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Past and Future of Diagnosis and Therapy of Transmissible Spongiform Encephalopathy

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1. Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, including Creutzfeldt-Jakob disease (CJD) in human, Bovine Spongiform Encephalopathy (BSE) in cow, and scrapie in sheep, represent diseases with complex and still poorly understood molecular mechanisms (Prusiner, 1998). The protein-only hypothesis postulates a possible pathogenic mechanism involving the prion protein. The α–helix rich normal prion protein (PrPC) is found to be infected by its β-sheet rich abnormal proteinase K (PK)-resistant form, PrPSc, and is converted into PrPSc (Prusiner, 1998). This infection will lead to the aggregation of PrPSc-based amyloid fibrils that accumulate in the peripheral and invade to the central nervous system and damage neurons.

In this chapter, we discuss current understanding of pathogenic mechanisms in this prion-only hypothesis based disease in Section 2. This understanding then leads to the discussions of the past and present developments in both diagnosis and therapy for these diseases in Section 3. Based on the understanding of pathogenic mechanisms and current diagnosis and therapeutic strategies, we propose an alternative strategy. The proposal considers an aptamer-based theranostic approach to detect and prevent the aggregation of amyloid plaques in Section 4. Note that aptamer is defined as short nucleic acid sequences, and designed through Systematic Evolution of Ligands by EXponential enrichment (SELEX) (James, 2000). It is hoped that this proposal will open a novel research direction and eventually lead to a better diagnosis and treatment of prion diseases in the future.

2. The infectivity of prion diseases

Numerous models have been developed to provide better understanding of the pathogenesis of the prion diseases. One of models advanced is the template-assistance model (Prusiner, 1998; Horiuchi & Caughy, 1999; Tompa et al., 2002). This model assumed that PrPC, which is normally more stable than PrPSc in isolation, would in the presence of PrPSc convert to the latter via a transient catalytic interaction with it. The implication is that a dimer of PrPSc's is energetically more stable than a system of non-interacting PrPC and PrPSc. This was supported by Morrissey and Shakhnovich's computational analyses (Morrissey & Shakhnovich, 1999). When there are other PrPSc's present, the initial

autocatalytic process would then lead to a propagation of PrPC to PrPSc conversion. Therefore, many studies have been focused on investigating possible mechanisms of this triggering event. For example, Zou and Cashman report in their studies that acidic environment increases the chance of triggering conversion (Zou & Cashman, 2002). Several groups have searched for the potential sites responsible for the conversion, referred to as hot spots, in PrPC (Guilbert, et al, 2000; Kuwata et al., 2007; Tseng et al., 2009).

Furthermore, experimental evidences suggest that the infectivity of BSE cross-strains may associate with a host-independent molecule (Somerville, 2002). Gale showed that a phospholipid is most likely to be this molecule through the strain thermostability studies (Gale, 2006). Recent studies of prion diseases in membrane environments suggest that Phosphatidylserine (PS) is a molecule capable of altering amyloid aggregation pathways and increasing aggregation rates (Robinson & Pinheiro, 2010). This finding is further supported by the identification of the endosome recycling compartment as the potential conversion site (Marijanovic et al., 2009; Thellung et al., 2011).

In addition to exploring pathogenic mechanisms, another fundamental issue is how PrPSc based amyloid aggregation leads to the development of clinical symptoms and neuropathology. Chiesa et al. have designed a series of experiments based on Tg(PG14) mice models to resolve this issue (Chiesa et al., 2000). They demonstrated that one of the most obvious symptoms in neuropathology is massive loss of cerebellar granule cells due to apoptosis. Apoptosis is a vital, highly regulated, natural process that contributes to the development and maintenance of human and animal cells and tissues (Kerr et al., 1972; Weinberg, 2007). Apoptosis plays multiple roles in the normal development of organisms extending from embryonic development to the maintenance of normal cell homeostasis (Reed & Tomaselli, 2000; Elmore, 2007; Rastogi et al., 2009). Chiesa et al.'s discovery is supported by a recent study by Thellung et al. (Thellung et al., 2011), in which SH-SY5Y human neuroblastoma cells were used. They observed that accumulation of human PrPSc90-231 in membrane triggers lysosome dependent apoptosis and leads to neuronal cells death.

These insights seem open a route that differs from modern approaches as will be discussed below and which could lead to novel detection and "treatment" methods of prion diseases. However, before we propose this alternative solution, we first need to understand how modern approaches work including their advantages and shortcomings.

3. Past and current developments of diagnosis and treatment of prion diseases

3.1 Diagnosis

According to the WHO manual for surveillance of human TSEs (WHO, 2003), current diagnosis methods can be categorized into two groups based on pathological characteristics of TSEs at microscopic and macroscopic levels. First, because of the diverse etiology of prion diseases in humans and animals, many studies have focused on the search for common mechanisms of these diseases at the molecular (microscopic) level in order to detect their onsets. The key question in this search is to ask if one can distinguish PrPSc from PrPC based on pathological changes and how this can be accomplished. The crux of the matter hinges on the conventional belief that specific prion protein types correlate with phenotypes

of the disease. Second, several pathological characteristics of TSEs at the brain (macroscopic) level are generally recognized, can one distinguish signals or patterns generated by diseased brains from those present in normal brains. The key to correct diagnosis of TSEs then depends on the specificity and sensitivity of sensors

Regarding to the first group of diagnoses, three characteristics of PrPSc including prion protein gene (PRNP) codon 129 polymorphisms (Quadrio et al., 2011), Tyr-Tyr-Arg exposure (Paramithiotis et al., 2003) and over-expression of brain protein 14-3-3 (P14-3-3) (Hsich et al., 1996) were found to be distinctive from PrPC. Regarding to first characteristic, codon 129 was shown to be responsible for determining the variants of PK-resistant PrPSc in CJD (Parchi et al., 1996, 1999). Note that codon 129 encodes for two amino acids, methionine (M) and valine (V). A phenotypic influence in inherited prion diseases associated with codon 129 has been investigated and summarized in (Kovacs et al., 2002; Capellari et al., 2011). Regarding to second characteristic, Paramithiotis et al. showed that the Tyr-Tyr-Arg motif in PrP, which is a hydrophilic-like fragment, will be exposed to solvent when PrPC is converted into PrPSc. Regarding the third characteristic, P14-3-3 has been studied because of its potential as a bio-marker (Hsich et al., 1996). Basically, they found the presence of proteins 130 and 131, which are P14-3-3 type proteins, in cerebrospinal fluid from patients with CJD and little amount in normal patients. This result has led to the development of P14-3-3 immunoassays to aid in the diagnosis of prion diseases.

Regarding the second group, several noninvasive tools such as electroencephalography (EEG) and magnetic resonance imaging (MRI) have been investigated to detect abnormal onsets of prion diseases in brain besides biopsy. Tschampa et al. have shown that periodic sharp wave complexes appear in neuronal electric signals measured by non-invasive EEG in patients with CJD. These complexes are most likely the consequence of parvalbumin-positive neurons being reduced in the majority of thalamic nuclei (Tschampa et al., 2002). Namely, the damage to these neurons determines the generation of this typical clinical feature of CJD. World Health Organization (WHO) has standardized a protocol using EEG in diagnosis in 1998 (WHO, 2003). For MRI-related methods such as fluid attenuation inversion recovery (FLAIR) and diffusion-weighted MRI imaging (DWMRI), these are still relatively new techniques that have been introduced (Appel et al., 2011). These methods are based on the detection of abnormal signals from the basal ganglia, thalamus and cortex of patients. However, since most studies have investigated using only small samples it is still impossible to firmly confirm the usefulness of MRI in this context. Nevertheless, it was shown that MRI may be able to better locate abnormal patterns in deep gray matter while EEG may be more sensitive in the cortex. Both methods may have a complementary role in diagnosing prion diseases.

In summary, there are three tools commonly considered to aid in the diagnosis of prion diseases at present. Regarding codon 129 (genetic analysis), there are more and more studies that support the findings of a reproducible phenotypic spectrum of CJD variants and that the codon 129 genotype acts as the main determinant of the disease phenotype. Namely, DNA sequencing of the coding region of the PRNP may detect the onset of the disease. Regarding P14-3-3 (immunoassay), although it is not specific to prion diseases, it is a marker of neuronal damage and has been available *in vivo*. Regarding the periodic sharp wave complexes (EEG analysis), it indicates a footprint of neuronal electric signals from neurons damaged by PrPSc aggregation. It has been found that incorporation of non-invasive EEG

analysis and P14-3-3 detection increases the sensitivity of detection and improves diagnostic criteria (Zerr et al., 2000). Furthermore, Quadrio et al. suggest that conducting genetic analysis focusing on PRNP in addition to the combination of P14-3-3 detection and EEG analysis provides an extensive strategy to better diagnose prion diseases because these methods are sensitive to specific variants of PrP (Quadrio et al., 2011). Because the current molecular-based diagnoses hinge on the specificity and sensitivity of distinguishing between PrPC and PrPSc, the variants of PrP lower the success rate of these methods. Searching for more universal footprints of these variants will be one way to better detect the onset of prion diseases. Note that there are many other methods not mentioned here including in vivo biomarkers in blood and urine and tissue-based analysis which are currently under investigation (refer to (WHO, 2003; Quadrio et al., 2011) for more details).

3.2 Treatments

3.2.1 Rationale

Although there are no effective therapies for prion diseases yet, one can still summarize the current strategies as being divided into three categories based on various concepts of choosing therapeutic targets. Note that the discussions below are based on the assumption that the blood-brain barrier penetration issue is overcome. Moreover, an additional issue in treating prion diseases, administration methods was shown by Doh-ura et al. (Doh-ura et al., 2004) to be equally crucial as designing better inhibitors. These authors have demonstrated intra-ventricular administration of pentosan polysulfate (PPS, an FDA approved drug originally designed for anti-malaria treatments) through an infusion device can inhibit PrPSc aggregation. However, based on our expertise in drug discovery, we will limit our discussion to addressing the issue of effective inhibitor design in this section.

Based on the prion hypothesis, researchers in this field have focused on three targets, which include PrPC, PrPSc and PrPSc-producing cells. First, regarding the selection of PrPC as a target, Mallucci et al's study provides strong evidence in support of this choice (Mallucci et al., 2007). They showed that when PrPc is depleted at an early stage of the disease in mouse, damaged neuronal functions are recovered. Furthermore, the recovery is independent of PrPSc aggregation, which implies novel mechanisms of neurotoxicity and therapeutic possibilities. Second, for choosing PrPSc as a target, the idea is simply to stop PrPSc aggregation. Many studies pointed out that PrPSc is replicated and accumulated in the lympho-reticular system during the initial stage of extra-cerebral prion infection (Mabbott & MacPherson, 2006). Furthermore, because Korth et al. discovered a unique epitope in PrPSc (Korth et al, 1997), this finding makes PrPSc a promising target to prevent prion infection at an early stage through immunotherapy. Third, for choosing PrPSc-producing cells as a target, the goal is to stop the infection in the peripheral nervous system and to prevent the plaque from being transmitted to the central nervous system. Several studies suggest dendritic cells as a potential target to inhibit neuronal invasion because of the role of dendritic cells in up-regulating follicular dendritic cells (FDCs), in which PrPSc are replicated and accumulated in diseased mice (Huang et al., 2002; Brown et al., 2009).

Various bio-materials including antibodies, chemical compounds and aptamers have been investigated as inhibitors for these three targets. For example, targeting PrPC includes anti-PrP antibody development (Fe´raudet-Tarisse et al., 2010) and PPS (Doh-ura et al., 2004) etc.

Targeting PrPSc includes anti-PrP monoclonal antibody development (Mab) IgM (Paramithiotis, 2003) and 6D11 (Sadowski, 2009) and RNA aptamer design (Weiss et al., 1997; Proske et al., 2002; Rhie et al., 2003). For PrPSc-producing cells, cytotoxic T cells, antibody raised based on dendritic cells are used (Rosset et al. 2009). Although the results obtained from the immunotherapy focused on either PrPC, PrPSc or PrPSc-producing cells remain controversial, the immunotherapy is still a widely investigated strategy and has been shown to have an anti-prion effect in cellular and animal models (Paramithiotis et al., 2003; Sadowski et al., 2009; Fe´raudet-Tarisse et al., 2010). Because of the controversial issues in immunotherapy, many other groups continue searching for novel small molecule inhibitors including those either mimicking negative inhibition of prion replication by Perrier et al. (Perrier et al., 2000) or those targeting protease-resistant PrP by Charvériat et al. (Charvériat et al., 2008).

Since currently immunotherapy is the most developed and promising treatment, we will only focus on this modality in order to learn more about the key issues involved and to gain better understanding of the features of prion diseases revealed through these studies as discussed in the following sections.

3.2.2 Immunotherapy

Basically, immunotherapy hinges on inducing anti-PrP antibodies in appropriate hosts and utilizes these anti-PrP antibodies to activate the host's immune system's response against PrPSc aggregation. Several approaches of generating antibodies are employed including either bacterially-expressed full length PrP, PrP-PrP polyproteins, synthetic PrP peptides etc. However, studies have shown these approaches result in a low anti-PrP titer and immune-effects are moderate (Heppner & Aguzzi, 2004).

Nevertheless, immunotherapy still attracts the attention of many investigators. While much effort has been made to determine more factors responsible for prion conversion as potential drug targets, as mentioned previously, even more work is focused on targeting either PrPC, PrPSc or PrPSc-producing cells. A strategy that applies anti-PrP antibodies directly against PrPC has been shown to delay PrPSc peripheral accumulation (Fe´raudet-Tarisse et al., 2010). Fe´raudet-Tarisse et al. (2010) further studied pharmacokinetics and pharmacodynamics of several anti-PrP antibodies to examine their immunotherapeutic effects in mice. Another strategy in immunotherapy is to target PrPSc peripheral replication in the lympho-reticular system before the central nervous system (Bessen et al. 2009). The follicular dendritic cells are the major target of prion infection. Sadowski et al. showed that Mab 6D11 raised against PrPSc fibrils from brains of terminally ill CD-1 mice infected with 139A strain (Kascsak et al, 1987) prevents infection of FDC-P1 cells and removes PrPSc from the infected FDC-P1/22L cell line (Sadowski et al., 2009). However, Sadowski et al. also showed that time-limited treatment, which is more practical from the clinical point of view, using Mab 6D11 is not capable of preventing prion diseases. This result is also supported by the findings of Fe´raudet-Tarisse et al. (Fe´raudet-Tarisse et al., 2010). Therefore, one can conclude that the currently available anti-PrP antibodies are likely to only temporarily control PrPSc aggregation.

In summary, several issues hinder the practical implementation of immunotherapy in prion diseases. It includes a low anti-PrP antibody titer and moderate immuno-effects.

Furthermore, additional issues such as the expressed full length PrP approach are discussed below. First, the products failed to recognize the cell-surface PrPC, which is believed to directly associate with prion conversion, in wild type mice (Heppner & Aguzzi, 2004) and second, the full length antibodies when encountering the blood-brain barrier, are unable to cross it and enter into brain (Campana et al., 2009). The molecular mechanisms in the issue of low titer and moderate immuno-effects require more studies for better understanding. The latter issue may be resolved based on the work of Campana et al., in which they proposed a protocol to develop antibody fragments for prion diseases (Campana et al., 2009).

To develop a more promising immunotherapy for prion diseases in the future, it will require further investigations into various key areas. As shown in the works (Heppner & Aguzzi, 2004; Sadowski et al., 2009; Fe´raudet-Tarisse et al., 2010), these key areas include understanding the mechanisms of antibody resistance, identification of better antibodies against either PrPC or PrPSc (or other immune effectors such as dendritic cells (Rosset et al. 2009)) and designing proper administration methods (not addressed here) etc.

4. Aptamer-based theranostic approach to targeting phosphatidylserine

4.1 Logic

In the diagnosis and treatment of prion diseases, as mentioned previously, two questions need to be answered, i.e.: (a) what are "good" factors that would allow detectors to distinguish between PrPC and PrPSc and (b) what are appropriate therapeutic targets. In diagnosis, Protein P14-3-3 was shown to be a marker for neuronal damage and its use in detecting the onset of prion diseases has been demonstrated *in vivo* (Hsich et al., 1996). The question emerges if there are any other markers one can utilize to detect neuronal damage due to PrPSc aggregation? In terms of therapy, we have previously stated that three targets, PrPC (Mallucci et al., 2007), PrPSc (Huang et al., 2002; Brown et al., 2009) and PrPSc-producing cells (Bessen et al. 2009) are commonly considered as proper targets that could be used to prevent the disease. However, there are no effective prophylactic or therapeutic treatments available at present (Fe´raudet-Tarisse et al., 2010). Even for immunotherapy, its effects remain controversial (Heppner & Aguzzi, 2004). Since this controversial issue is yet to be resolved, we argue that targeting conversion factors rather than either PrPC, PrPSc, or PrPSc-producing cells may emerge as a viable strategy to avoid this problem and treat prion diseases.

As discussed in Sec. 2, Gale has shown that PS is the host-independent molecule associated with enhancing PrPSc aggregation (Gale, 2006). Robinson and Pinheiro have recently reported that PSs alter amyloid aggregation pathways by increasing aggregation (Robinson & Pinheiro, 2010), which suggests an alternative approach for therapeutic treatment of prion diseases. Namely, we may be able to delay or even stop PrPSc aggregation by directly inhibiting the interactions between PSs and PrPSc amyloid.

Furthermore, Chiesa et al and Thellung et al. have shown intracellular PrPSc aggregation in membranes triggers apoptosis (Chiesa et al, 2000; Thellung et al., 2011). Since we know that an early marker of apoptosis is the redistribution of PS between inner and outer plasma membranes (PS externalization) (Reutelingsperger et al., 1995; Blankenberg, 2009), PS externalization is specific to apoptotic cells with the exception of activated platelets and

erythrocytes. Therefore, it is an attractive target for apoptosis detection (Blankenberg, 2008, 2009; Smrz et al., 2008) and as means of providing an early indication of the success or failure of therapy for prion diseases.

4.2 Aptamer design

Recently, aptamers have attracted great attention and may provide a viable alternative in the diagnosis and treatment of prion diseases (Weiss et al., 1997; Proske et al., 2002; Rhie et al., 2003). Aptamers are in many aspects, such as binding specificity and strong affinity, equivalent to antibodies. There are several advantages of using aptamers over antibodies. First, the selection of aptamers using SELEX can target any system while identification of antibodies depends on animal systems. Second, once an aptamer is selected, it can be easily synthesized and manipulated.

Based on their characteristics, aptamers appear to be a viable material, other than antibodies, to be implemented as either inhibitors or detectors of cellular events such as apoptosis. Aptamers are selected through SELEX to bind to specific bio-molecular targets including small molecules, proteins, nucleic acids, phospholipids as well as complex structures such as cells, tissues, bacteria and other organisms. Because aptamers have strong and specific binding affinity through molecular recognition and low toxicity, they are generally recognized as having potential therapeutic and diagnostic clinical applications (Nimjee et al., 2005; James, 2000). SELEX consists of a number of rounds of *in vitro* selection in which the RNA/DNA pool is incubated with the binding target. In practice, multiple rounds of selection and expansion are required in SELEX before unique tightly binding sequences can be identified. Additionally, isolated aptamers will often need to be re-engineered to reduce their sequence length and impart additional favorable biological properties. These issues pose a challenge for the efficient identification of correct aptamers.

Despite the issues involved in the use of SELEX, several groups have successfully identified RNA aptamers to bind specifically to either PrPC (Weiss et al., 1997; Proske et al., 2002) or PrPSc (Rhie et al., 2003). Proske et al. have utilized PrPC protein comprising amino acid residues 90-129, which belong to a short epitope (90-141) as the target. The resulting RNA aptamer has been shown to recognize the full-length PrPC and to reduce PrPSc formation in prion-infected neuroblastoma cells. On the other hand, Rhie et al. have selected RNA aptamers targeting scrapie-associated fibrils. They have shown an at least 10-fold higher binding affinity for PrPC. Although this aptamer has not been tested for its ability to inhibit the prion conversion in either cell lines or animal models, the studies in a cell-free model system do show the inhibition of the conversion due to the presence of this aptamer.

Even though we cannot dismiss the applicability of the above two examples in prion diseases, the issues involving SELEX still impede practical implementations of aptamer structures in various directions in both diagnosis and therapeutics. We have developed an information-driven theoretical approach, called Entropic Fragment Based Aptamer design (EFBA), in order to resolve these issues and design aptamers based solely on the structural information regarding molecular targets. Basically, our approach is based on a seed-and-grow strategy to determine the aptamer that has the highest probability to interact with the target of interest. Details of the approach and its validation are discussed in (Tseng et al., 2011).

Because of the molecular characteristics of aptamers and our validated aptamer design algorithm, we can apply the algorithm to computationally design aptamer-based theranostic agents that can specifically bind to any targets that are associated with either prion conversion or PrPSc aggregation. Thereafter, we can computationally and experimentally investigate its binding properties and apply the results to improve the designed sequence.

4.3 Aptamer-based theranostic approach targeting PS

4.3.1 Strategy

As we know, PS is shown to increase PrPSc aggregation, which is independent of the host, and PrPSc aggregation triggers apoptosis. Furthermore, when apoptosis is initiated, an early indication of this process is PS externalization. Therefore, we propose to design an aptamer simply targeting PS to inhibit interactions between PrPSc and PS. The inhibition may also delay accumulation. Besides, we can monitor the changes of PS externalization to not only detect the onset of the disease but also to estimate therapeutic effects.

The advance of aptamer design of our algorithm allows us to computationally design an aptamer-based theranostic agent aimed at prion diseases. The application of aptamers will likely be free from difficulties of using other materials such as a well-studied apoptotic probe, annexin V, a small peptide (Blankenberg, 2009). For example, annexin V has a high uptake in normal tissues, especially liver and kidney, a long biological half-life in non-target tissues, a high radiation burden for ^{111}In and ^{124}I analogs and laborious radiochemistry for the labeling (Boersma et al., 2005). Therefore, an aptamer attached with a fluorescence tag that specifically binds to PS can both inhibit the interactions between PrPSc aggregation and PS and detect the changes of PS externalization at the same time using fluorescence imaging.

In the following, we will only demonstrate the application of our aptamer design algorithm in designing a DNA aptamer to bind specifically to PS. Furthermore, wet lab investigations in both imaging properties and therapeutic effects will not be presented here because of resource limitations at present.

4.3.2 The PS DNA-aptamer

In our earlier work (Tseng et al., 2011), the design of a PS aptamer has been briefly addressed. Basically, we start with the molecular model of PS, which was manually constructed using the MOLDEN program (Schaftenaar & Noordik, 2000). The resulting structure was then minimized with the GAMESS-US (Schmidt et al., 1993) program using the AM1 semi-empirical method (Dewar & Dieter, 1986; Dewar et al., 1985). The PS structure was further equilibrated using molecular dynamics simulation for 1 ns for DNA aptamer design. Two top sequences, namely 5′-AAAAGA-3′ (PS-aptamer I) and AAAGAC (PS-aptamer II), were selected from our design for experimental binding assays and *in silico* experiments (*in silico* results are not shown here). The structure of the PS-aptamer I in new ribbons format and the potential location of its binding site are shown in the left panel of Fig. 1. Note that the aptamer structure has not been energy equilibrated yet.

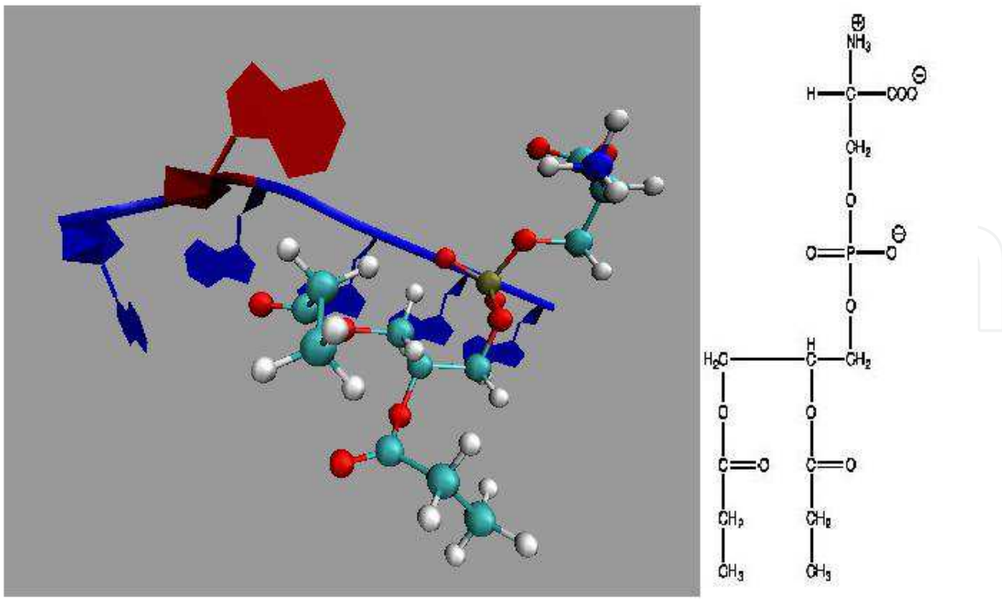

Fig. 1. The tertiary structure (on the left) of the designed PS-aptamer I generated using VMD (Humphrey et al., 1996) and the two-dimensional structure (on the right) of PS are shown. Note adenine is colored by blue and guanine is red.

Two liposomes were prepared in order to study both the designed PS aptamer binding affinity and specificity. The first one uses DPPC and DPPS at a 10:1 DPPC/DPPS molar ratio and the second one uses DPPC alone. In both cases cholesterol exists at the DPPC/cholesterol molar ratio of 2:1. The latter served as a control without PS available for binding. The results are shown in Fig. 2. The low fluorescent level shown in the right-hand panel indicates that the PS-aptamer II binds either poorly or not at all to DPPC. Conversely, the left–hand panel shows a relatively high fluorescence level while DPPS is present. It suggests that both DNA aptamers bind specifically to DPPS. Second, although both DNA aptamers bind to DPPS, the results show that the PS-aptamer II has a higher fluorescence level than the PS-aptamer I when the concentrations of DNA aptamers are increased beyond 0.165 nmol. This suggests that the PS-aptamer II has a relatively better or stronger binding affinity than PS-aptamer I.

Since the fluorescence studies did not show any saturation point for both aptamers, it is definitely required to further re-engineer this PS aptamer template to enhance its binding affinity before one can consider implementing it to become a theranostic agent. Furthermore, more studies in model systems and cell lines, are required to further investigate the theranostic effects of this aptamer. Nevertheless, given an appropriate drug delivery technique such as the exosome-based drug delivery technique (Alvarez-Erviti et al., 2011), which has the ability to carry therapeutic agents including nucleic acid across the blood-brain-barrier, to deliver PS aptamer into cells, it is likely that this PS aptamer-based theranostic agent will open new possibilities in the development of a successful therapy for prion diseases.

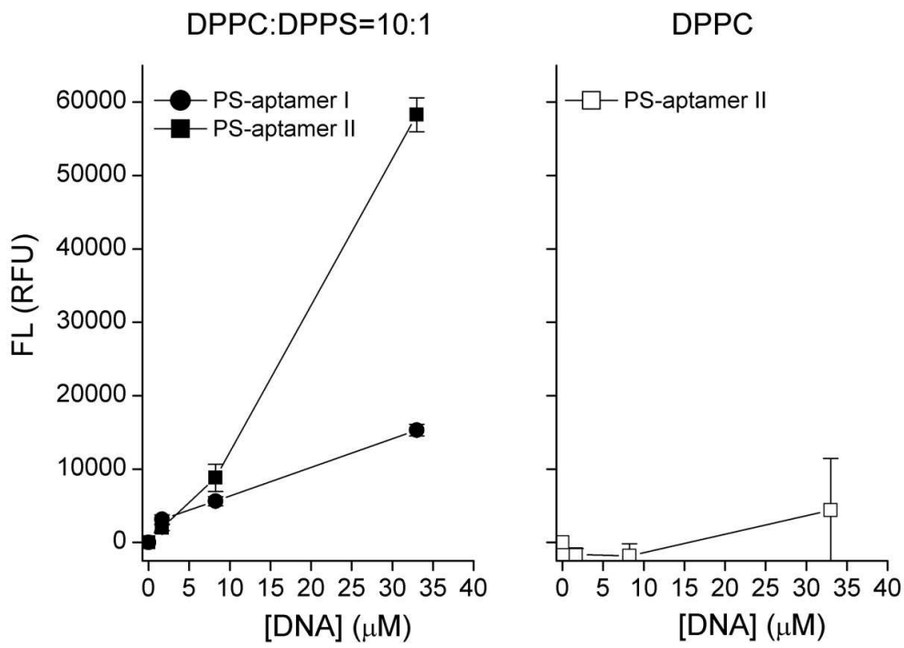

Fig. 2. Fluorescence (FL) measured in relative fluorescence units (RFU) versus DNA aptamer concentration. Left panel: selective binding of two designed DNA aptamers with liposomes containing PS. Right panel: low non-specific binding of designed DNA aptamer with liposome containing only PC.

5. Conclusions

We have reviewed current understanding of infectivity of prion diseases and recent developments in the area of diagnosis and therapeutic strategies involving various types of biochemical and chemical agents. In particular, we discussed the advantages and challenges involved in three tools, genetic (codon 129), P14-3-3 in the cerebrospinal fluid and EEG analysis, in diagnosis and a widely investigated treatment, immunotherapy. Based on these insights into the search for a detector to diagnose the onset of prion diseases and treatments aimed at either stopping prion conversion or PrPSc aggregation, a prion conversion factor, PS, has recently attracted our attention. In those studies, PS has been shown to increase PrPSc aggregation. This aggregation then activates neuronal cell apoptosis, which in turn triggers PS externalization as an early marker. We argue that these two discoveries may suggest a potential theranostic target.

An aptamer-based theranostic approach targeting PS is proposed here. Based on our recently proposed aptamer design algorithm, a promising result in designing a specific PS binding aptamer is demonstrated. A series of wet lab studies will be designed to validate and implement this proposal including both imaging of apoptosis events and inhibition of PrPSc aggregation. Finally, given this PS DNA aptamer template, we hope this proposal will trigger a ripple effect in the research community and lead to a solution which will assist us in diagnosing and preventing prion diseases.

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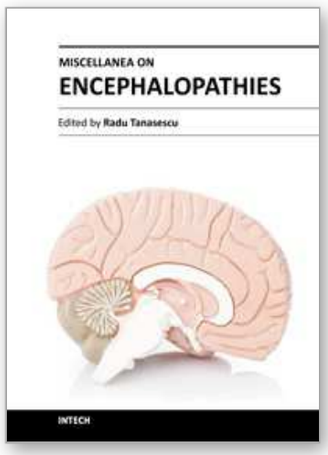

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