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1
2 **Therapeutic approaches targeting the prion receptor LRP/LR**

3
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14
15
16 **Abstract**

17 Transmissible spongiform encephalopathies known as prion diseases are a group of
18 fatal neurodegenerative disorders that affect both humans and animals. The generally
19 accepted principle of the disease is that the conversion of the cellular prion protein (PrP^c) into
20 the disease associated isoform PrP^{Sc} leads to spongiform degeneration of the brain and
21 amyloid plaque formation. Until now no therapy leading to potential alleviation or even cure
22 of the disease exists. It is important to develop therapeutic approaches for the treatment of
23 TSEs since these infections are inevitably fatal and, especially in the case of vCJD, they
24 affect youngsters. Besides current conventional therapeutic strategies, this review summarizes
25 new therapeutic tools targeting the prion receptor LRP/LR.

26

27 *Key words:* Bovine spongiform encephalopathy; 37kDa/67kDa laminin receptor; prion;
28 transmissible spongiform encephalopathy; therapy
29

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29 1. Introduction

30 Prion diseases or TSEs are neurological disorders associated with the aggregation of a
31 pathologic isoform of a host-encoded prion protein (PrP). Conversion of the cellular prion
32 protein (PrP^c) into the disease-associated form PrP^{Sc} leads to conformational changes
33 resulting in aggregation and accumulation. Deposition of this abnormal protein takes place
34 mainly in the brain and the lymphoreticular system, accompanied with neuronal vacuolation
35 (spongiosis) and neuronal death. After extremely long incubation times, affected individuals
36 show progressive neurological symptoms terminating in death. Conventional therapeutic
37 approaches use anti-prion compounds which can prolong incubation times but do not lead to a
38 cure. It has been demonstrated that prion propagation *in vitro* needs the laminin receptor
39 (Leucht et al., 2003). Approaches downregulating LRP/LR are a promising alternative
40 strategy for the treatment of prion diseases.

41

42 2. Prion diseases in humans and animals

43 Prion diseases involve rapid neurological decline, accompanied by neuronal loss and
44 spongiform changes caused by accumulation of the aggregated and misfolded prion protein.
45 The most common type of human prion diseases, termed Creutzfeld-Jakob disease (CJD), can
46 be classified into four categories: sporadic (sCJD), inherited/familial (fCJD), iatrogenic
47 (iCJD) and variant (vCJD). Whereas it has been suggested that the latter results from
48 ingestion/consumption of BSE-contaminated food (Bruce et al., 1997), familial disorders
49 (fCJD) are the inheritance of autosomal-dominant mutations within the *Prn-p* locus.
50 Transplantation of tissues or injection of hormones originating from individuals suffering
51 from CJD or the use of contaminated surgical instruments result in the iatrogenic form of
52 CJD. Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), its
53 sporadic form (sFI) and kuru are other human prion diseases.

54 Animal TSEs have been observed in different species: Bovine spongiform
55 encephalopathy (BSE) in cattle, scrapie in sheep and goat, chronic wasting disease (CWD) in
56 cervids such as deer, elk or captive mule and feline spongiform encephalopathy (FSE) in cats
57 or mink spongiform encephalopathy (TME). In addition, some exotic diseases were observed
58 including exotic ungulate encephalopathy (EUE) and primate spongiform encephalopathy
59 (PSE). Transmission of BSE to pigs has been experimentally proven (Wells et al., 2003).
60 Recently, new forms of TSEs with unusual characteristics e.g. an atypical scrapie case
61 (Nor98) (Benestad et al., 2003) have been discovered. Apart from the existing species barrier,
62 the different modes of transmission are not yet understood.

63

64 **3. Prions and different forms of prion proteins**

65 The term prion was defined by Stanley Prusiner as a “small proteinaceous particle that
66 resists inactivation by procedures which modify nucleic acids”, suggesting that a new agent
67 exists beside the commonly known pathogenic organisms such as bacteria, virus or fungi
68 (Prusiner, 1982). The infectious “agent”, the prion, and the exact infectious mechanism for
69 prion disorders is just as little understood as the mechanism by which they kill neurons.

70 PrP^c is a ubiquitous membrane-bound glycoprotein attached to the cell surface by a
71 glycosylphosphatidylinositol (GPI) anchor, expressed in many tissues and cell types. Its
72 conversion leads to the disease-associated form PrP^{Sc}, which exhibits a higher β -sheet
73 content correlating with a high tendency to form aggregates. PrP^{Sc} is characterized as
74 insoluble and partially resistant to proteases (Cohen and Prusiner, 1998). Digestion of PrP^c
75 with proteases results in the truncated form PrPres (a 27-30kDa fragment) demonstrating
76 insolubility in aqueous and organic solvents as well as in non-ionic detergents. Additionally,
77 it is completely resistant to proteases. PrP^{Sc} and PrP27-30 both have the tendency to form
78 amyloid fibrils.

79

80 4. The 37kDa/67kDa LRP/LR as the receptor for PrP^c

81 In a yeast two-hybrid screen we identified the 37kDa laminin receptor precursor
82 (LRP) as an interaction partner for the prion protein (Rieger et al., 1997). Further *in vitro*
83 studies on neuronal and non-neuronal cells proved that both the 37kDa LRP and the 67kDa
84 high affinity laminin receptor function as the receptor for the cellular prion protein
85 (Gauczynski et al., 2001). Direct and indirect heparan sulphate proteoglycane (HSPG)-
86 dependent binding domains on LRP/LR and on PrP^c have been identified suggesting that
87 HSPGs act as co-factors or co-receptors for PrP^c (Hundt et al., 2001). It has been suggested
88 that the 37kDa LRP is the precursor of the 67kDa form which was first isolated 1983 from
89 melanoma cells due to its high binding capacity to laminin (Rao et al., 1983). The relationship
90 between the 37kDa precursor form and the mature 67kDa isoform is still unknown.
91 Regarding the function of LRP/LR, the 37kDa LRP appears to be a multifunctional protein
92 involved in the translational machinery (Auth and Brawerman, 1992) and has also been
93 identified as a ribosome-associated protein termed p40 (Makrides et al., 1988). LRP has also
94 been localized in the nucleus, where it is closely associated with nuclear structures (Sato et
95 al., 1996) and binds to DNA through connections with histones H2A, H2B and H4 (Kinoshita
96 et al., 1998). The 37kDa/67kDa LRP/LR has been described as a receptor for laminin, elastin
97 and carbohydrates (Ardini et al., 1998), as well as a receptor for Venezuelan equine
98 encephalitis virus (VEE) (Ludwig et al., 1996), Sindbis virus (Wang et al., 1992) and Dengue
99 virus (Tio et al., 2005). In addition, studies have been carried out in order to detect the
100 isoforms that are present in the central nervous system and that bind PrP. Several maturation
101 states of the receptor were identified, including a 44kDa, 60kDa, 67kDa and a 220kDa form.
102 All of these isoforms were able to bind PrP, suggesting a physiological role for the laminin
103 receptor/PrP interaction in the brain (Simoneau et al., 2003). Although LRP consists of a

104 transmembrane domain (amino acid residue 86-101, (Castronovo et al., 1991)) it is abundant
105 in the cytoplasm (Romanov et al., 1994). In mammalian cells both the 37kDa LRP and the
106 67kDa LR are present in plasma membrane fractions (Gauczynski et al., 2001).

107

108 **5. The role of LRP/LR in PrP^{Sc} propagation**

109 LRP/LR not only acts as a receptor for the cellular prion protein but also for the
110 infectious PrP^{Sc} and PrP27-30, an N-terminal truncated version of PrP^{Sc} (Gauczynski et al.,
111 2006). The importance of LRP/LR in PrP^{Sc} propagation was verified using a polyclonal anti-
112 LRP/LR antibody termed W3 which was able to block and prevent the binding of PrP^{Sc} and to
113 clear scrapie-infected neuroblastoma cells (ScN2a) from PrP^{Sc} (Leucht et al., 2003).

114 LRP/LR-dependent binding of PrP^c and PrP^{Sc} to the cell surface (either alone or
115 together with other co-factors) is accompanied by internalisation which is thought to occur in
116 clathrin-coated pits. After this receptor-mediated endocytosis the conversion of PrP^c
117 molecules into the disease-associated form probably takes place in endosomes, lysosomes or
118 endolysosomes. Heparan sulphates also play an essential role in prion uptake and cell
119 infection (Horonchik et al., 2005) suggesting that the LRP/LR and heparan sulphates act
120 presumably in synergy for PrP^{Sc} binding and internalisation.

121 The fact that LRP/LR is present in higher amounts in several organs and tissues of
122 scrapie-infected mice and hamsters suggests a correlation between LRP/LR levels and PrP^{Sc}
123 propagation (Rieger et al., 1997). Furthermore, expression studies revealed distribution of the
124 laminin receptor in the intestinal epithelial/brush border confirming that the prion protein
125 uptake and therefore the infection is mediated and supported by this receptor (Shmakov et al.,
126 2000). After oral exposure, TSE agents accumulate in lymphoid tissue, spleen, lymph nodes,
127 tonsils, appendices and Peyer's patches. For this reason prion particles have to cross the
128 intestinal epithelial barrier. Besides the proposition that M-cells are responsible for the uptake

129 of prions (Heppner et al., 2001) has also been suggested that enterocytes are involved in this
130 process, due to the fact that bovine prions are rapidly endocytosed in the presence of LRP/LR
131 (Morel et al., 2005). By preincubating the human enterocytes with the polyclonal anti-
132 LRP/LR antibody, endocytosis of PrP^{BSE} was reduced.

133 Distribution studies in adult rats revealed that the 67kDa LR form is greater in brain
134 regions classically associated with prion-related neurodegeneration, whereas the 37kDa form
135 was detected in a subclass of interneurons known to be particularly sensitive to abnormal
136 prion accumulation and cell death during the early stages of CJD (Baloui et al., 2004).

137

138 **6. Conventional therapeutic strategies for the treatment of TSEs**

139 In recent years, various studies gave evidence that substantial neuropathological
140 changes (e.g. nerve cell degeneration) already occur prior to the onset of symptoms and might
141 be related to PrP^{Sc} accumulation. Accordingly, any effective intervention must aim to start
142 directly after inoculation. Unfortunately, no diagnostic tests are available to detect the disease
143 prior to the onset of symptoms, except for individuals carrying pathogenic mutations within
144 the *Prn-p* gene. Inhibition of PrP^{Sc} formation is the most studied target and can be achieved
145 through (i) inhibition of PrP^c synthesis or prevention of its transport to the cell surface, (ii)
146 stabilization of the PrP^c structure to make its conformational change unfavourable, (iii)
147 destruction of PrP^{Sc} aggregates, (iv) reversion of PrP^{Sc} to a protease-sensitive form, (v)
148 inhibition of the prion protein receptor(s).

149 A series of compounds efficiently interfere with PrP^{Sc} accumulation, such as Congo
150 red (Ingrosso et al., 1995) and analogs (Demaimay et al., 1997), certain cyclic tetrapyrroles
151 (Priola et al., 2000) and sulphated polyanions. Although many other compounds have been
152 identified, only flupirtine, an analgetic, had beneficial effects on cognitive function for human
153 CJD patients (Otto et al., 2004). The anthracycline 4'-iodo-4'-deoxy-doxorubicin (IDX) was

154 able to delay clinical signs of the disease and prolong the survival time in scrapie-infected
155 hamsters (Tagliavini et al., 1997). It was also shown that quinacrine reduces the protease
156 resistance of PrP peptide aggregates and is able to inhibit the *in vitro* conversion of the
157 normal prion protein (PrP^c) to the abnormal form (PrP^{Sc}) (Barret et al., 2003).
158 Chlorpromazine was reported to increase incubation time in mice after intracerebral, but not
159 intraperitoneal, injection (Roikhel et al., 1984), but was less effective in cell culture than
160 quinacrine (Korth et al., 2001). Unfortunately, most substances that inhibit PrP^{Sc} formation
161 show only significant effects when administered long before the clinical onset of the disease.
162 Since no effective therapy for clinically affected TSE patients is available these diseases are
163 inevitably fatal.

164 PrP-specific antibodies, a promising alternative tool in TSE treatment, counteract
165 prion propagation both *in vitro* and *in vivo* (Buchholz et al., 2006). In a murine model,
166 treatment with this monoclonal antibody delayed the development of prion disease (White et
167 al., 2003). Application of monoclonal antibodies raised against recombinant PrP also resulted
168 in a reduction of PrP^{Sc} levels in infected mouse neuroblastoma cells (Pankiewicz et al., 2006).
169 Single chain antibodies (scFv) directed against PrP^c revealed an anti-prion effect in
170 neuroblastoma cells (Donofrio et al., 2005).

171

172 **7. Therapeutic approaches targeting LRP/LR**

173 *7.1. Trans-dominant negative LRP mutants*

174 Recently, it has been shown that an LRP mutant encompassing the extracellular
175 domain of LRP/LR (LRP102-295:: FLAG) might act in a trans-dominant negative way as a
176 decoy by trapping PrP molecules (Fig.1, I) (Vana and Weiss, 2006). *In-vitro* studies revealed
177 that the LRP mutant is able to reduce the PrP^{Sc} formation in scrapie-infected neuronal cells
178 (Vana and Weiss, 2006) and might therefore represent a promising novel tool in TSE therapy.

179

180 *7.2. RNA interference and antisense RNA*

181 A further strategy used to influence the PrP^{Sc} propagation level is the knockdown of
182 LRP/LR by siRNA and antisense RNA technology. This has already successfully been shown
183 for PrP using *Prn-p*-specific sequences. Thus, the transfection of siRNAs corresponding to
184 the murine *Prn-p* triggered specific *Prn-p*-gene silencing in scrapie-infected neuroblastoma
185 cells. This caused a rapid loss of their PrPres content (Daude et al., 2003). Accordingly, it
186 was shown that transfection of either LRP antisense RNA or LRP-specific siRNAs in scrapie-
187 infected neuronal cells results in downregulation of LRP/LR expression and prevention of
188 PrP^{Sc} propagation (Fig.1, II) (Leucht et al., 2003).

189 However, a permanent effect of knockdown of disease-relevant genes using RNAi has
190 been achieved using a lentivirus-mediated gene transfer (Ralph et al., 2005; Raoul et al.,
191 2005). This shows that a lentivirus-based RNAi gene therapy strategy using HIV-derived
192 vectors expressing LRP-specific siRNAs is another innovative approach in TSE treatment.

193

194 *7.3. Antibodies directed against the LRP/LR*

195 The PrP binding capacity of LRP offers strategies in therapeutic approaches against
196 prion diseases. The curative effect of the polyclonal anti-LRP/LR antibody (W3) on scrapie
197 infected N2a cells recommends anti-LRP antibodies as therapeutic tools for the treatment of
198 prion diseases (Leucht et al., 2003), especially since (i) this antibody prevents the binding of
199 infectious prions to mammalian cells (Fig.1, III) (Gauczynski et al., 2006) and (ii)
200 endocytosis of PrP^{BSE} by enterocytes mediated by the LRP/LR is inhibited after treatment
201 with W3 (Morel et al., 2005).

202 Since a polyclonal antibody format is not suitable for a therapy in animals or humans
203 the development of single-chain antibodies directed against LRP/LR provides a promising

204 alternative therapeutic strategy. Smaller (30 kDa) and with better tissue penetration, they can
205 be delivered via passive immunotransfer for example intracerebrally into the brain region
206 where massive prion propagation takes place. So far, no immune response or side reactions
207 have been observed after application of scFvs. To circumvent the problem of the short half-
208 life in organisms a permanent delivery of single chain antibodies directed against LRP/LR
209 may be achieved by gene therapeutic strategies employing AAV-based or lentiviral vector
210 systems.

211

212 *7.4. Polysulphated glycans*

213 Polysulphated glycans such as heparan mimetics (HM) or pentosan polysulphate
214 interfere with the binding of the infectious PrP²⁷⁻³⁰ to the LRP/LR-HSPG complex and are
215 therefore another promising therapeutic strategy (Fig. 1, IV) (Gauczynski et al., 2006).
216 Treatment of scrapie-infected mice with pentosan polysulphate resulted in a prolonged
217 incubation time and even in the cure of two mouse strains infected with two different scrapie
218 strains (Farquhar and Dickinson, 1986). Moreover, it has been shown that GAGs (Hijazi et
219 al., 2005), especially heparan sulphate, (Horonchik et al., 2005) also act as receptors for the
220 infectious PrP^{Sc}. Polysulphated glycans such as SP54 and pS3 (phycarin sulphate) also show
221 an inhibitory effect on the binding of PrP²⁷⁻³⁰ to LRP hyperexpressing BHK cells
222 (Gauczynski et al., 2006). Both pentosan polysulphates and heparan sulphate mimetics are
223 able to prolong the incubation time in rodent models and interfere with PrP^{Sc} propagation in
224 neuronal cells due to the inhibition of the LRP/LR dependent binding of prions to target cells.

225

226 **8. Conclusions**

227 So far, there is no TSE treatment available that is able to cure affected individuals.
228 Alternative therapeutic strategies targeting LRP/LR might be promising since it acts as the

229 receptor for PrP^c and PrP^{Sc}. Molecules targeting the LRP-PrP interaction such as LRP
230 mutants, LRP/LR-specific antibodies and polysulphated glycans or tools downregulating the
231 LRP/LR levels such as siRNAs and antisense RNAs represent alternative therapies for the
232 treatment of prion diseases.

233

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239

240

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378 **Figure legends:**

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380 **Fig. 1** Molecules inhibiting the PrP-LRP interaction and reducing the LRP/LR levels.
381 LRP/LR (orange) is able to bind both the cellular prion protein (green circle) and the
382 infectious PrP^{Sc} (green rectangle). After binding to the LRP/LR-HSPG complex the prion
383 protein becomes internalized into endo-/lysosomal compartments where the conversion of
384 PrP^c to PrP^{Sc} might take place. Prevention of the binding to LRP/LR is achieved by (I)
385 trapping PrP^{Sc} by a LRP mutant encompassing the extracellular domain (LRP102-295),
386 downregulation of LRP/LR by (IIa) siRNAs and (IIb) antisense RNA directed against LRP
387 mRNA, (III) anti-LRP/LR antibodies competing with PrP for LRP binding sites and (IV)
388 heparan mimetics interfering with the binding of PrP^{Sc} to the LRP/LR/HSPG complex.