiotics (66) are compounds that hamper the conversion of PrP^c to PrP^{Sc}. Furthermore, compounds that enhance chaperone activities or the proteosomal degradation of the misfolded protein as well as interfere with the misfolding pathway (67) may be employed as potential tools in prion therapy.

7. THERAPEUTIC INTERVENTIONS UTILIZING RNA INTERFERENCE FOR PRION DISEASE TREATMENT

The presence of cellular prion proteins, PrP^c, is imperative for the propagation of infectious, prion disease causing, PrP^{Sc}. It is a known fact that transgenic mice in which the Prnp gene has been knocked out ($Prp^{0/0}$ mice) are resistant to prion diseases (28) and those in which the protein's expression is reduced exhibit slowed disease progression (58). RNA interference (RNAi) strategies, such as those employing lentiviral PrP specific short hairpin RNAs (shRNAs) have been successful in suppressing the prion protein expression and thus presents an approach for prion disease treatment (58, 59). Similarly, suppression of 37-kDa/67-kDa LRP\LR expression in scrapie infected neuronal cells (ScN2a and ScGTI) (45) inhibits PrP^{Sc} accumulation and has been achieved upon the transfection of the aforementioned cell lines with antisense LRP mRNA and small interfering RNAs (siRNAs) directed against the LRP mRNA (45). In addition, transgenic mice (tgN(NSEasLRP) with reduced 37-kDa/67-kDa LRP\LR levels in the hippocampal and cerebellar brain regions did not exhibit abnormal behaviour (60).

Lentiviral siRNA delivery allows down-regulation of disease associated genes in dividing and embryonic stem cells (58). Lentivector-mediated RNAi was employed to generate chimeric mice expressing lower levels of PrP. In highly chimeric Scrapie infected mice, survival was significantly prolonged (58). Microinjection of lentiviral vectors expressing small interfering RNAs directed against LRP mRNA significantly prolonged the pre-clinical phase in Scrapie infected mice (61). Both reports confirm the cellular prion protein and its 37-kDa/67-kDa laminin receptor as important players in prion pathogenesis and recommend the lentiviral mediated gene transfer system for RNAi expression.

8. LRP102-295 MUTANT AS A DECOY RECEPTOR FOR THE TREATMENT OF PRION DISORDERS

Investigations employing Baby Hamster Kidney (BHK) cells which express and secrete an LRP-mutant which contains merely the extracellular domain of 37-kDa/67-kDa LRP/LR (LRP102-295::FLAG) demonstrate that these cells exhibit reduced PrP27-30 binding (62). The mutant receptor lacks the transmembrane binding domain (aa86-101) and is thereby secreted into the extracellular matrix. However, the mutant still contains the direct PrP binding domain (aa161-180) (12) and is therefore still able to bind to infectious prion proteins (62). The transdominant negative decoy mutant traps PrP^{Sc} molecules and reduces PrP^{Sc} propagation in Scrapie infected neuronal cells (62). Due to the promising results in cell culture, hemizygous transgenic mice were generated expressing the LRP102-295::FLAG mutant in the brain. After Scrapie infection the transgenic mice revealed a significant prolongation of the incubation time compared to wild-type mice (63). Interestingly, at the terminal stage of disease the transgenic mice revealed significantly reduced PrPres levels compared to wild-type mice (63). The results recommend the laminin receptor decoy mutant as an alternative therapeutic vehicle for treatment of prion disorders.

9. OTHER STRATEGIES FOR PRION DISEASE THERAPY

Numerous substances have been investigated as potential tools in prion therapy, and yet despite promising results in vivo commercial therapeutics is non-existent (for review (1-4). Many such substances share a common strategy: hamper the conversion of PrP^{c} to PrP^{Sc} (64). These include: Curcumin, a non-toxic component of turmeric which has the ability to penetrate the BBB (65), polyene antibiotics such as Amphotericin B which indirectly hinder the conversion process by altering the properties of membrane micro-domains (66), Porphyrins, polyamines and anthracyclines (64). Drugs such as geldanamycin, which augments chaperone activity and may thereby reduce levels of the misfolded PrP^{Sc} and Congo Red, binds to β-sheets and interferes with the misfolding pathway, have also been investigated in this regard (67). However, the efficacy of these compounds is low or questionable.

Researchers have also tried to stimulate innate immunity in their attempts to develop effective therapeutics for prion diseases. (68) employed cytidyl guanosyl oligodeoxynucleotides (CpG-ODNs) to stimulate innate immunity. This treatment was effective in prolonging incubation times but the side effects were severe and may have resulted in the destruction of Follicular dendritic cells (FDCs) (64).

10. THE ROLE OF THE PRION PROTEIN AND ITS RECEPTOR IN OTHER NEURODENERATIVE DISEASES

Alzheimer's disease (AD) is the most prevalent type of dementia to affect the aging population. In 2006 this neurodegenerative disease affected more than 25 million people worldwide and it is thought that this number will quadruple within the next 40 years (69). The incidence of AD increases with age, hence, the majority of patients are over 65 years old when disease onset begins. Initial symptoms include memory loss (especially short term) and within a number of years, disease progression leads to dementia that involves both behavioural and cognitive spheres of the brain. This leads to extrapyramidal symptoms, slowed and involuntary movements, uncontrollable speech and loss of bodily functions ultimately leading to death (70).

The molecular mechanisms underlying this neurodegenerative disease involve the formation of neurofibrillary tangles and amyloid plaques (also referred to as senile plaques) on neuronal tissue in the brain, which leads to the degeneration of this tissue. Two main factors have been associated with AD, namely the amyloid beta (A-beta) peptide and tau proteins. Tau is a phospho-protein that functions in the stabilization of microtubules. However, hyperphosphorylation of tau proteins leads to neurofibrillary tangles within neurons and these tangles are implicated in the onset of AD (Figure 4) (71). The amyloid plaques associated with this neurodegeneration are composed of the A-beta peptide that is released during the cleavage of the amyloid precursor protein (APP). There are two different cleavage pathways for membrane bound APP, namely; the non-amyloidogenic and amyloidogenic pathways. In the non-amyloidogenic pathway, the N-terminal of APP is cleaved by alpha-secretase to release sAPP-alpha into the extracellular matrix. The remaining membrane bound C-terminal is subsequently cleaved by gamma-secretase and the cleaved fragment, the soluble p3 peptide, is shed into the extracellular matrix. In the amyloidogenic pathway, the beta-secretase acts instead of the alpha-secretase to cleave the N-terminal of the APP at a different site. The fragment released is thus sAPP-beta. Once the remaining c-terminal is cleaved by the gamma-secretase, a 4-kDa insoluble peptide, A-beta, is released into the extracellular matrix instead of p3 (Figure 5) (72). These A-beta peptides then aggregate to form the amyloid plaques on neuronal tissue that are characteristic of Alzheimer's disease.

Prion proteins (PrP) are proteins that are found in the brain. Though their function is not completely understood, misfolded forms of cellular prion proteins (PrP^{c}) lead to TSEs such as scrapie in sheep, BSE (mad cow disease) in cattle and Creutzfeldt-Jacob disease among others in humans (14, 34). A connection has also been established between prion proteins and AD. Several factors suggest that prions have a protective role against AD. Firstly, it has been found that mutations within the gene for PrP^{c}, such as the $Y125^{stop}$ and $Q160^{stop}$ mutations, result in early onset AD, suggesting the PrP^{c} has a role in preventing or delaying the onset of this disease (73, 74). Subsequent in vitro studies further confirmed this, as, when PrP^{c} was over expressed in neuronal cell lines, the amount of A-beta released by neuronal cells was significantly reduced. Conversely when PrP^{c} was down-regulated with the use of siRNA, the levels of A-beta increased significantly (75). Co-immunoprecipitation studies revealed that this is due to

Figure 4. Paired helical fragments (PHF)-tau containing dystrophic neurites (black) and between 8 and 10 neurofibrillary tangles (NFTs; black). The Alzheimer brain is characterized by two types of lesions, amyloid-beta-containing plaques and neurofibrillary tangles (NFTs). The Gallyas silver impregnation method visualizes the flame-shaped NFTs. In addition it picks up the dystrophic neurites which form a halo around the amyloid plaques. Hence, this method is an indirect means to visualize an amyloid plaque such as the one shown in the center of the micrograph. The micrograph is the courtesy of Prof Juergen Goetz, Sydney, Australia.

an interaction between the beta-secretase and PrP^c, whereby the beta-secretase is in some way inhibited by the presence of PrP^c. The exact mechanism of this inhibition remains elusive as the catalytic ability of the beta-secretase is not reduced and the amount of this enzyme expressed on the surface of the cell remains the same. It is thus probable that PrP^c somehow interferes with the initial steps in the proteolytic processing of APP. This inhibition was found to be most successful when PrP^c is localized in cholesterol-rich lipid rafts and interacting with glycosaminoglycans (75).

The receptor for both infectious and non-infectious prion proteins is 37-kDa/67-kDa LRP/LR. This receptor interacts with PrP^c directly and indirectly via heparan sulfate proteoglycans (12). Since PrP^c plays an important role in the regulation of A-beta secretion, it is possible that the receptor for PrP^c (37-kDa/67-kDa LRP/LR) may also be involved in regulating the pathways involved in the development of Alzheimer's disease.

11. 37-kDa/67-kDa LRP/LR AND CANCER

Cancer is one of the leading causes of mortality worldwide and was found to be responsible for approximately 13% of all deaths worldwide in 2007 (http://www.who.int/cancer/en). Predictions have shown an increased mortality rate resulting from this disease with an estimated 12 million deaths by 2030 (http://www.who.int/cancer/en). The most striking feature of this disease is that it affects people of all ages, races, genders and economic status. Several types of cancer have been identified with the most common in the Western World being lung, breast, prostate and colon cancers (http://info.cancerresearchuk.org). However, this differs to developing countries where the prominent cancer types include cervix and stomach cancer (Figure 6). This variance is due to progress in diagnostic techniques, which is often unaffordable to those in poorer regions. Early diagnosis is possible with the help of magnetic resonance imaging (MRI) and leads to an overall decrease in mortality. Another aspect that plays an influential role in reducing the number of fatalities includes the discovery and consequently the spread of awareness regarding risk factors for cancer. Currently several modes of treatment are available such as surgery, chemotherapy, radiation therapy, immunotherapy and monoclonal antibody therapy. In cases when the tumor becomes metastatic, treatment becomes challenging since various tissues are affected.

Cancer is a disease whereby mutations result in uncontrolled cell growth by either unregulated cell proliferation or the lack of apoptosis. These cells then continue to grow and mutate with the aid of oncogenes or tumor suppressor genes that act by altering cell cycle checkpoint regulators and the DNA repair system (76). It has been shown that changes in the general apoptotic process could have a significant effect on the progression of tumors, suggesting the inactivation of apoptosis at the time of tumor progression (76). The acquired capability of evading apoptosis can be attained through various strategies, with the most common being that of mutations caused by the p53 tumor-suppressing gene (76). Loss of pro-apoptotic regulators occurs as a result of this mutation. It is suspected that most cancer types are able to induce changes that allow for the evasion of apoptosis (Figure 7). A report has hinted that with the use of small interfering RNAs 37-kDa/67-kDa LRP/LR can be inhibited and consequently result in the induction of apoptosis in various cells (23). It was shown that silencing of 37-kDa/67-kDa LRP/LR induces apoptosis in Hep3B cells (23). Thus LRP/LR supports cancer by inhibiting apoptosis.

The degree of metastasis of neoplastic cells is highly dependent on the interaction with 37-kDa/67-kDa LRP/LR, this in turn affects the general laminin mediated basement attachment and consequently local degradation and cell movement. Expression of the mature 67-kDa form of the receptor is found on most normal cells, the immature 37-kDa form had been noted as being an oncofetal antigen. A direct link between the over-expression of this form and an increased invasiveness has been identified (23).

This trend was found particularly in gastric cancer (77), colon carcinoma (78), colorectal carcinoma (79), cervical (80), breast (81), lung (82), ovary (83), pancreatic (84) and prostate cancer (85). This discovery has lead to the suggestion that the expression level of 67-kDa laminin receptors could be a reliable method of prognosis for metastatic tumors (84, 85). Furthermore the over-expression of this receptor could provide an opportunity for cancer therapy directed at this site.

Human fibrosarcoma cells that were pre-treated with an anti-LRP antibody showed significantly reduced

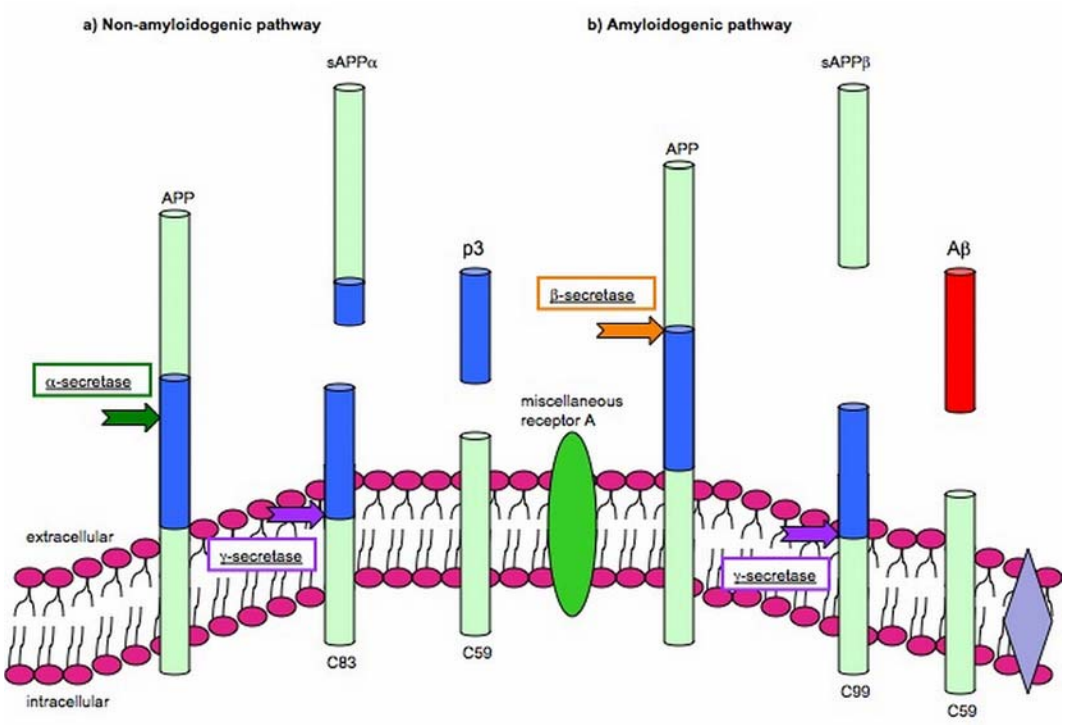

Figure 5. Overview of APP cleavage pathways. **(**a) The non-amyloidogenic pathway is shown. This pathway makes use of the alpha-secretase enzyme belonging to the ADAM (a disintegrin and metalloprotease) family. Alpha-secretase proteolytically cleaves the APP (amyloid precursor protein) to release sAPP-alpha, which is shed into the extracellular matrix. The remaining fragment of the APP (the C-terminal) remains membrane bound and is subsequently cleaved by the gamma-secretase. The gamma-secretase is composed of four sub-units: the catalytic domain is composed of the presenilins (aspartyl proteases) and the remaining subunits are composed of anterior-pharynx defective 1 (APH1), nicastrin and presenilin enhancer (PEN-2). The cleavage of C–terminal of APP by gamma-secretase results in the shedding of p3, a soluble 3-kDa peptide. (b) The amyloidogenic pathway is shown, where beta-secretase cleaves APP to release sAPP-beta. Beta-secretase (beta-site APP cleaving enzyme, BACE1) cleaves at a different site to alpha-secretase, so that when the gamma-secretase cleaves the remaining C-terminal, the fragment that is shed is the insoluble A-beta peptide. The A-beta fragment is 4-kDa in size and accumulates to form amyloid plaques on neuronal tissue which causes the neurodegeneration associated with Alzheimer's disease.

lung metastases compared to untreated cells in a murine model (86). In vivo studies demonstrated that suppression of 37-kDa LRP expression lead to a reduction in the proliferation and tumor formation of lung cancer cells (6).

Most forms of prion disease therapy directed at 37-kDa/67-kDa LRP/LR have the potential to be utilized in tumor intervening therapy. Studies done using human fibrosarcoma cells (HT1080) have illustrated that the blocking or down-regulation of the 37-kDa/67-kDa LRP/LR receptor minimized the invasive potential of these cells (5). Interference of the laminin-37-kDa LRP interaction results in a hindered invasion. Ways in which this can be achieved include anti-37-kDa/67-kDa LRP/LR scFv antibodies, full length format IgG, as well as heparan mimetics and pentosan polysulfate (5). The success of this brings forward the possibility of developing alternative methods capable of reducing the metastatic nature of cancer. Interestingly, current research has shown a definite link between 37-kDa/67-kDa LRP/LR and the maintenance of cell viability (23). An increased level of 37-kDa/67-kDa LRP/LR found on the neoplastic cell line HT1080 (5) in conjunction with the fact that 37-kDa/67-kDa LRP/LR inhibits apoptotic processes (23) indicates that the receptor plays a critical role in the progression of cancer and the development of metastatic tumors.

12. 37-kDa/67-kDa LRP/LR AND VIRAL DISEASES

Viral entry into host's cells is dependent on its interaction cell surface receptors. Indeed, various distinct protein receptors have been identified as initial virus attachment sites for several viruses. For example, the CD4 receptor together with its chemokine co-receptors, CCR5 and CXCR4, have been shown to act as attachment sites for the human immunodeficiency virus (HIV) (87). This AIDS causing retrovirus plays a major role in the worldwide and in South Africa and the virus host cell interaction represents a promising target for therapeutic interventions (88) and might supplement conservative targets such as reverse transcriptase (89-91) (for review: (92)), integrase (93), protease (94) and the budding event (95) (for review: (96, 97)). The complement receptor CR2 acts as a receptor for the Epstein-Barr virus that is associated with infectious mononucleosis and the development of cancer (98). Wang et al. (19) demonstrated that Hamster cells with up-

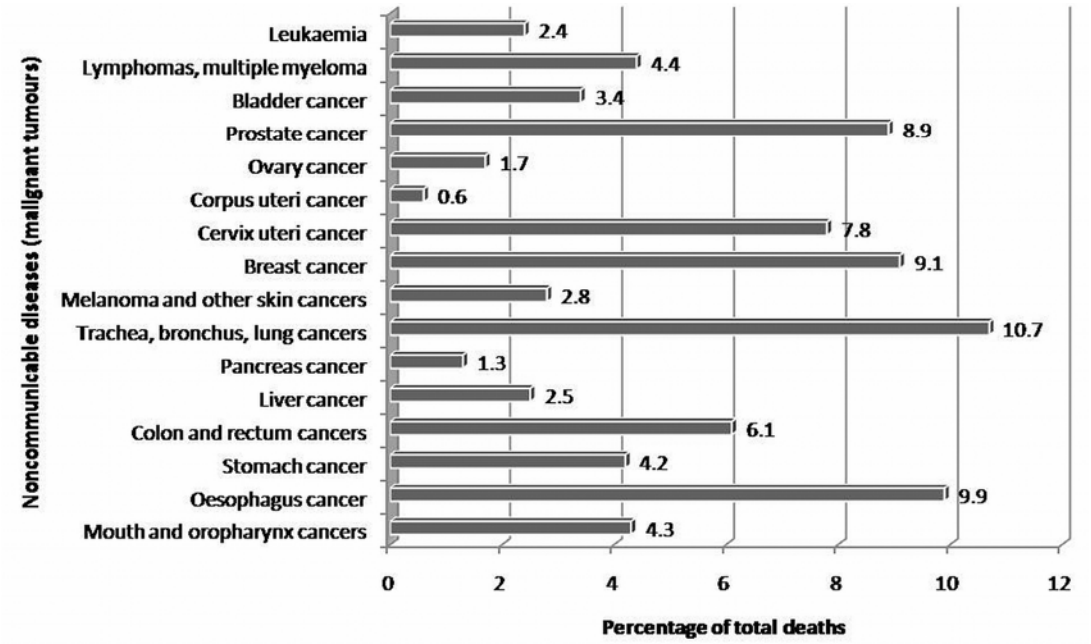

Figure 6. Estimated cancer deaths per 100,000 people by cause, and member state, 2004, Republic of South Africa (RSA) (http://www.who.int/cancer/en/).

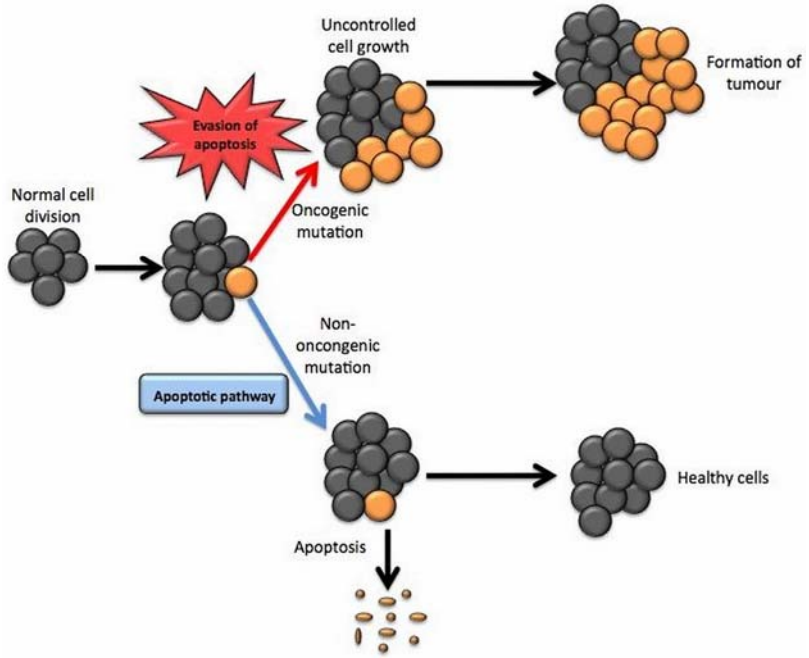

Figure 7. Comparison of normal (lower panel) and tumor (upper panel) cell division/proliferation. Healthy cells are displayed in grey and mutated cells in yellow. With the activation of oncogenes apoptosis is evaded and the tumor progresses. Non-oncogenic mutations induce apoptosis and allow for the continued proliferation of healthy cells.

regulated levels of the laminin receptor were more prone to infection by the Sindbis virus as compared to cells that were expressing lower amounts of the receptor. This led to the identification of the laminin receptor as a receptor for the Sindbis virus. The 37-kDa/67-kDa LRP/LR was also identified as a receptor for (i) dengue virus, which is the causative agent of dengue fever and dengue hemorrhagic fever, in human liver cells (18), porcine kidney cells (99) and mosquito cells (100), respectively, and (ii) for several AAV subtypes (21). The ability of 37-kDa/67-kDa LRP/LR to act as a receptor for various viruses and other pathogens suggests that LRP might also be involved in the attachment and internalization of other viruses as a receptor or co-receptor. Thus, it is imperative to investigate the potential

interaction of the 37-kDa/67-kDa LRP/LR with other viruses that cause fatal human diseases.

13. PERSPECTIVE

The 37-kDa/67-kDa LRP/LR is a key player in major cellular processes and is involved in a many diseases such as metastatic cancer, neurodegenerative diseases and viral infections. Recent findings describing a major role of the receptor in signal transduction processes, apoptosis and cell cycle progression together with the proven finding that 37-kDa/67-kDa LRP/LR plays critical roles in invasion and cell adhesion, two key components in metastatic tumors recommends the receptor as an alternative target for therapeutic intervention in many important metastatic cancer types worldwide. The 37-kDa/67-kDa LRP/LR functions moreover as a receptor for prions and recent research indicates that blocking or down-regulating the receptor results in prolonged incubation times (pre-clinical phases) and/or survival. Since 37-kDa/67-kDa LRP/LR interacts with the misfolded prion protein, which has a proposed protective effect in Alzheimer's disease, a possible role of the receptor in other neurodegenerative protein misfolding diseases such as Morbus Alzheimer might be conceivable. Since the receptor acts as a cell surface receptor for a series of different viruses (e.g. Sindbis, Dengue and Adeno-associated virus), targeting the receptor might represent an alternative therapeutic intervention for the treatment of viral diseases. Especially antibodies targeting 37-kDa/67-kDa LRP/LR might become effective alternative tools for the treatment of all kind of diseases associated with or caused by the non-integrin laminin receptor 37-kDa/67-kDa LRP/LR.

14. ACKNOWLEDGEMENTS

Vusi Mbazima, and Bianca Da Costa Dias contributed equally to this work. We thank the Deutsche Forschungsgemeinschaft (DFG) grant WE 2664/2-1, Germany and the National Research Foundation (NRF), Republic of South Africa (RSA) for financial support. We thank Prof Juergen Goetz, Director, Alzheimer's and Parkinson's Disease Laboratory, Brain and Mind Research Institute, University of Sidney, Australia, for providing a micrograph of neurofibrillary tangles.

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Abbreviations: aa: amino acid; A-beta: amyloid beta protein; AD: Alzheimer's diseases; APP: amyloid precursor protein; AAV: Adeno-associated virus, BACE: beta-site APP cleaving enzyme; BBB: blood brain barrier; BSE: Bovine Spongiform Encephalopathy; CJD: Creutzfeldt-Jakob Disease; CWD: Chronic Wasting Disease; GAG: Glycosaminoglycan; LRP: laminin receptor precursor; LR: laminin receptor; MRI: magnetic resonance imaging; LRP/LR: Laminin receptor precursor/laminin receptor; PHF: paired helical fragments; PrP: prion protein; PrP^{c}: cellular prion protein; PrP^{Sc}: scrapie form of prion protein; PrP27-30: protease resistant prion protein; GPI: glycosylphosphatidylinositol; TSE: Transmissible Spongiform Encephalopathy; PPS: pentosan polysulfate; WHO: World Health Organisation.

Key Words: AAV, Adeno-associated virus, alphaviridae, adhesion, Alzheimer's disease, Apoptosis, Cancer, Cell Cycle, Dengue virus, ECM, HIV, human immunodeficiency virus, laminin receptor, lentiviral vectors, LRP/LR, metastasis, prion, PrP, signal transduction, Sindbis virus, Venezuelean Equine Encephalitis virus, Zoonosis, Cell Adhesion, Amyloid, Review

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