

Progress report. Investigating the neuroprotective function of Hsp27

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Hsp27 is expressed in many cell types and tissues at specific stages of development and differentiation. It is an ATP independent chaperone, its main function is to bind misfolded proteins hereby block the formation of protein aggregates. The protein can protect cell against stressful stimuli through its several other properties. For example it has been shown to modulate apoptosis since it can interact with and inhibit key components of the apoptotic signaling pathway. Hsp27 can also increase the resistance of cells to oxidative injuries as it reduce lipid peroxidation, protein oxidation and f-actin architecture disruption.

In order to study the neuroprotective effects of Hsp27 *in vivo*, transgenic mouse lines overexpressing the human Hsp27 were generated. The human Hsp27 protein expressed at high level in the brain of the transgenic mice demonstrated by western blot analysis. The cellular localization of the expression of transgenic protein in the brain was detected by immunohistochemistry. We found strong expression of the transgene in the cerebellum, hippocampus and cerebral cortex.

The neuroprotective effect of Hsp27 protein was investigated in acute and chronic ethanol administration. In the acute administration studies animals received an intraperitoneal injection of 20% ethanol (2g/kg) than behavioural studies were performed to analyse motor coordination and muscle strength. Five different behavioural tests were performed and in three of the tests statistical analysis using one-way analysis of variance (ANOVA) revealed a significant difference in the performance of transgenic and wild type mice. In the inverted screen test all of the wild type animals showed ataxia compared to the transgenic group, where it was significantly less (57%) ($p=2,925E-4$). In the beam walking test 86% of wild type mice fell off the beam, while in the transgenic group only 53% ($p=0,031$). In the footprint analysis we found significant difference in the stride length between the transgenic and wild type groups ($p=0,002$ for the forelimb and $p=5,6036E-6$ for the hindlimb).

In the chronic ethanol treatment drinking water was replaced by a 20% of ethanol solution for five weeks. At the end of the treating period 10 μ m frozen sections were made from the brain of the animals. Neurodegeneration was detected by Fluoro-Jade C staining. We detected less degenerated neurons in the brain of the transgenic mice compared to wild type mice especially in the cerebellum, hippocampal region and cerebral cortex.

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The role of dADA2b adaptor proteins in dSAGA histone acetyltransferase complex

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In eukaryotes the genetic material is present in a compact chromatin structure consisting of DNA and histone proteins. Histone acetyltransferase (HAT) complexes play a role in chromatin structure modifications which might lead to changes in gene expression. The GCN5 protein is the catalytic component of several multiprotein HAT complexes which modify chromatin structure by acetylating specific lysine residues at the N-terminal tails of histone H3 and H4. Many of the GCN5-containing HAT complexes also contain ADA-type adaptor proteins, which play roles in modulating HAT activity and specificity.

In *Drosophila melanogaster* our group has described two ADA2 proteins (dADA2a and dADA2b) in two GCN5-containing HAT complexes, ATAC and dSAGA which have different histone specificities. The dADA2b-containing dSAGA complex is involved in the post-translational modification of nucleosomal histone H3 at lysine (K)9 and K14. Furthermore, analysis of the *dAda2b* gene revealed that by alternative splicing it gives rise to two mRNAs (dAda2bS and dAda2bL). Despite the detection of two different forms of dAda2b message in earlier studies, the production and function of the two protein isoforms have not been studied.

In my thesis work I have shown that *dAda2b* produces at least two protein isoforms, dADA2bL and dADA2bS during the course of development and demonstrated that dADA2b proteins are most abundant in pupae, in the developmental stage when in the absence of the proteins *dAda2b* null mutants die.

My further studies included generation of *dAda2b* mutations which eliminate the production of either or both dADA2b isoforms in order to characterize dADA2b function in dSAGA complexes. Functional studies of *dAda2b* null mutant reported so far demonstrated the essential function of *dAda2b* gene in *Drosophila* development and the requirement for histone H3K9 and K14 acetylation. Genetic studies showed that neither dADA2b isoform alone could provide a complete restoration of *dAda2b* function, suggesting that both are required for development. On the other hand, either dADA2b isoform can productively participate in dSAGA complexes and render those at least partially active in histone H3 acetylation.

To study the molecular consequences of the loss of H3 K9 and K14 acetylation we compared the mRNA profiles of wild-type and *dAda2b* mutant animals in late L3 larval and in P4 pupal stages by cDNA microarray. Global gene expression profiling indicates that the

expression of less than 5% of the genes is significantly changed in *dAda2b* null mutants at both time points analyzed. We observed a relatively small overlap between the two stages in both mutant and wild-type samples. More than half of the affected genes are up-regulated, of which the high number of defense-related genes is particularly striking.

To demonstrate whether dSAGA has a direct role in the regulation of those genes which showed altered transcription levels in *dAda2b* mutant, we performed chromatin immunoprecipitations (ChIP) to detect the presence of dSAGA-specific histone H3 acetylation marks on these genes. Interestingly, we found that *dAda2b* mutation affects the H3K9ac level by a different extent at dSAGA-dependent and independent genes. In *dAda2b* mutants the histone acetylation levels are decreased both at dSAGA up-regulated, and at dSAGA down-regulated genes, while in the promoters of dSAGA independent genes a high level of histone H3K9ac is maintained.

Our data support the notion that different dADA2 proteins contribute to the functional versatility of multiprotein complexes and demonstrate that the loss of dSAGA specific H3 K9 and K14 acetylation affects (up- or down-regulates) the expression of a rather small subset of genes but does not cause a general transcription deregulation. We envision that the loss of *dAda2b* function results in a global decrease in H3 acetylation which can lead to an increase or to a decrease in the transcription level of genes.

Zsindely N, Pankotai T, Ujfaludi Zs, Lakatos D, Komonyi O, Bodai, L, Tora L, Boros I. The loss of histone H3 lysine 9 and lysine 14 acetylation due to dSAGA-specific *dAda2b* mutation influences the expression of only a small subset of genes. Submitted to Nucleic Acids Research, in revision.

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