



Research Article

Computational Profiling of the Interaction Between Prion and SOD1

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Abstract

Prion and SOD1 are implicated in a number of cascades of cellular reactions. Though prion is presumed to have multiple cellular functions, the absence of a crystallized form of mutant prion protein and the challenge in finding therapeutic options intimidates the scientific community from disseminating absolute picture of prion protein and prion diseases pathomechanism. In the same way, SOD1 is a critical protein that involves in a many cellular functions. SOD1 is mainly known for its active role in cellular oxidative stress. Superoxide dismutase activity, metal and protein binding and free radical detoxification are of well characterized activities of SOD1. Both proteins are highly expressed in brain regions specifically in frontal cortex (BA9), Cerebellar hemisphere, NA (Basal ganglia), cerebellum and the like. These proteins assumed to synergistically work in protecting the cell from oxidative stress. Prion binds and provides copper to SOD1 to facilitate the removal of free radicals. Here, ClusPro Server was used to predict the protein-protein interaction between SOD1 and PrNP. Based on ClusPro outputs and free energy values, model 0 was considered as the best possible interaction model of SOD1 and PrP. PyMol Interface Residue script was also used to filter and find all the presumable residues both in SOD1 and prion. Accordingly, some domains of SOD1 were identified to actively interact with prion. Therefore, it is dare to conclude the presence of close connection between prion and SOD1 beyond just functional interaction.

Keywords

Computational; Disease; Interaction; Prediction; Prion; SOD1

Introduction

A number of proteins interact with other proteins to give rise to vital biological function. The interplay of proteins form signals in cellular activities both in health and disease. Likewise, Prion and SOD1 are implicated in a number of cascades of cellular reactions. So far, there is no certainty in defined biological functions of Prion. However, many in vivo and in vitro studies showed that cellular prion involves in many signaling processes[1] such as programmed cell death, removing radioactive oxygen species, neural development, myelination, and neural synaptic potentiation[2-6]. At the same time, the mutated/ truncated form of Prion protein is discovered to cause fatal neurodegenerative diseases. Among the known prion-related neurodegenerative diseases CJD is relatively widely studied lethal disease [7-9]. Other forms of prion disease are autosomal dominant

familial diseases which are equally debilitating [10]. Despite it is rare; there are a number of reasons why prion disease needs a great deal of attention. Among the many reasons the knowledge gap in defining the exclusive cellular function of the Prion protein, the absence of a crystallized form of mutant prion protein and the challenge in finding therapeutic options intimidates the scientific community from disseminating absolute picture of prion protein and prion diseases pathomechanism. Similarly, SOD1 is a critical protein that involves in a number of cellular functions. SOD1 is known for its active role in cellular oxidative stress. Superoxide dismutase activity, metal and protein binding and free radical detoxification activities of SOD1 are well characterized[11,12]. A number of mutations were implicated in the SOD1 genes which are related to the commonest motor neuron disease called ALS[13,14]. A study reported that there was no CCS gene and ALS [15].

The misfolds of proteins such as prion and SOD1 are the principal cause for neuronal death which causes many clinical neurodegenerative diseases[16,17]. Despite there are unlimited sources of information and significant number of studies concerning Prion, SOD1, and diseases caused by the respective proteins, the physical interaction of Prion and SOD1 is not yet clearly established. The primary factor that triggers for the formulation of the hypothesis as the existence of bilateral interaction of Prion and SOD1 is their roles in lethal neurodegenerative disease especially motor impairment. Besides, their colocalization and tissue-specific expression support the logical assumption that these proteins may physically interact. Moreover, previous studies showed that the change in expression level and/ or up-regulation and down-regulation of SOD1 in Prion knockouts and knock down experiments. However, similar studies reported that the expression level of SOD1 doesn't affect by the Prion knockout[18,19]. Thus, in this computational study, we tried to triangulate data from different data sources to establish strong evidence that the two proteins are physically interacting intracellularly to perform a given biological function.

Material and Methods

Data sources

NCBI, genecards and Psort databases were particularly used to identify different motifs of the secondary structure of PrP and SOD1 protein. The bulk information about tissue specific expression of proteins of interest was extracted from GTEXportal. Wolfpsort online data server was principally used as a source of information related to subcellular localization of the proteins along with other cited sources. Protein Data Bank, ClusPro 2.0 Server, PyMol and PyMol script tools were also used in the stepwise docking process of PrP and SOD1.

The docking analysis and selection of interface residues

To predict the protein-protein interaction between SOD1 and Prion the ClusPro Server, a widely used tool for protein-protein docking analysis, was used. The relevant proteins were identified from Protein Data Bank (SOD1=6F6P, Prion=5YJ5) and uploaded to ClusPro 2.0 Server. ClusPro 2.0 works based on PIPER docking algorithm and various formulas to predict the best interaction model of proteins of interest as described before[20,12]. According to

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ClusPro outputs and free energy values, model 0 was considered as the best possible interaction model of SOD1 and prion that assumed to satisfy the finest docking model. PyMol Interface Residue script was used to filter and find all the involving residues both in SOD1 and Prion and presented as follows.

Result and Discussion

Prion and SOD1 proteins structural overview

Prion protein is expressed by the PRNP gene located on 20 p13 containing 2 exons where the second exon contains coding sequences. In human prion proteins consists of 253 amino acids. A cellular Prion constitutes an N terminal signaling peptide, octarepeat and a cytoplasmic tail [21]. The cellular PrP secondary structure is made of 1 sheet, 2 Beta hairpin, 2 Strands, 4 Helices, 4.Helix-Helix interacts, 2 Beta turns, 3 Gamma turn and 1 disulphide bond while SOD1 is consists of 1 Beta Sheet, 4 Beta Hairpins, 3 Beta Bulges, 8 Strands, 1 Helix, 13 Beta Turns,1 Gamma Turn,1 Disulphide[22]. The possible cleavage sites of PrP and SOD1 are between 22/ 23 and 17/18 respectively. The C-terminal peptide is located inside the cell in prion[23].Additionally, prion protein undergoes multiple posttranslational modifications such as glycosylation at multiple positions[24].

SOD1 is a 154 amino acids length protein expressed by the SOD1 gene located on 21q22.11 which consists of 5 exons. It is known for its enzymatic activities. SOD1 seems to have no N terminal signal peptide [23] Like that of Prion, SOD1 is also undergoing several posttranslational modifications (PTM). Major PTMs of SOD1 are Palmitoylation, Succinylation, Ubiquitination and Glycosylation that makes SOD1 specifically adopt multifunctional catalytic property[24].

Tissue-specific expression of SOD1 and Prion

Both proteins are highly expressed in brain regions specifically

in frontal cortex (BA9), Cerebellar hemisphere, NA (Basal ganglia), cerebellum, ACC (BA24), Putamen (Basal ganglia), Hippocampus, amygdala, and substantiate nigra (Figures 1 and 2). Despite Peripheral nervous system is more enriched in Prion, there is also a high expression of SOD1 [25]. The localization of both proteins in such specific regions makes Prion and SOD1 the best candidates to potentially interact with each other. Moreover, such brain regions are often known for their motor function i.e. highly populated with motor neural cells which are involved in involuntary movements [26,27]. As a result, neuronal lesion in such areas would cause motor impairments [28]. It is confirmed that the misfolding of both proteins cause motor derangements independently [29,30].Thus, it may be possible to speculate that both of these proteins are actively involved in each other's functions especially in maintaining normal motor neural activities.

Subcellular localization of SOD and Prion

In the above descriptions, Prion and SOD1 are expressed in specific brain regions. Both Prion and SOD are predicted to localize in a number of cellular organelles (Figure 3). However, based on the physiochemical nature of the Prion and SOD1 their localization was predicted to be mainly cytosol and extracellular space respectively. These proteins assumed to synergistically work in protecting the cell from oxidative stress. Prion binds to and provides copper to SOD1 to facilitate the removal of free radicals [31-33]. This is one of the evidences that Prion and SOD1 have strong bilateral functional interactions. Despite they are not evenly concentrated in the same organelle; the presence of both proteins in some particular subcellular organelle and spaces could create a room for the proteins to interact on demand.

Predictive interaction of Prion and SOD1

Protein-protein interactions are crucial for resolving cellular function and organization. To understand biological process and

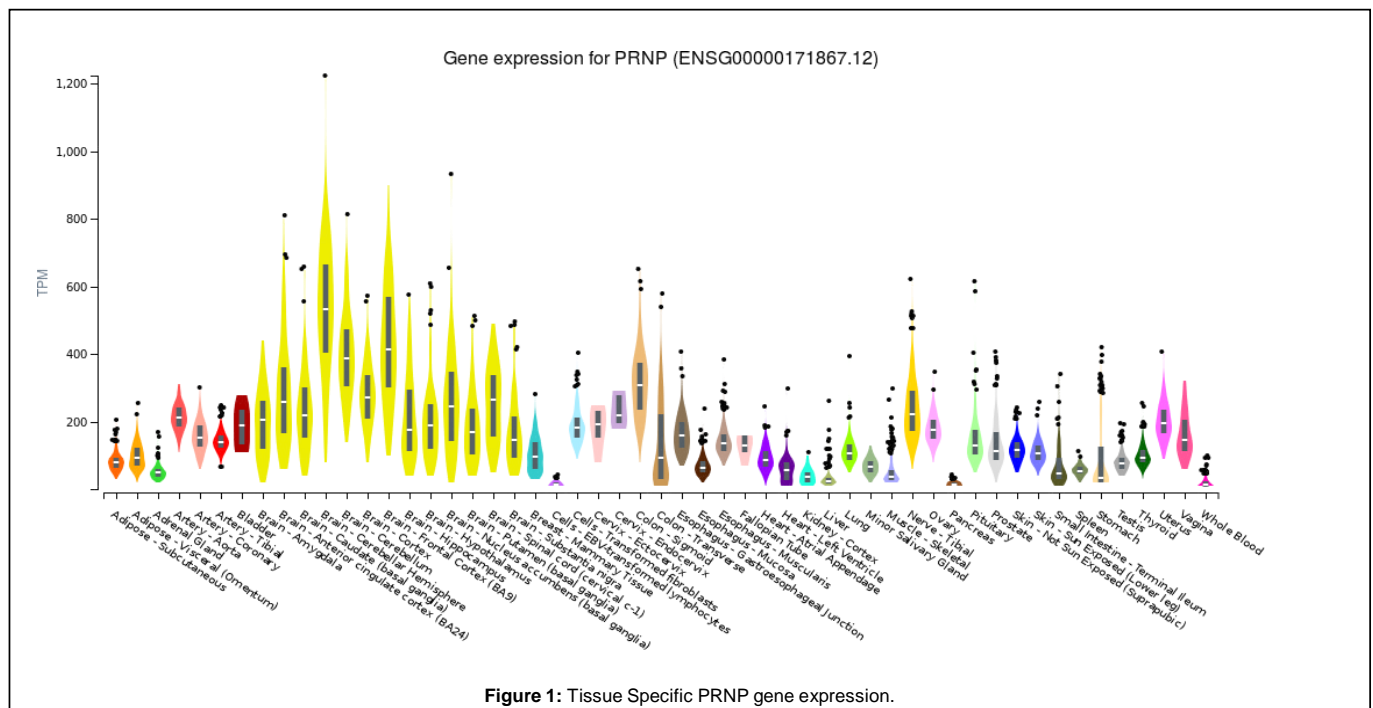
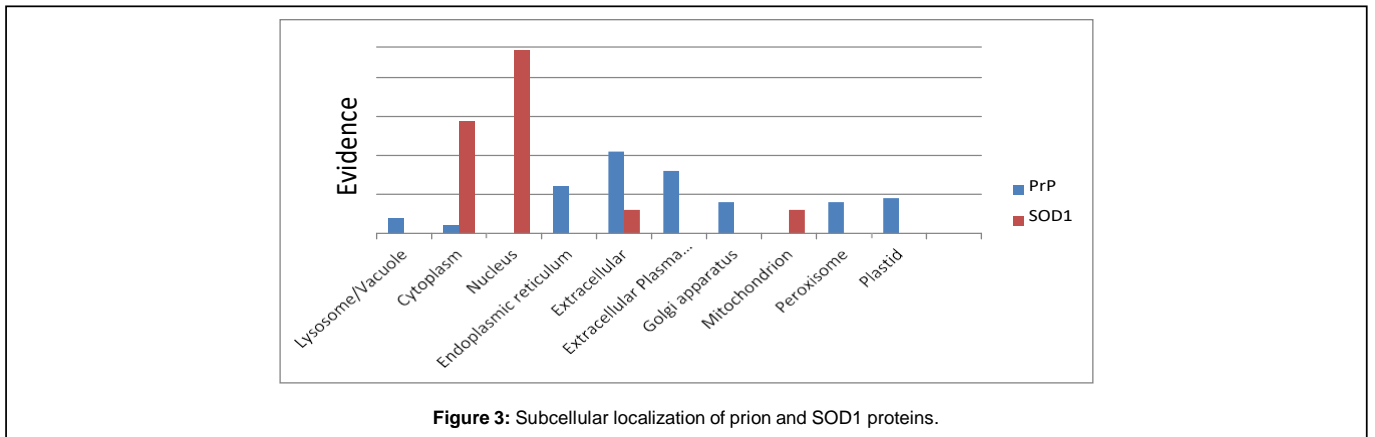
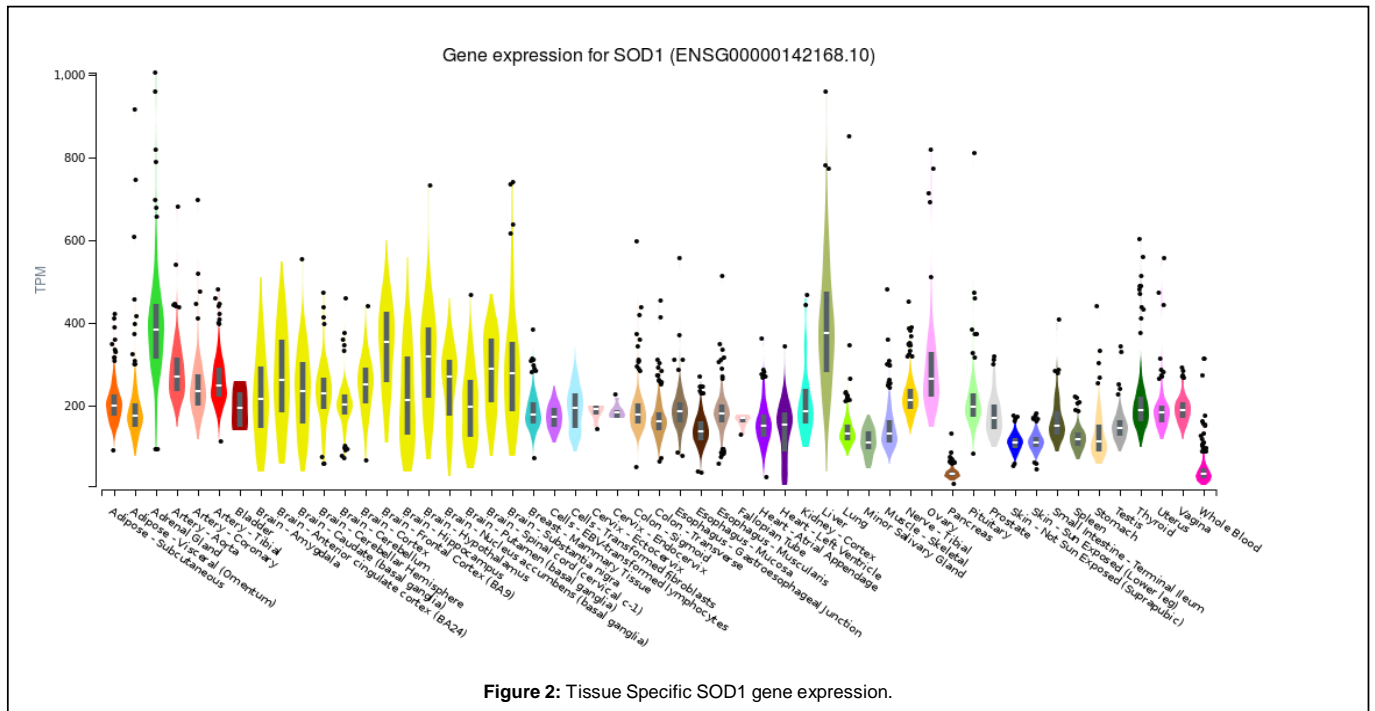


Figure 1: Tissue Specific PRNP gene expression.



pathophysiology of diseases, it is worth considering the relationship between molecules. Although there are many in vivo approaches to resolve interaction puzzle of diseases pathways which are mostly carried in wet lab procedures, there are various technical limits to discover and follow up each molecule’s pathway and interactive. Despite the current state of the art technology, it is still challenging to create environment that absolutely mimic the natural complex of proteins at a certain physiological condition. Thus, computational docking methods provide clean, easy and tremendously big data about the most possible docking model of two molecules. Here, by utilizing several bioinformatics techniques, we aim to provide dataset for further wet lab procedures.

In this study, we found and listed the critical interacting positions which are taken as best candidates for the predicted interaction under Table1. Chain A of PrP is multi-interacting with Chain D, E and F of SOD1 respectively. Beside listed amino acids which are involving interaction, Figures 4 and 5 shows us interaction map of those two molecules. Only three chains of SOD1 were interacting with PrP, with

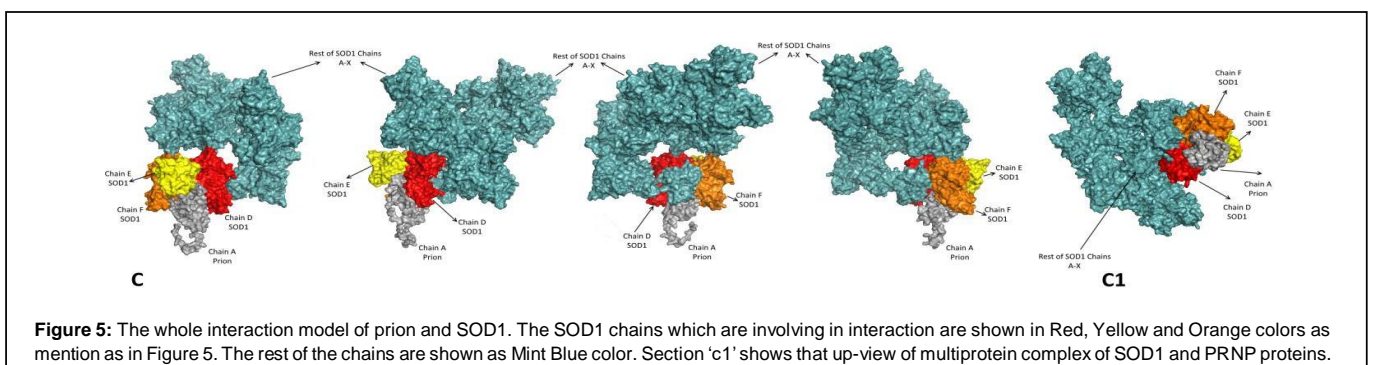
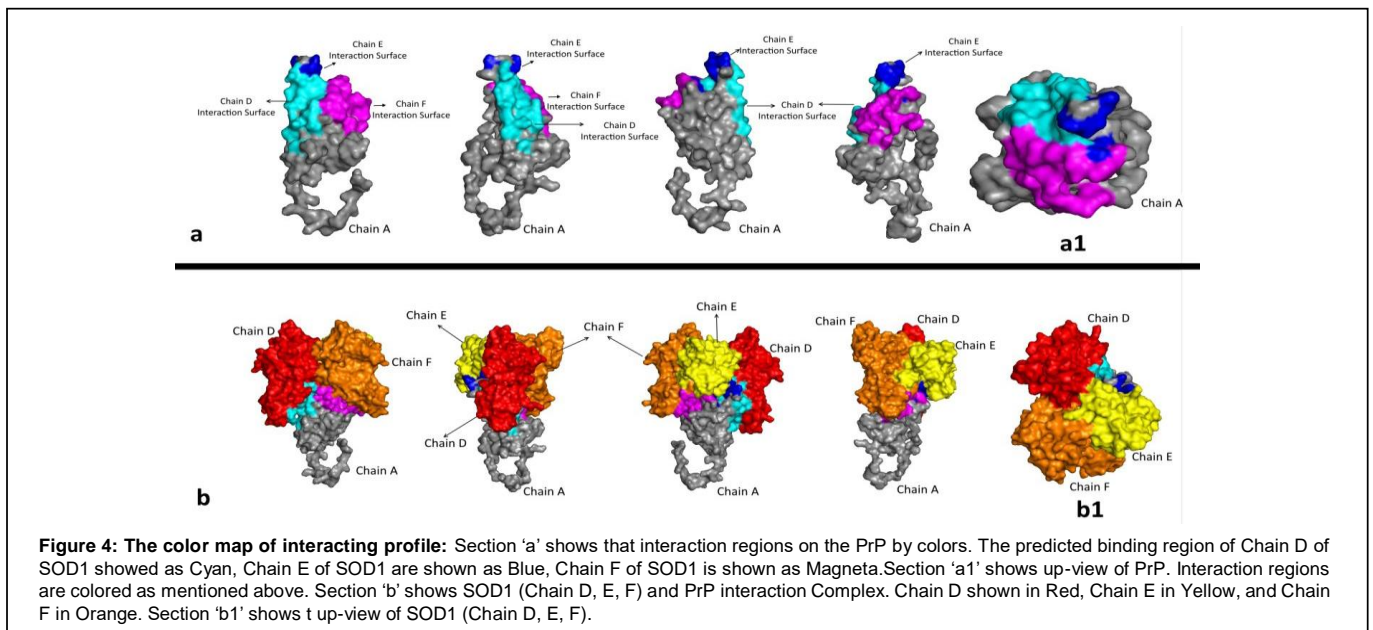
a very large molecule and have number of other residues. As listed in Table 1, between 122nd to 137th amino acids of PrP are might be involving in physical interaction [34,35].

Concluding Remarks

Previous works showed that there is strong functional interaction between prion, CCS and SOD1. It was clearly justified that CCS is a principal molecule in the functional maintenance of SOD1 by providing Cu. In Parallel, the internalization of PrP is possible upon Cu binding. Such internalization facilitates the effective transfer of Cu to SOD1. Hence, the distinctive localization of PrP and SOD1 does not affect the interaction of those proteins. The above mentioned specific SOD1 domains which are predicted to interact with PrP are actively involved in oxidative reduction process. Therefore, it is dare to conclude the presence of close connection between prion and SOD1 beyond just functional interaction. Yet, it is recommended to further investigate the predicted interaction with advanced methods.

PRION	SOD1	PRION	SOD1	PRION	SOD1
Residue name/Position	Residue name/Position	Residue name/Position	Residue name/Position	Residue name/Position	Residue name/Position
MET/129	D/THR/10	GLU/168	E/PRO/62	VAL/122	F/GLU/43
LEU/130	D/LEU/11	TRY/169	E/ASN/65	GLY/123	F/HIS/45
GLY/131	D/GLN/40	TYR/225	E/PRO/66	GLY/124	F/GLU/47
SER/132	D/ASP/41	ARG/228	E/LEU/67	LEU/125	F/ASP/48
ALA/133	D/GLU/43	SER/230	E/SER/68	GLY/126	F/MET/50
MET/134	D/HIS/45	SER/231	E/ARG/69	GLY/127	F/GLN/82
SER/135	D/GLU/47		E/GLU/77	TRY/128	F/LEU/83
ARG/136	D/ASP/48		E/ASP/109	MET/129	F/GLN/84
PRO/137	D/LEU/52		E/HIS/110	LEU/130	F/ASN/85
MET/154	D/HIS/54			GLY/131	F/LEU/236
ASN/159	D/GLY/81			TYR/162	F/PHE/237
TRY/163	D/GLN/82			ARG/164	F/GLN/238
MET/166	D/LEU/83			PRO/165	PRO/240
GLN/217	D/GLN/84			ASP/167	F/GLN/242
ARG/220	D/ASN/85			TYR/169	
GLU/221	D/THR/109			LYS/185	
GLN/223	D/ARG/112			GLN/186	
ALA/224	D/LEU/236				
GLN/227	D/PHE/237				
ARG/228					

Table 1: Interface Surface of Chain A (Prion) and Chain D, E, & F (SOD1).



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