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Cytoprotective and toxic functions of the prion protein family

Summary of Ph.D. thesis

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Szeged
2016

Publications related to the thesis:

I. **Nyeste, A**., Bencsura, P., Vida, I., Hegyi, Z., Homolya, L., Fodor, E., and Welker, E. (2016) Expression of the Prion Protein Family Member Shadoo Causes Drug Hypersensitivity That Is Diminished by the Coexpression of the Wild Type Prion Protein. J. Biol. Chem. 291, 4473–86

II. Cingaram, P. K. R., **Nyeste, A**., Dondapati, D. T., Fodor, E., and Welker, E. (2015) Prion Protein Does Not Confer Resistance to Hippocampus-Derived Zpl Cells against the Toxic Effects of Cu2+, Mn2+, Zn2+ and Co2+ Not Supporting a General Protective Role for PrP in Transition Metal Induced Toxicity. PLoS One. 10, e0139219

Other publications:

I. Schäfer, B., Orbán, E., Borics, A., Huszár, K., **Nyeste, A**., Welker, E., and Tömböly, C. (2013) Preparation of semisynthetic lipoproteins with fluorescent cholesterol anchor and their introduction to the cell membrane with minimal disruption of the membrane. Bioconjug. Chem. 24, 1684–1697

II. Tóth, E., Huszár, K., Bencsura, P., Kulcsár, P. I., Vodicska, B., **Nyeste, A**., Welker, Z., Tóth, S., and Welker, E. (2014) Restriction enzyme body doubles and PCR cloning: on the general use of type IIs restriction enzymes for cloning. PLoS One. 9, e90896

Introduction

Prion protein

Prion protein (PrP) is ubiquitously expressed as a cell surface glycoprotein, showing the highest expression levels in neurons of the brain and spinal cord. The mature protein consists of two major regions: a globular C-terminal domain and a flexible N-terminal tail.

PrP is best known for its role in the transmissible spongiform encephalopathies (TSEs), a group of rare, incurable and fatal neurodegenerative diseases that affect several mammalian species, including humans. The infectious agent of the TSEs is mainly composed of PrP^{Sc}, an abnormal conformational isoform of the cellular prion protein, or PrP^{C}, furthermore the key event of TSE pathogenesis is the conversion of the host's own PrP^{C} molecules into the pathological PrP^{Sc} isoform where PrP^{Sc} molecules serve as template for the conversion process.

Several pieces of evidence support a hypothesis suggesting that the TSE-associated neurodegeneration is caused neither by the loss of a vital function of the PrP^{C} nor by PrP^{Sc} merely gaining a novel, toxic function, but probably by the **subversion or corruption of a non-toxic function of the prion protein** that leads to neurotoxic phenotype. Therefore investigating the numerous functions of the WT PrP and its mutants – especially those activities that participate in toxic or protective processes – might help to elucidate the role of this protein in the mechanism of neurodegeneration in TSE.

Protective activities of the prion protein

Several functions associated to the prion protein are related to protection against certain stressors. In cell culture and animal models PrP expression protected from the excitotoxicity of glutamate, kainate and NMDA. It was shown that PrP has a superoxide dismutase activity, providing protection against oxidative stress. Furthermore,

according to certain data in literature, immortalized cells of hippocampal origin expressing prion protein were more resistant to apoptosis induced by oxidative stress and also showed higher viability and lower autophagy-marker expression levels during serum deprivation.

Toxic activities of the prion protein

PrP acquires toxic properties if certain regions of its N-terminal tail is deleted. When PrP transgenes, missing a short alanine, glycine and valine-rich stretch, called hydrophobic domain (or HD), or an overlapping segment, called the "Central Region" (or CR), are expressed in mice on a PrP knockout background, the host mice develop neurodegeneration. Similarly, these mutants (called PrPΔHD or PrPΔCR, respectively) cause increased degree of cell death when expressed in primary cultures, or neuronal stem cells. Interestingly, infectious matter or PrP^{Sc} isn't formed in either model.

In immortalized cell lines no such robust effect has been detected, but a peculiar phenotype was discovered in a wide range of cells expressing such mutant PrP-s: PrPΔCR caused hypersensitivity to a handful of drugs from the antibiotic classes aminoglycosides (G418 and hygromycin), and glycopeptides (bleomycin and Zeocin) when it was overexpressed on PrP knockout background. Kinetic studies also revealed that the mutant protein increased the cellular uptake of both G418 and Zeocin. Furthermore in whole cell patch clamping experiments it was demonstrated that ΔCR and related PrP mutants induced random, spontaneous inward cationic currents.

Curiously, the presence of wild type PrP interfered with all of these toxic functions of PrPΔHD and PrPΔCR, reducing or completely eliminating the toxic effects in a dose dependent manner.

Shadoo protein

Shadow of the prion protein (or Shadoo, Sho), the latest discovered member of the prion protein family, is also expressed in the CNS and is considered as a paralog of the prion protein. Shadoo is an unstructured protein, resembling to the flexible N-terminal tail of PrP. The two proteins have similar domain organization, even though no extended sequence similarity exists between them. Both mature Shadoo and prion proteins begin with a positively charged N-terminal cluster, followed by repeated sequences (octarepeats in PrP, $(RXXX)_8$ motif in Sho and they have similar hydrophobic regions).

Shadoo shows not only structural similarity to the N-terminal region of PrP, but also functional analogy to wild type PrP. Just like WT-PrP, Shadoo is able to eliminate the toxic phenotypes of both Doppel and the Shmerling, or ΔHD mutant PrP-s in primary or immortalized cell cultures. Furthermore, akin to WT-PrP, Shadoo exhibits protective effect against glutamate-induced excitotoxicity in cell cultures emphasizing the neuroprotective feature of Sho that is also characteristic of PrP bearing an intact N-terminal part.

Whether Shadoo provides any protection against the drug hypersensitivity or eliminates the ionic currents caused by PrPΔCR has not been studied before our work.

Aims of this study

As several evidences underline the hypothesis, that a toxic or protective function of the prion protein play role in TSE associated neurodegeneration, we studied the toxic and protective effects associated to the prion protein family.

Our goal was to establish a model system where such activities of the prion protein family can be studied. We investigated experimental constructs of PrP-s in immortalized human and non-human mammalian cell lines that were either prnp knock-out or had low endogenous PrP expression.

We studied two models:

In the first model we investigated the role of the prion protein in the effects of serum deprivation on cells and tried to answer the following questions:

1. Does wild-type PrP confer any protection against serum deprivation induced apoptosis?
2. Does PrPΔCR increase the sensitivity of the cells to serum deprivation?
3. Does PrPΔCR expression decrease the viability to cells maintained in normal condition?

In the second model system we studied the PrPΔCR-induced Zeocin and G418 hypersensitivity found by the Harris-group. We postulated that overlapping protective function of Shadoo and WT-PrP from literature may have the consequence that Shadoo can also eliminate PrPΔCR-induced Zeocin and G418 hypersensitivity. Surprisingly we have found that Shadoo protein not only doesn't eliminate the PrPΔCR-induced antibiotic-sensitivity, but instead induces such sensitivity.

Based on this surprising finding have characterized further this effect of the Shadoo protein.

In this topic we tried to answer the following questions:

1. Can WT-PrP eliminate also the antibiotic-sensitivity caused by Shadoo protein?
2. Which regions of Shadoo protein is required for the sensitizing activity?
3. Similarly to PrPΔCR, does Shadoo induce hypersensitivity by increasing the initial drug uptake or by another mechanism?

Experimental methods

- The plasmid vectors encoding the various PrP and Shadoo DNA constructs that were used for the experiments were prepared using standard cloning techniques.
- Our experiments were performed in immortalized mammalian cell lines (Zpl2-1, a PrP knockout mouse hippocampal cell line; HEK293, a human embryonic kidney cell line, and SH-SY5Y, a human neuroblastoma cell line)
- We established the stable expression of PrP or Shadoo constructs using either Sleeping Beauty transposon-based gene delivery system, or 3rd generation lentiviral gene delivery system. A fluorescent green (EGFP) or red (mCherry) reporter was always co-introduced into the genome along with the PrP or Shadoo transgenes. EGFP or mCherry positive cells were gathered using fluorescent-activated cell sorting (FACS).
- We have worked with non-cloned cells where the randomized sites of the integrations eliminated the potential risk of positional effects. Expression of the reporters were checked regularly by fluorescent microscopy and when needed populations were re-sorted using FACS. Expression of PrP and Shadoo transgenes were checked using immunoblotting.
- In the serum deprivation model, after 2 or 3 days of serum deprivation the ratios of dead, early apoptotic and living cells were quantified in the whole population using annexin-V (marker of early-apoptotic cells) and 7-aminoactinomycin-D (7-AAD, marker of late apoptotic and necrotic cells) staining with flow cytometry.
- In the drug hypersensitivity model we measured the viabilities of the PrP or Sho transgene expressing cells after 48 hours treatment with various drugs (Zeocin, G418 or puromycin), using MTT or PrestoBlue assays.
- The initial Zeocin uptake was measured by visualizing the Zeocin-induced double stranded breaks after an hour of Zeocin treatment. Positive nuclei were quantified using High Content Screening.

Results

1. We established the stable expression of various PrP and Shadoo transgenes and fluorescent reporter genes in an immortalized mouse (Zpl2-1) and two human cell lines (SH-SY5Y and HEK293). The expression of the transgenes and the reporter genes were linked, which allowed us to study the activities of untagged proteins.
2. In the serum deprivation experiments, after the exclusion of early apoptotic and necrotic cells using annexin-V and 7-AAD staining, we detected no higher viability in cells overexpressing wild type PrP compared to parental PrP knockout cells. While the stable expression of PrPΔCR caused no lethal phenotype, the presence of the mutant PrP transgene slightly sensitized Zpl2-1 cells to the effects of serum deprivation.
3. In the drug hypersensitivity model we found that Shadoo – unlike in other PrPΔCR/PrPΔHD toxicity models, where it acts as a wild type PrP analogue – provided no protection against the increased drug sensitivity caused by PrPΔCR. This lack of protection was not caused by a relatively lower Shadoo expression level compared to PrPΔCR. We measured this phenomenon in multiple (SH-SY5Y and HEK293) cell lines.
4. Instead, in this model we detected that Shadoo behaved as a PrPΔCR-analogue. It also increased the sensitivity of the cells to Zeocin and G418, but not to puromycin, similarly to PrPΔCR. The severity of the drug hypersensitivity was related to the expression level of Shadoo.
5. We found that the co-expression of wild type prion protein (WT PrP) with Shadoo eliminated the drug hypersensitivity caused by the latter, similarly as PrP did to the same activity of the PrPΔCR.

6. After one hour of Zeocin treatment we found that Shadoo increased the initial Zeocin uptake of cells, similarly to PrPΔCR. However this effect was less pronounced in Shadoo-expressing cells than in PrPΔCR-expressing ones.
7. We found that the deletion of the N-terminal $(RXXX)_8$ domain of the Shadoo or the substitution of the arginines in this region with glutamines eliminated the drug sensitizing activity of the protein completely, which suggested that the $(RXXX)_8$ domain bears an essential role in this function of the Shadoo.
8. The deletion of the hydrophobic domain (HD) of the Shadoo protein didn't eliminate the drug hypersensitivity caused by Shadoo, but the substitution of the Shadoo HD with PrP HD interfered with the drug sensitizing activity of the Shadoo. These findings suggest a non-essential but modulatory role of the HD in the drug sensitizing phenomenon.

Conclusions

Serum deprivation model

1. We detected no protection provided by WT PrP against the apoptosis caused by serum deprivation.
2. While PrPΔCR expression wasn't lethal or toxic in Zpl2-1 cells, it made the cells slightly more sensitive to serum deprivation than control cells were.

Drug hypersensitivity model

3. We found that Shadoo induces hypersensitivity to Zeocin and G418 but not to puromycin in cells overexpressing the protein.
4. Our results suggest that the mechanisms behind Shadoo and PrPΔCR-induced drug hypersensitivities are closely related.
   4.1. Shadoo and PrPΔCR sensitizes cells to identical antibiotics (Zeocin and G418 but not puromycin) in a dose dependent manner.
   4.2. Wild type PrP interferes with the G418 and Zeocin hypersensitivity caused by both Shadoo and PrPΔCR in SH-SY5Y and HEK293 cell cultures.
   4.3. Shadoo increases the initial drug uptake of the cell akin to PrPΔCR but with a lower efficiency.
5. We identified a region of the mature Shadoo protein, the $(RXXX)_8$ domain whose presence is essential for the drug-sensitizing activity of the Shadoo protein.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor, Ervin Welker DSc for the guidance he provided me and for teaching me the importance of controls and what it means to be a scientist. I would also like to thank him for his patience and encouragement during my PhD years and for providing opportunity to work in his group.

I am most grateful to Petra Bencsura, György Várady and István Vida, for without their skill and invaluable assistance this story would be much shorter.

I am thankful for Katalin Német and her group for the assistance they provided with the lentiviral work. Using lentiviruses instead of transfection saved me from going prematurely grey.

I am grateful to Nóra Kucsma and Elfrieda Fodor for offering advice and helping hand when I was in need.

I would like to give my special thanks to Ervin Welker, Ágnes Telbisz, Szilvia Pravda and Eszter Tóth for the patient and critical reading they provided, their help was invaluable in turning this into the thesis you now hold.

And last but not least, would like to thank my past and present colleagues, fellow students, family and friends whose support helped to overcome the hardships of Life and Science.