LOCALIZATION OF UBIQUITIN-PROTEASOME SYSTEM COMPONENTS AND THE HSP72 IN CONTROL AND NEURODEGENERATIVE BRAINS

PhD theses

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Made in the Department of Anatomy- Cell and Developmental Biology, ELTE TTK

2007 Budapest, Hungary
1. INTRODUCTION

In the developed countries, in parallel with the increasing of average age and the aging of the society, neurodegenerative diseases with serious damage of central nervous system (CNS) like Alzheimer and Parkinson diseases cause elevated number of medical, social, moreover economical problems. Besides of the great volume of selective neuronal loss in the CNS, the disturbance of cellular protein-homeostasis is typical in these disorders: proteins with abnormal conformation and susceptible to aggregation accumulate in extra- or intracellular protein inclusions. That is why these syndromes are often called „conformational diseases / foldopathies / folding diseases” or recently „ubiquitin catabolic disorders” and both the ubiquitin-proteasome system (UPS) and molecular chaperones take important part in their pathogenesis.

Molecular chaperones are proteins that bind to and stabilize an otherwise unstable conformation of another protein. Through controlled binding and release, they facilitate its correct folding, oligomeric assembly, transport to a particular subcellular compartment and disposal by degradation. The 26S proteasome (PS) is a multicatalytic protease complex found in all eucaryotic cells. Its main function is the degradation of misfolded, damaged and short-lived proteins marked with the small molecular weight ubiquitin. The function of the two molecular systems is antagonistic: molecular chaperones help to protect proteins but UPS degrades damaged or currently unnecessary proteins. However, the work of the two systems serves one final aim: to support the cellular protein homeostasis. This is also proved by the close cooperation between them: if molecular chaperones unable to refold the native conformation of a damaged protein they pass it to the proteasome.

Despite both molecular chaperones and the UPS are in the focus of the molecular cell biology research we do not know comprehensive studies about the \textit{in vivo} cellular and regional distribution of the Hsp72 molecular chaperone and certain components of the UPR either in case of control or neurodegenerative brains. In my PhD work our aim was to localize the Hsp72 and several components of the UPS on light and electron microscopy level. We used perfusively fixed rat brains and \textit{post mortem} samples from control and neurodegenerative brains. Neurodegenerative samples derived from Alzheimer and Creutzfeldt-Jakob diseased (CJD) brains – both of them are conformational disorders. Alzheimer disease is the most common neurodegenerative syndrome. CJD is related to prion diseases that are unique because their transmissibility. Their etiology and molecular pathology are interesting also for basic research.
2. AIM OF PhD WORK

- To test the specificity of antibodies and to introduce epitope retrieval methods with which we would be able to localize several UPS-components on rat brains and control and neurodegenerative human brains.

- To localize ubiquitin, 20S proteasome and 19S RC ATPases in control human and rat brains.

- To localize the Hsp72 molecular chaperon, ubiquitin, 20S proteasome and 19S RC ATPases in AD and CJD brains. To compare the regional and cellular localization patterns of these proteins with the distribution of pathologically affected neuronal populations.

3. METHODS USED

- Cell fractionating and Western blotting

- Light microscopy immunohistochemistry

- Fluorescent double labeling and confocal microscopy

- TUNEL-method (in situ cell death detection)

- Postembedding immuno-electronmicroscopy

- Morphometric and statistical methods
4. MAIN NEW RESULTS

- We have introduced epitope retrieval methods with which we were able to localize several UPS-components on rat brains and control and neurodegenerative human brains.

- According to our immunohistochemical analysis, the 19S RC ATPases S4 and S7 localize mainly in the nucleus whereas 19S RC ATPases S6a, S6b and S10b localize mainly in the cytoplasm both in human and rat brain samples (subunit S6a also localizes in some nuclear bodies, see next item). However, the intracellular distribution of S8 subunit alters in the two species: it is mainly nuclear in rat brains and predominantly cytoplasmic in human brains.

- In rat brain a subset of nuclear bodies is immunoreactive for ubiquitin, 20S proteasome and S6a both in neurons and glial cells.

- The intensity of immunostaining for ubiquitin, proteasomal subunits, and Hsp72 varies in different anatomical regions both in diseased and control brains. Areas with weaker immunolabeling correspond to affected areas in CJD and AD.

- According to the semiquantitative score, the protected cerebellar Purkinje cells exhibit stronger Hsp72 immunoreactivity in CJD cases than in controls. We have not seen such an effect in case of UPR-components.

- In diseased cases, antibodies for 20S, S4, S6b, S7 and ubiquitin intensely immunolabel neuronal nuclei of vulnerable cells in affected areas.
5. CONCLUSIONS

- Prominent nuclear immunolocalization of members of the ubiquitin-proteasome system gives further morphological evidence of the additional function of proteasomal ATPases in transcription regulation and/or DNA repair.

- The UPS immunopositive nuclear bodies we describe here are strongly reminiscent of clastosomes. However, our results suggest, that these structures are widespread in glial cells and in many types of neurons in rat brain even in animals without any pretreatment.

- We demonstrate that immunostaining intensity of ubiquitin, proteasomal regulatory ATPases, and the inducible stress protein, Hsp72, varies among regions in a similar manner in non-diseased, AD and CJD brains. Cell populations where the intensity of immunostaining for these proteins is higher in both control and disease cases are more protected against cell death in disease cases. By contrast, neuronal populations where the signal intensities are lower in control cases overlap with affected cell populations exhibiting frequent positive TUNEL-reaction in AD and CJD. These results provide further evidence for Hsp72 playing a role in neuroprotection and suggests that components of the UPS may also be involved in the process of neuroprotection in less vulnerable cells. Vulnerability of similar anatomical regions is common in biochemically different neurodegenerative diseases. Here we demonstrate that neuronal populations showing low expression of UPS and Hsp72 components might represent the "weakest-link" in the human brain being more prone to certain types of neurodegeneration (e.g. AD, CJD).

- In addition, nuclear redistribution and accumulation of UPS components may reflect their involvement in DNA repair mechanisms and/or cell death machinery in CJD and AD.
6. ACKNOWLEDGEMENT

I would like to say many thanks to the following persons who all helped me much during my PhD work:

*Dr. Lajos László & Dr. Gábor G. Kovács,*

and

*Dr. Péter Lőw,*

*Dr. Herbert Budka,*

*Dr. R. John Mayer,*

*Dr. Carlos Gorbea,*

*Dr. István Kurucz,*

*Dr. Moszkovkin Georgij,*

*Dr. Erzsébet Fellinger,*

*Dr. Ferenc Müller,*

*Dr. György Bagdy,*

*Dr. Ádám Vannay,*

*Gergő Botond,*

*Rómeo D. Andó,*

*Eszter Kirilly,*

and:

*Eszter Bíró,*

*Helga Flicker,*

*Miklósné Druskó and*  
*Zsofia Pálfia.*

Thank you to Prof. Dr. Miklós Sass to let me complete my PhD work in the Department of Anatomy- Cell and Developmental Biology, ELTE TTK. Many thanks to all colleagues in the Department of Anatomy- Cell and Developmental Biology (ELTE TTK) and in the Laboratory of Neurochemistry and Neuropsychopharmacology (National Institute of Psychiatry and Neurology, Budapest).
7. THESE TESISSES BASED ON THE FOLLOWING SCIENTIFIC PAPERS:


8. OTHER SCIENTIFIC PAPERS


### 9. MAIN POSTERS IN SCIENTIFIC CONGRESSES


Ádori Cs, Kovács GG, Lőw P, László L: Intracellular redistribution of components of ubiquitin-proteasome-system correlates with neuronal vulnerability - a study on immunolocalization of ubiquitin and proteasome; 59th Harden/EMBO Conference - The Ubiquitin Proteasome System in Health and Disease, Royal Agricultural College, Cirencester, UK; 6 - 10 September 2004.

10. LECTURES IN SCIENTIFIC CONGRESSES


Adori Cs: Immunolocalization of the components of ubiquitin-proteasome-system (UPS) in Alzheimer and Creutzfeldt-Jakob disease; BrainNet Europe International Conference, San Servolo Island, Venice, Italy; June, 2006

11. BOOK CHAPTERS


12. EDUCATIONAL PAPERS

Ádori Cs: Borrelia Burgdorferi kimutatása Sopron környékén gyűjtött kullancsokból Természet Világa, 1995. április, diákmedléklet.