Poly-L-histidine inhibits prion propagation in a prion-infected cell line

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ABSTRACT
Transmissible spongiform encephalopathies (TSEs) are a group of lethal neurodegenerative diseases involving the structural conversion of cellular prion protein (PrP\(^C\)) into the pathogenic isoform (PrP\(^\Sc\)). For which no effective treatment is currently available. Previous studies have implicated that a polymeric molecule with a repeating unit, such as pentosane polysulfate and polyamidoamide dendrimers, exhibits a potent anti-prion activity. Here, by screening a series of poly-(amino acid)s in a prion-infected neuroblastoma cell line (GT\(^{Pr\Sc}\)), we identified poly-L-His as a novel anti-prion compound with an IC\(_{50}\) value of 1.8 µg/mL (0.18 µM). This potent anti-prion activity was specific to a high-molecular-weight poly-L-His and absent in monomeric histidine or low-molecular-weight poly-L-His. Solution NMR data indicated that poly-L-His directly binds to the loop region connecting Helix 2 and Helix 3 of PrP\(^C\). Poly-L-His, however, did not inhibit prion propagation in a prion-infected mouse when administered intraperitoneally, suggesting that the penetration of blood-brain barrier and/or the chemical stability of this polypeptide must be addressed before its application in vivo. Taken together, this study revealed the potential use of poly-L-His as a novel treatment against TSEs.

Introduction
Transmissible spongiform encephalopathies (TSE), or prion diseases, are a group of fatal neurodegenerative diseases that affect human and animals [1]. The pathogenesis of TSEs is strongly associated with misfolding of cellular prion protein (PrP\(^C\)) into its proteinase-K resistant isoform (PrP\(^\Sc\)). PrP\(^\Sc\) can propagate TSE in an infectious manner by converting endogenous PrP\(^C\) into PrP\(^\Sc\). TSE occurs in one-in-a-million individuals per year in human that causes neurodegenerative symptoms and death within 23 months. No effective treatment is currently available in clinical use, despite the extensive effort in developing an anti-prion agent over the last decades [2].

Previous studies have implicated that a polymeric molecule with a repeating unit, such as pentosane polysulfate [3], polyamidoamide dendrimers [4,5], and phosphorothioate oligonucleotides [6], exhibits a potent anti-prion activity in preclinical trials. Although the mechanistic basis of the anti-prion activity remains controversial (for example, see [7]), these studies are consistent in showing that the repeating unit in these molecules is an essential part of the anti-prion activity [3,4,6]. Several studies have also indicated that electrostatic charge in the polymeric structure is an important factor in determining its anti-prion activity [3,7]. These studies led us to hypothesize that poly-(amino acid)s, charged molecules with repeating units, is a potential molecule for inhibiting prion propagation. In fact, several preceding studies have reported anti-prion activity in positively charged poly-(amino acid)s, such as poly-L-Lys [8–10] and poly-L-Arg [11].

In this study, we systematically examine a series of poly-(amino acid)s in a prion-infected neuroblastoma cell line in order to expand the repertory of potential anti-prion compounds in vitro. We identified poly-L-His as a novel anti-prion compound with a half inhibitory concentration (IC\(_{50}\)) of 18 µg/mL (0.18 µM). This study also explored molecular basis underlying the anti-prion activity of poly-L-His and examined its application in vivo.

Methods
Reagents
Poly-(amino acid)s with heterogeneous degree of polymerization were purchased from Sigma-Aldrich (Table 1). A poly-L-His with homogeneous degree of polymerization (n = 2, 4, 8, 16, and 32) was chemically synthesized by Peptide Institute (Japan).
Cell-based anti-prion assay

Anti-prion activity of poly-L-(amino acid)s was studied using GT\textsuperscript{FK} cells as described previously [12]. Briefly, GT\textsuperscript{FK} was maintained and grown at 37°C in 5% CO\textsubscript{2} in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 50 U/ml penicillin G sodium, and 50 µg/ml streptomycin sulfate. 1.5 \times 10^5 cells were cultured in a 6-well plate for 24 hours and subsequently treated with poly-(amino acid)s for 72 hours. The cells were lysed in Triton X-100-deoxycholate buffer containing 150 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, and 50 mM Tris-HCl (pH 7.5). After 1 min of centrifugation at 10,000 g, the supernatant was collected and treated with proteinase-K as described previously [13]. Proteinase-K resistant PrP\textsuperscript{Sc} was detected using M-20 antibody (Santa Cruz Biotechnology). Western blotting was performed using a standard procedure.

Binding assay using solution NMR

Binding assay using solution NMR. \textsuperscript{15}N-uniformly labeled recombinant mouse PrP (residues 90–231) was expressed in an E.coli system and purified as described previously [14]. 0.5 mM PrP was dissolved with 20 mM sodium acetate buffer and 10% D\textsubscript{2}O in the absence or presence of 1 mM poly-L-His. The solution pH was precisely adjusted to 4.5 by adding a few drops of HCl or NaOH. The solution was transferred to a Shigemi tube and \textsuperscript{1}H,\textsuperscript{15}N-HSQC spectra of \textsuperscript{15}N-PrP were acquired at 20°C using Bruker 800 MHz NMR spectrometer. The NMR peaks were assigned and analyzed as described previously [14].

In vivo anti-prion assay

Anti-prion activity of poly-L-His was evaluated in vivo as described previously [14]. Briefly, 10% FK-infected mice brain homogenate was inoculated into 4-week-old male mice via an intracerebral route. At 4 weeks after inoculation, the mice were intraperitoneally administered with 10 mg/kg poly-L-His or saline. The treatment was repeated at 1-week intervals until death. Body weight and survival of the mice were examined every 1 week. Survival data were statistically analyzed using BellCurve (Social Survey Research Information Co., Ltd.).

Results

Poly-L-His inhibits prion propagation in vitro

To examine anti-prion activity of poly-(amino acid)s, we used a neuroblastoma cell line (GT1) chronically infected with Fukuoka-1 strain (designated as GT\textsuperscript{FK}) [15]. This cell line gives rise to five proteinase K-resistant bands at the positions of approximately 10, 15, 20, 23, and 28 kDa (Figure 1(a)), whose assignment has not been established [12,14,16,17]. Eight of 12 commercially available poly-(amino acid)s, which were water-soluble at a concentration of 1 mg/mL (Table 1), were administered into GT\textsuperscript{FK} at the final concentration of 10 µg/mL. After 3 days of administration, the level of proteinase K-resistant isoform (PrP\textsuperscript{Sc}) in GT\textsuperscript{FK} was evaluated using western blotting against a C-terminal region of PrP (M-20 antibody). Among the eight poly-(amino acid)s examined, poly-L-His exhibited the most potent inhibitory activity against prion propagation (Figure 1(a,b)). No cytotoxicity was observed in the cell treated with poly-L-His as judged by microscopy and the total protein level in cell lysate (data not shown). Poly-L-Lys and poly-L-Arg, which were previously suggested as anti-prion compounds, inhibited prion propagation in a statistically significant manner (p < 0.05, in t-test), but their effects were significantly weaker than that of poly-L-His.

To quantitatively evaluate the anti-prion activity of poly-L-His, we next examined the concentration-dependency using 0–100 µg/mL poly-L-His (Figure 1(c)). A plot of PrP\textsuperscript{Sc} level vs. poly-L-His concentration exhibited

![Table 1. Poly-amino acids used in this study.](image)

| Poly-amino acids       | CAS number | Sigma product number | Molecular weight | Solubility |
|------------------------|------------|----------------------|------------------|------------|
| Poly-DL-Ala            | 25,281–63-4| P9003                | 1,000–5,000      | Soluble    |
| Poly-L-Thr             | 82,822–12-6| P8077                | 5,000–15,000     | Soluble    |
| Poly-L-Asn             | 28,088–48-4| P8137                | 5,000–15,000     | Soluble    |
| Poly-L-Lys             | 25,988–63-0| P8954                | 500–2,000        | Soluble    |
| Poly-L-His             | 26,062–48-6| P9386                | 5,000–25,000     | Soluble\textsuperscript{2} |
| Poly-L-Ile             | 34,464–35-2| P3329                | 500–5,000        | Insoluble  |
| Poly-L-Trp             | 27,813–82-7| P4647                | 1,000–5,000      | Insoluble  |
| Poly-Gly               | 25,718–94-9| P8791                | 500–5,000        | Insoluble  |
| Poly-L-Pro             | 25,191–13-3| P2254                | 1,000–10,000     | Soluble    |
| Poly-L-Arg             | 26,982–20-7| P4663                | 5,000–15,000     | Soluble    |
| Poly-L-Gln             | 26,247–79-0| P1943                | 750–5,000        | Soluble    |
| Poly-L-Leu             | 25,322–63-8| P5762                | 3,000–15,000     | Insoluble  |

\textsuperscript{1}Solubility in phosphate-buffer saline (pH 7.4) at the concentration of 1 mg/mL; \textsuperscript{2}Poly-L-His was soluble in PBS when the solution pH was decreased from 7.4 to 5.5 by the addition of 6 M HCl.
a sigmoidal curve with a half inhibitory concentration (IC$_{50}$) of 1.8 µg/mL (Figure 1(d)). Given that the average molecular mass of poly-L-His was 10 kDa as judged by SDS-PAGE (data not shown), the IC$_{50}$ value was roughly equal to 0.18 µM. This value was comparable to those of anti-prion compounds previously reported, such as GN8 (1.4 µM) [14], tetrapyroles (0.5–2 µM) [18,19], quinacrine (0.4 µM) [20], 6-chloro-1,2,3,4-tetrahydroacridine (0.2 µM) [21], and phosphorothioate oligonucleotides (few nM) [6], suggesting that poly-L-His is a potent inhibitor against prion propagation.

**Polymerization of l-his is essential for the anti-prion activity**

We next examined whether the polymerization degree of histidine (n) would affect the anti-prion activity. Since the poly-L-His used in the Figure 1 experiments was heterogeneous in terms of the polymerization degree (n = 30–130, see Table), we synthesized a series of homogeneous poly-L-His with a defined degree of polymerization (n = 1, 2, 4, 8, 16, and 32). As shown in Figure 2(a,b), when administrated at a fixed molar concentration (10 µM), the anti-prion activity was gradually increased by increasing n from 1 to 32. To rule out the possibility that the positive correlation between n and anti-prion activity arises simply from the increased weight concentration of poly-L-His (for example, the weight concentrations of 1- and 32-mers were 1.55 and 49.6 µg/mL, respectively), the same experiment was repeated using a fixed weight concentration of poly-L-His (28 µg/mL). As shown in Figure 2(c,d), the same trend was maintained even at the fixed weight concentration. This result confirmed that the polymerization degree of poly-L-His is positively correlated with its anti-prion activity. Thus, consistent with previously reported anti-prion compounds such as pentosane polysulfate [3,4,6], the polymerization of L-His is an essential factor in determining its anti-prion activity.

**Poly-l-his directly binds to cellular prion protein**

We next examined how poly-L-His inhibits prion propagation. Poly-L-His has a unique property among the eight poly-(amino acid)s examined; it binds to metal ions at a few nanomolar affinity. Taken together with the fact that copper binding to PrP markedly influences the efficacy of pathogenic conversion toward PrP$^{Sc}$ [22], we initially hypothesized that metal chelation by poly-L-His might underlie the anti-prion activity. To test this hypothesis, we conducted a simple experiment by treating GT$^{FK}$ cell with 0–800 µM EDTA. If the metal chelation hypothesis is valid, then EDTA, which is a metal chelator with a micromolar affinity, should also act as an anti-prion compound in this assay system. However, as shown in Figure 3(a,b), no significant
difference in the level of PrP<sup>Sc</sup> was detected when the cell was treated with EDTA. This result ruled out the possibility that poly-L-His inhibits prion propagation through metal chelation.

A previous study examining anti-prion mechanism of a positively charged poly-(amino acid), poly-L-Lys, indicated that a negatively charged surface of the Helix 2-Helix 3 region of PrP<sup>C</sup> (residues 169–230) is the specific site for poly-L-Lys binding [8]. In fact, the Helix 2-Helix 3 region has been suggested as an initiation site for the structural conversion of PrP toward PrP<sup>Sc</sup> [23–26] and is a major target for many anti-prion compounds [14,27–30]. Given that the major population of poly-L-His is positively charged in acidic environments, in which the pathogenic conversion is believed to occur [31,32], the same mechanism can be applied to the case of poly-L-His.

Our initial attempt to detect interaction between PrP<sup>C</sup> and poly-L-His using surface plasmon resonance was unsuccessful, because poly-L-His binds to the gold surface of sensorchip. Therefore, we instead employed solution NMR technique and examined chemical shift perturbation induced by poly-L-His. As shown in Figure 4(a,h), <sup>15</sup>N-heteronuclear single quantum coherence (HSQC) spectrum of recombinant PrP<sup>C</sup> was acquired in the presence or absence of poly-L-His, and a chemical shift perturbation was examined by superimposing the two spectra. This experiment was performed under a mildly acidic condition (pH 4.5), because (1) both PrP<sup>C</sup> and poly-L-His were less soluble at neutral pH and (2) the population of positively charged poly-L-His is maximized at an acidic pH. We observed significant chemical shift perturbation in several (but not all) peaks in the NMR spectrum, suggesting that poly-L-His specifically binds to a specific region of PrP<sup>C</sup>. An assignment of NMR peaks indicated that most of the perturbed peaks (Δδ > 0.10) were mapped onto the loop region connecting Helix 2 and Helix 3 (residues 180–200) (Figure 4(b,c)). As indicated in the previous study [8], this loop region composes a negatively charged patch to serve as a binding surface for poly-L-Lys. These results indicated that poly-L-His and poly-L-Lys share a common mechanism in inhibiting prion propagation which involves direct binding to a negatively charged surface of PrP<sup>C</sup>. This also indicates that poly-L-His might inhibit prion propagation by sterically blocking the pathogenic conversion toward PrP<sup>Sc</sup> [8].

**Poly-l-his did not inhibit prion propagation in a prion-infected mouse**

A mouse intracerebrally inoculated with Fukuoka-1 was used to examine in vivo activity of poly-L-His. Four
weeks after the inoculation, the mouse was intraperitoneally administered with 10 mg/kg poly-L-His. The treatment was repeated every 1 week until death of mice. As shown in Figure 5(a), no significant prolongation of survival time was observed in a group treated with poly-L-His (p > 0.05 in log-rank test). We also found no significant improvement in body weight compared to control group (Figure 5(b)). These data indicate that poly-L-His is unable to inhibit prion propagation via an intraperitoneal route, and further optimization in administration protocol is required to achieve in vivo efficacy.

**Discussion**

This study investigated a series of poly-(amino acid)s as a potential inhibitor of prion propagation. Our screening of 12 poly-(amino acid)s identified poly-L-His as a novel anti-prion compound against Fukuoka-1 strain. The strong anti-prion activity (IC$_{50}$ = 1.8 µg/mL) was observed only in high-molecular-weight poly-L-His, but not in low-molecular-weight poly-L-His or monomeric L-histidine. While poly-L-His is the third poly-(amino acid) that exhibits anti-prion activity, following poly-L-Lys [8–10] and poly-L-Arg [11], it represents the most potent inhibitory activity against Fukuoka-1 strain among the three poly-(amino acid)s. Although strain dependence of the anti-prion activity remains to be demonstrated, the mechanism of action (i.e. direct binding to PrP$_{Sc}$) indicate that poly-L-His might inhibit prion propagation in strain-independent manner. In addition, consistent with a previous study [33], poly-L-His was not cytotoxic in the concentration range of 0–100 µg/mL. These results promise a potential use of poly-L-His as a novel treatment against TSEs. A further optimization of poly-L-His as an anti-prion compound is possible through screening hetero-poly-(amino acid) involving His, Arg, and Lys, such as (His-Arg)$_n$ and (His-Lys)$_n$.

Unfortunately, poly-L-His did not extend the lifetime of prion-infected mice when administrated intraperitoneally (Figure 4). This result indicates that poly-L-His is unable to inhibit prion propagation in vivo using a standard administration protocol. Potential factors that prevent its in vivo application include (1) toxicity related to the chelating activity of poly-L-His (such as iron-deficiency anemia), (2) proteolytic cleavage of the polypeptide chain, and (3) low penetration of the blood-brain barrier. These factors must be addressed to achieve a successful in vivo inhibition of prion propagation using poly-L-His. Although any optimization of administration protocol was not attempted in this study, the first problem can be simply solved by co-administration of essential metals. The second problem can be also solved by using poly-D-His, which is resistant to proteolytic cleavage [8]. An attachment of a cell-penetrating peptide, such as poly-L-Arg and TAT, to the C- or N-terminus of poly-L-His would improve the penetration of the blood-brain barrier [34]. Implanting an intraventricular infusion device is an alternative approach to directly deliver poly-L-His to the brain [35]. We therefore believe that poly-His will inhibit prion propagation in vivo in future studies after optimization of administration protocol. However, we could not completely rule out at this

**Figure 3.** Effect of metal chelation on the level of proteinase K resistance PrP (PrP$_{Sc}$). (A) Western blotting probing the level of PrP$_{Sc}$ in GT1$^{FK}$ cell after 3 days treatment of EDTA. No inhibition of cell growth was observed up to 400 µM EDTA as judged by microscopy and total protein amount in cell lysate (data not shown). N.C., negative control (PBS); P.C., positive control (1.8 µM GN8). (B) A relative level of PrP$_{Sc}$ derived from the band density in each lane of Figure 3A. Error bars represent standard deviation (n = 3).
stage the possibility that poly-L-His has no benefit as a treatment of TSEs; it might only reduce the level of proteinase-K resistant PrPSc, but not the level of infectious PrPSc in vivo [36].

Interestingly, three poly-(amino acid)s that inhibit prion propagation in vitro, poly-L-His, poly-L-Lys, and poly-L-Arg, are all positively charged at mildly acidic pH, at which the pathogenic conversion is believed to occur [31,32]. This indicates that these three poly-(amino acid)s all share a common anti-prion mechanism involving an electrostatic interaction between positive charges of the poly-(amino acid) and negative charges of a target molecule at an acidic environment. Our NMR experiment and a previous study consistently show that poly-L-His and poly-L-Arg can directly bind to a negative charged surface of PrP C [8].

This indicates that the target molecule of the poly-(amino acid)s might be PrPSc, in which the poly-(amino acid)s might serve as an steric block against the pathogenic conversion toward PrPSc. However, a careful interpretation is necessary, because the extent of target specificity of poly-(amino acid)s remains elusive. In fact, Ryou and colleagues recently proposed that poly-L-Arg and poly-L-Lys bind to plasminogen activator [11], a molecule that might promote the pathogenic conversion of PrP [37]. A further experiment using pull-down assay coupled with mass spectroscopy is required to identify the target molecule of the poly-(amino acid)s and to further understand the anti-prion mechanism.
Disclosure statement

No potential conflict of interest was reported by the authors.

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Notes on contributor

R.H. designed this study and wrote the manuscript. K.Y. and M.F. performed in vitro experiments, and analyzed the data. A.E. and M.F. performed in vivo experiments. K.K. supervised this study.

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References

[1] Colby DW, Prusiner SB. Prions. Cold Spring Harb Perspect Biol. 2011 Jan;3(1):a006833. PubMed PMID: 21421910; PubMed Central PMCID: PMC3003464.

[2] Giles K, Olson SH, Prusiner SB. Developing therapeutics for PrP prion diseases. Cold Spring Harb Perspect Med. 2017;7(4):a023747.

[3] Caughey B, Raymond GJ. Sulfated polyanion inhibition of scrapie-associated PrP accumulation in cultured cells. J Virol. 1993;67(2):643–650.

[4] Supattapone S, Nguyen H-OB, Cohen FE, et al. Elimination of prions by branched polyanimes and implications for therapeutics. Proc Natl Acad Sci. 1999;96(25):14529–14534.

[5] Supattapone S, Wille H, Uyechi L, et al. Branched polyanimes cure prion-infected neuroblastoma cells. J Virol. 2001;75(7):3453–3461.

[6] Kocisko DA, Vaillant A, Lee KS, et al. Potent antiscrapipe activities of degenerate phosphorothioate oligonucleotides. Antimicrob Agents Chemother. 2006 Mar;50(3):1034–1044. PubMed PMID: 16495266; PubMed Central PMCID: PMC1426446. eng.

[7] Caughey B, Caughey W, Kocisko D, et al. Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics: A common mechanism for anti-TSE compounds? Acc Chem Res. 2006;39(9):646–653.

[8] Xu Z, Adrover M, Pastore A, et al. Mechanistic insights into cellular alteration of prion by poly-D-lysine: the role of H2H3 domain. Faseb J. 2011 Oct;25(10):3426–3435. PubMed PMID: 21697549; eng.

[9] Lee H-M RC. Targeting of poly (L-lysine) to organs that propagate prions. J Bioact Compat Polym. 2014;29(5):432–444.

[10] Jackson KS, Yeom J, Han Y, et al. Preference toward a polylysine enantiomer in inhibiting prions. Amino Acids. 2013;44(3):993–1000.

[11] Waqs M, Lee H-M, Kim J, et al. Effect of poly-L-arginine in inhibiting scrapie prion protein of cultured cells. Mol Cell Biochem. 2017;428(1–2):57–66.

[12] Hosokawa-Muto J, Kamatari YO, Nakamura HK, et al. Variety of antiprion compounds discovered through an in silico screen based on cellular-form prion protein structure: correlation between antiprion activity and binding affinity. Antimicrob Agents Chemother. 2009;53(2):765–771.

[13] Nishida N, Harris DA, Vilette D, et al. Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. J Virol. 2000;74(1):320–325.

[14] Kuwata K, Nishida N, Matsumoto T, et al. Hot spots in prion protein for pathogenic conversion. Proc Natl Acad Sci U S A. 2007 Jul 17;104(29):11921–11926. PubMed PMID: 17616582.

[15] Milhavet O, McMahon HE, Rachidi W, et al. Prion infection impairs the cellular response to oxidative stress. Proc Natl Acad Sci. 2000;97(25):13937–13942.

[16] Mashima T, Nishikawa F, Kamatari YO, et al. Antiprion activity of an RNA aptamer and its structural basis. Nucleic Acids Res. 2013 Jan;41(2):1355–1362. PubMed PMID: 23180780; PubMed Central PMCID: PMCPMC3553944. eng.

[17] Kimura T, Hosokawa-Muto J, Kamatari YO, et al. Synthesis of GN8 derivatives and evaluation of their antiprion activity in TSE-infected cells. Bioorg Med Chem Lett. 2011 Mar 1;21(5):1502–1507. PubMed PMID: 21277202; eng.

[18] Nicoll AJ, Trevitt CR, Tattum MH, et al. Pharmacological chaperone for the structured domain of human prion protein. Proc Natl Acad Sci. 2010;107(41):17610–17615.

[19] Caughey WS, Raymond LD, Horiuchi M, et al. Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. Proc Natl Acad Sci U S A. 1998 Oct 13;95(21):12117–12122. PubMed PMID: 9770449; PubMed Central PMCID: PMCPMC22794. eng.

[20] Doh-Ura K, Iwaki T, Caughey B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol. 2000;74(10):4894–4897.

[21] Bongarzone S, Tran HNA, Cavalli A, et al. Parallel synthesis, evaluation, and preliminary structure-activity relationship of 2, 5-Diamino-1, 4-benzoquinones as a novel class of bivalent anti-prion compound. J Med Chem. 2010;53(22):8197–8201.

[22] Mullhauser GL. Copper binding in the prion protein. Acc Chem Res. 2004;37(2):79–85.

[23] Adrover M, Pauwels K, Prigent S, et al. Prion fibrillization is Mediated by a native structural element that comprises helices H2 and H3. J Biol Chem. 2010 Jul 2;285(27):21004–21012. 10.1074/jbc.M110.111815. PubMed PMID: WOS:000279228600064; English.

[24] K-I Y, Matsumoto T, Kuwata K. Critical region for amyloid fibril formation of mouse prion protein:
unusual amyloidogenic properties of the helix 2 Peptide. Biochemistry. 2008;47(50):13242–13251.

[25] Singh J, Udgaonkar JB. Molecular mechanism of the misfolding and oligomerization of the prion Protein: current understanding and its implications. Biochemistry. 2015 Jul 28;54(29):4431–4442. PubMed PMID: 26171588; eng.

[26] Honda R, Kuwata K. Evidence for a central role of PrP helix 2 in the nucleation of amyloid fibrils. PubMed PMID: 29401635 FASEB J. 2018;327:3641–3652.

[27] Ferreira N, Ascari L, Hughson A, et al. A promising antiprion trimethoxychalcone binds to the globular domain of the cellular prion protein and changes its cellular location. Antimicrob Agents Chemother. 2018;62(2):e01441–17.

[28] Zhou S, Liu X, An X, et al. Molecular dynamics simulation study on the binding and stabilization mechanism of antiprion compounds to the “Hot Spot” region of PrPC. ACS Chem Neurosci. 2017;8(11):2446–2456.

[29] Pagadala NS, Bjorndahl TC, Joyce M, et al. The compound (3-[5-[(2, 5-dimethoxyphenyl) amino]-1, 3, 4-thiadiazolidin-2-yl]-5, 8-methoxy-2H-chromen-2-one) inhibits the prion protein conversion from PrPC to PrPSc with lower IC50 in ScN2a cells. Bioorg Med Chem. 2017;25(20):5875–5888.

[30] Hyeon JW, Choi J, Kim SY, et al. Discovery of novel anti-prion compounds using in silico and in vitro approaches. Sci Rep. 2015;5:14944.

[31] Borchelt D, Taraboulos A, Prusiner S. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J Biol Chem. 1992;267(23):16188–16199.

[32] Swietnicki W, Petersen R, Gambetti P, et al. pH-dependent stability and conformation of the recombinant human prion protein PrP(90–231). J Biol Chem. 1997 Oct 31;272(44):27517–27520. 10.1074/jbc.272.44.27517. PubMed PMID: WOS:A1997YD47300007; English.

[33] Putnam D, Zelikin AN, Izumrudov VA, et al. Polyhistidine-PEG:DNA nanocomposites for gene delivery. Biomaterials. 2003 Nov;24(24):4425–4433. PubMed PMID: 12922153; eng.

[34] Guidotti G, Brambilla I, Rossi D. Cell-penetrating peptides: from basic research to clinics. Trends Pharmacol Sci. 2017;38(4):406–424.

[35] Doh-Ura K, Ishikawa K, Murakami-Kubo I, et al. Treatment of transmissible spongiform encephalopathy by intraventricular drug infusion in animal models. J Virol. 2004;78(10):4999–5006.

[36] Sajnani G, Silva CJ, Ramos A, et al. PK-sensitive PrPSc is Infectious and shares basic structural features with PK-resistant PrPSc. PLoS Pathog. 2012;8(3):e1002547.

[37] Mays CE, Ryoo C. Plasminogen stimulates propagation of protease-resistant prion protein in vitro. FASEB J. 2010;24(12):5102–5112.