Searching for Factors Involved in Misfolding of the PrPc via In-silico Techniques

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Background and Motivation

Prion protein misfolding is responsible for the Transmissible Spongiform Encephalopathies (TSE) [1]. Experimental investigations suggest that the pathogenesis of prion diseases is characterized by the unfolding of normal prion (PrP0) followed by misfolding into an infectious scrapie isoform (PrPSc) [2].

We present novel in-silico results that identify potential factors that predispose PrP0 to misfold into PrPSc using computational methods. In-silico exploration of possible factors involved in the misfolding process is a crucial first step in understanding (and eventually treating) TSEs, as the number of possible factors is likely astronomical. In-silico techniques identify candidate factors for further wet-lab investigations, providing an enormous gain in efficiency compared to manual wet-lab searches through all the possible factors.

Methods and Results

Our research is based on a novel approach that contrasts mammalian and non-mammalian PrP proteins. Unlike mammalian prions, non-mammalian prions do not cause prion diseases. We have undertaken a systematic comparison of mammalian vs. non-mammalian PrP sequences (hereafter “the contrasts”), with a focus on the C-terminus domain. We used an array of computational techniques including multiple sequence alignment, exchange group similarities, feature selection methods and the AGADIR [11] helix-stability function.

Alignment based analysis

The findings of our analysis are shown as *H187Y, V189I, T191P, and V210I* with an underline in the above figure and details are given below:

- Alignment method identified six distinct point mutations that differentiate the contrasts: P137M, D144P, H187Y, V189I, T191P, and V210I.

- V210I, which is associated with CID in humans, was identified using the alignment.

- Exchange groups (conservative replacement through evolution) with identified five distinct point mutations that differentiate the contrasts: E1,137E, E1,42E, E1,44E, E1,83E, and E1,87E.

- Feature selection method identified that Lucine and Tryptophan residue positions (i.e. entries in the composition moment vector) also differentiate the contrasts.

- Positions 145 and 208 were identified as significant locations; from the literature, mutation 145VSG associated with GSS, and E200K is associated with CID.

Helix stability based analysis

Our analysis shows that:

- Helix stability of helix-1 is very low and also higher in mammalian prions than in non-mammalian prions.

- Helix stability of helix-2 is lower compared with helix-1 and helix-3 in mammalian prions. In addition, stability of helix-2 is more than twice as high in non-mammalian vs. mammalian prions.

- Helix-2 is much less stable compared with helix-3 and thus is likely to be the most susceptible to form β-sheets in the PrPSc.

Conclusions and Future Work

Our results, which were compared with previously reported research, confirmed some existing findings. Additionally, we uncovered several new hypotheses:

- Analysis with the use of exchange groups and alignment revealed three potential mutations: P137M, D144P and H187Y. The residues on these positions are conserved and their exchange groups are different between mammalian and non-mammalian PrP, even though relatively low sequence homology among the non-mammalian prions.

- Stability of helix-2 and helix-3 are very low compared with helix-1 in both mammalian and non-mammalian prions. In addition, helix-2 is much less stable compared with helix-3 and thus is likely to be the most susceptible to form β-sheets in the PrPSc.

- Using feature selection methods, we found that the absence of Lucine in helical regions of mammalians is also a major distinction from the non-mammalian prions.

- All of these new findings are candidate factors for conformational change from PrP0 to PrPSc. They are a new set of hypotheses that should be investigated via wet-lab experimentation or (at a minimum) molecular dynamics simulations. We plan to extend this research by applying multi-point mutation analysis and development of a method for prediction of the most likely β-sheet locations in the PrPSc.

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