PROJECT PROPOSAL for applicants for Ph.D. fellowships

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PROJECT SUMMARY
In the last year, our group investigated the neuroprotective role of a small heat shock protein, Hsp27. We have generated transgenic mice overexpressing the human Hsp27 protein and crossed them with APPswe/Pse1dE9 mouse strain, a validated model of Alzheimer’s disease (AD). Our results have shown that overexpression of Hsp27 protein might ameliorate certain symptoms of AD and open new avenues for therapeutic purposes. In the forthcoming years our aims are to reveal the molecular nature of the protective mechanism of Hsp27 protein in endothelial dysfunction, amyloid clearance and to study the membrane-lipid related pathophysiological alterations in AD model and ApoB-100 transgenic mice overexpressing human Hsp27.

BACKGROUND OF THE STUDY
Currently almost 30 million people are diagnosed with AD worldwide, with nearly 5 million new cases annually. As the disease lasts for several years and the population of world is continuously aging this neurodegenerative disease constitute a serious health problem for our societies. In the US direct health care of AD patients cost 200 billion USD in 2013. To date there is no cure for AD and the origin and development of this neurodegenerative disease are not completely understood. What is known, that the disease is accompanied by the formation of amyloid plaques and hyperphosphorylation of tau protein in the cortex and hippocampus, leading to axonal degeneration and neuronal loss. Eventually, these pathological changes are manifested in memory loss and dementia.

A subset of heat shock proteins (Hsps) is molecular chaperone that can be found in every organism and in almost all cell types. Under normal conditions they have an essential role in biosynthesis, transport, translocation, folding and assembly of other proteins as well. Moreover, they have protective role in apoptosis and oxidative stress. Several studies demonstrated that Hsp27 was upregulated during brain injury and diseases, for example in Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS) and also in acute inflammatory conditions such as stroke and epilepsy. Hsps are grouped according to their molecular weight. Hsp27 belongs to the small heat shock protein (sHsp) family, which are ATP-independent chaperones.

RELEVANT RESEARCH IN THE HOST LABORATORY
In the last year we have investigated the neuroprotective effect of a small heat shock protein, Hsp27. We generated a transgenic mouse strain overexpressing the human Hsp27 protein and crossed with APPswe/PS1dE9 mouse strain, a mouse model of Alzheimer’s disease (AD). One of the main characteristic of AD is the development of great number of amyloid plaques in the hippocampus and cortex. We counted significantly less amyloid...
plaques in the brain of AD model mice overexpressing Hsp27 compared to AD model mice. Using electrophysiological recordings we found that excitability of neurons was significantly increased and long-term potentiation (LTP) was impaired in AD model mice. By overexpression of Hsp27 these impairments were restored in triple transgenic mice. Finally, using a set of different behavioral tests, we found that spatial learning was impaired in AD model mice, however it was rescued by Hsp27 overexpression. These results suggest that overexpression of Hsp27 protein might ameliorate certain symptoms of AD and open new avenues for therapeutic purposes.

Behavioral tests (Barnes maze and Morris water maze) in APPSwexPse1 and APPSwexPse1xHsp27 transgenic mice

In another set of experiments we have also shown, that transgenic mice overexpressing the human ApoB-100 protein are hyperlipidemic and hyperlipidemia induces hyperphosphorylation of tau proteins (primarily at Ser262, Ser396, Ser199/202, Ser404 phosphosites), which in turn results in disruption of the neurotubular network and leads to the degeneration of neurones. Using electrophysiological recordings we demonstrated impairment of synaptic and cognitive functions in the hippocampal region of transgenic mice. We detected widespread degeneration and apoptosis of cortical and hippocampal neurons of 7 month old transgenic animals. These investigations strengthen our hypothesis that chronic hyperlipidemia might lead to the development of neurodegeneration.
Recently, we have found that transgenic mice overexpressing the human ApoB-100 protein showed increased permeability of the blood-brain barrier (BBB), oxidative stress and endothelial dysfunction in transgenic brains. Currently, we are investigating whether overexpressed Hsp27 can protect endothelial cells and prevent endothelial dysfunction in ApoB-100 transgenic mice.

SPECIFIC AIMS

In the forthcoming years our aim is to reveal the molecular nature of the protective mechanism of Hsp27 in a) endothelial dysfunction (by permeability studies, measurement of gene expression level of tight junction and inflammatory proteins, detection of apoptosis in endothelial cell), b) amyloid clearance (by perivascular detection of amyloids in different brain regions, monitoring the gene expression level of Aβ transporters and receptors) and c) membrane lipid-related pathophysiological alterations, using AD model and ApoB-100 transgenic mice overexpressing the human Hsp27 protein. Furthermore, we plan d) to monitor changes in the membrane-lipid composition during the protective mechanism of Hsp27 protein e) to investigate lipid modifications after docosahexaenoic acid (DHA) supplementation in transgenic brains f) to analyze the expression levels of other Hsp chaperones (especially Hsp70 and sHsps) in transgenic mice. We presume, that identification of small hsp inducer molecules are particularly important and we plan to monitor their effect in mouse models of neurodegenerative diseases.

MATERIAL AND METHODS

- Mouse genotyping using PCR

To study neuroprotection in multiple transgenic mice using:

- Immunohistochemistry
- Amyloid plaque-stainings
- Protein isolation and Western blottings
- Quantitative protein identification using ELISA
- RNA isolation and real-time QPCR
- Isolation of primary cell cultures derived from transgenic mice

SUGGESTED READINGS

Representative recent research grants
“Dementia, neurodegenerative diseases: early diagnosis, pathomechanisms and identification of new therapeutic targets” (TÁMOP, 2012-2014)
„The role of Hsp27 in neurodegenerative diseases from the aspects of membranes and lipids” (OTKA NN, 2014-2017)

Some of the latest students in the laboratory
Lénárt N, Ph.D., 2009-2014; “ApoB-100 induced hyperlipidemia and neurodegeneration”
Csibrány B, PhD, 2011-recent; “The cell protective role of Hsp27”
Nagy D, M.Sc., 2013- recent; “Hyperlipidemia induced endothelial dysfunction”
Dukay B, M.Sc., 2013- recent; “Neuroprotective role of Hsp27”