Ion G, Fajka-Boja R, Kovács F, Szebeni G, Gombos I, Czibula A, Matkó J, Monostori E (2006) Acid sphingomyelinase mediated release of ceramide is essential to trigger the mitochondrial pathway of apoptosis by galectin-1. Cell Signal 18(11):1887-1896

Kadri T(2005) Proteomic Study of Galectin-1 Expression in Human Mesenchymal Stem Cells Stem Cells And Development 14:204–212.

Kiss J, Kunstár A, Fajka-Boja R, Dudics V, Tóvári J, Légrádi A, Monostori E, Uher F (2007) A Novel Anti-Inflammatory Function of Human Galectin-1: Inhibition of Hematopoietic Progenitor Cell Mobilization. Exp Hematol 35:305–313.

Lazennec G (2008) Concise review: adult multipotent stromal cells and cancer: risk or benefit? Stem Cells 26(6):1387-1394.

Lepelletier Y, Lecourt S, Arnulf B, Vanneaux V, Fermand JP, Menasche P, Domet T, Marolleau JP, Hermine O, Larghero J (2010) Galectin-1 and Semaphorin-3A are two soluble factors conferring T cell immunosuppression to bone marrow mesenchymal stem cell. Stem Cells Dev 19(7):1075-1079.

Panepucci RA, Siufi JL, Silva WA Jr, Proto-Siquiera R, Neder L, Orellana M, Rocha V, Covas DT, Zago MA. (2004) Comparison of gene expression of umbilical cord vein and bone marrow-derived mesenchymal stem cells Stem Cells 22(7):1263-1278.

Roorda BD, ter Elst A, Kamps WA, de Bont ES (2009) Bone marrow-derived cells and tumor growth: Contribution of bone marrow-derived cells to tumor micro-environments with special focus on mesenchymal stem cells. Crit Rev Oncol Hematol 69(3):187-198.

Silva WA Jr, Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, Santos AR, Zago MA (2003) The profile of gene expression of human marrow mesenchymal stem cells Stem Cells 21(6):661-669.

Urbán VS, Kiss J, Kovács J, Gócza E, Vas V, Monostori E, Uher F (2008) Mesenchymal Stem Cells Cooperate with Bone Marrow Cells in Therapy of Diabetes. Stem Cells 26:244-253.

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Studying the chromatin structure of MDR1 gene in drug-sensitive and drug-resistant human cells

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The reason for failure of chemotherapy is often the development of multidrug resistance, which is caused by the elevated level of ABC (ATP binding cassette) type transporters. One of the most often described ABC transporter is encoded by the MDR1 (multidrug resistance 1) gene. It was earlier described that drug induced upregulation of MDR1 is associated with increased H3 acetylation level in discrete region of MDR1 locus. Therefore exploration of the epigenetic mechanisms that contribute to the development of multidrug resistance has great importance. Several human diseases may originate from impaired function of histone acetyl transferases (HATs); therefore, these enzymes will serve as novel molecular targets for therapy in the future.

We aimed to study the changes in specific histone acetylation upon MDR1 gene induction and to analyze the histone acetyl transferase complexes that are responsible for these modifications.

We characterized a drug resistant, MCF7-derived, breast carcinoma cell line named MCF-KCR. It was generated via long term treatment of MCF7 cells with doxorubicin. We examined the level of MDR1 gene and mRNA in these cell lines. We found that the MDR1 gene level is 17 fold while the mRNA level 23000 fold elevated in the drug-resistant cells compared to the parental cells. These data suggest that epigenetic upregulation of MDR1 transcription is more important in developing the drug resistance than gene amplification. Importantly, the level of MRP1 (multidrug resistance protein 1) mRNA was not elevated in the drug resistant cells.

We showed by immunoblotting that the global H3 acetylation is elevated in the MCF-KCR cells. In addition, we examined the histone acetylation pattern in the regulatory regions (two promoters) and the coding region of the MDR1 gene by employing chromatin immunoprecipitation. Our data reveal an interesting acetylation map. With the use of acetylated residue specific antibodies we found that the acetylation level of H3K9 is about 100 fold elevated in the downstream promoter region and in the first exon in the drug resistant subline compared to the drug sensitive cells. H3K4, H3K14 and H4K12 are also slightly increased in the downstream promoter region and in the first exon of the MDR1 gene in those cells that overexpress MDR1 mRNA (Toth et al. 2009).

When we treated the cells with trichostatin A (TSA), a histone deacetylase inhibitor, MDR1 expression increased, while that of the other genes examined did not change in the drug sensitive cells. In contrast, MDR1 mRNA level did not change in the drug resistant cells upon TSA treatment. To try to down-regulate the acetylation and, along with that, the expression of the MDR1 gene, we treated the cells with a novel HAT inhibitor (HATi II) that strongly inhibits p300 and CBP (CREB binding protein) and weakly inhibits PCAF (p300/CBP associated factor) and GCN5 (homolog of yeast general control nonderepressable). Importantly, expression of MDR1 was further increased in the drug resistant MCF-KCR cells, while it did not change in the parental MCF7 cells. Next, we knocked down the level of PCAF, GCN5 and Ada2b (homolog of yeast alteration/deficiency in activation 2b) mRNA by transfecting specific siRNAs. The latter is a component of GCN5 containing multisubunit HAT complexes, such as hTFTC/hSTAGA. Interestingly, PCAF downregulation resulted in a reduction of the MDR1 mRNA level in MCF7 cells but not in the MCF-KCR cells. MDR1 mRNA level did not change in the drug resistant subline and decreased only slightly in drug sensitive cells upon GCN5 or Ada2b knockdown. These data suggest that MDR1 expression can not be easily reduced by simple inhibition of HATs in the drug resistant cells, probably because histone acetylation is highly deregulated in these cells.

Volume 54(1):59-73, 2010
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Toth M, Molnar J, Boros IM, Balint E. Examination of the histone acetylation pattern in the regulatory regions of the human MDR1 gene causing multidrug resistance. EMBO Conference on Chromatin and Epigenetics, 13-17 May, 2009, Heidelberg, Germany. Poster 214.

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Functional interplay between factors involved in transcription in *Drosophila melanogaster*

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Regulation of transcription, the synthesis of RNA from a DNA template, is one of the most important steps in control of cell growth and differentiation. Gene regulation occurs in the context of chromatin where recruitment of chromatin remodeling complexes such as ATP-dependent remodeling complexes and histone modifying enzymes (HAT, HDAC, HMT, etc.) represents a crucial step in gene transcription (Svejstrup 2004). We are interested in studying the physical and functional interaction between proteins involved in transcription regulation such as the RNA PolII subunit Rpb4, the transcription factor p53, and adaptor proteins of the chromatin modifier complexes.

The RNA polymerase II is composed of a ten-subunit core and a two-subunit dissociable subcomplex comprising the forth and the seventh largest subunits, Rpb4 and Rpb7. In *Drosophila*, Rpb4 is a product of a bicistronic gene together with the ATAC histone acetyltransferase complex constituent ADA2a (Pankotai et al. 2010). The alignment of Ada2a and Rpb4-related sequences indicated that the two proteins share the same transcription unit. Comparison of the related protein and nucleotide sequences revealed that Ada2a and Rpb4 coding region are present in similar organization in 12 *Drosophila* species, other then *D. melanogaster*; however a similar gene organization in other organisms cannot be identified. We investigated the mechanism by which the two mRNAs are generated. From RT-PCR analysis we concluded that the shift between Ada2a and Rpb4 mRNA formation takes place by splice acceptor site selection. Ada2a protein has another distinct homolog in *Drosophila*, Ada2b which is present in the SAGA complex. Although the two proteins contain the same conserved domains and their interacting partners are rather similar, they are part of two distinct HAT complexes. We questioned what confers the complex specificity of the Ada2 adaptors, more precisely which regions are responsible for their interaction with different transcriptional coactivators. Firstly, different Ada2a/Ada2b or Ada2b/Ada2a hybrids proteins were generated by joining PCR fragments corresponding to functional domains of one and the other ADA2. All the hybrid plasmids were generated using the Gateway system and were successfully tested on western blot for their expression in *Drosophila* S2 cells. Since the chimeric structure of the proteins did not disturb their expression in S2 cells, plasmids suitable for embryo injections were generated. Our goal is to see if their expression will restore one or the other ADA2 function. The coding region of hybrid proteins were inserted into a P-element containing vector allowing site specific insertion and injected into *Drosophila* embryos. Two out of six plasmids are ready for *in vivo* analysis in *Ada2a* and *Ada2b* mutant background.

Previous studies have shown that HATs, beside their ability to relax chromatin, can regulate many other factors through acetylation, including transcription factors (Wang et al. 2001). In mammalian system it was shown that the Gcn5-containing acetyltransferase complex (STAGA) plays a role in p53-dependent gene activation. Ada2b and Gcn5L proteins are identified as direct interacting partners of the p53 transcription factor (Gamper and Roeder 2008). According to previous studies *Drosophila* p53 is a functional homolog of mammalian p53 (Ollmann et al. 2000). We investigated whether the adaptor proteins of the HAT complex have any effect on p53 transcriptional activity. A plasmid containing three copies of *rpr* consensus binding site upstream of the luciferase gene was constructed. The reporter plasmid was cotransfected into S2 cells with different constructs expressing Ada2a, Ada2b or Ada3 proteins and performed luciferase assays. There was no significant change in transcriptional activity of the p53 responsive element. Although it was previously described that hAda3 can increase p53 trancriptional activity (Wang et al. 2001), perhaps in *Drosophila* S2 cells the efficiency of this interaction requires additional factors which can promote a significant response. We also tested plasmid vectors containing *Drosophila melanogaster* p53 (Dmp53) and a point mutant form of it ($Dmp53^{K302R}$) in reporter assays. Expression of either the wild type or the mutant variant increased the activity of the reporter gene 20 fold compared to control. No significant change was observed in the level of transcriptional activation ability of the wild type and the mutant variant of Dmp53. Future *in vivo* studies are planned to understand the mechanism by which p53 is activated.

Gamper AM, Roeder RG (2008) Multivalent binding of p53 to the STAGA complex mediates coactivator recruitment after UV damage. Mol Cell Biol 28:2517-27.

Ollmann M, Young LM, Di Como CJ, Karim F, Belvin M, Robertson S, Whittaker K, Demsky M, Fisher WW, Buchman A, Duyk G, Friedman L, Prives C, Kopczynski C (2000) Drosophila p53 is a structural and functional homolog of the tumor suppressor p53. Cell 101(1):91-101.

Pankotai T, Ujfaludi Z, Vamos E, Suri K, Boros IM (2010) The dissociable RPB4 subunit of RNA Pol II has vital functions in Drosophila. Mol Genet Genomics 283:89-97.

Svejstrup JQ (2004) The RNA polymerase II transcription cycle: cycling through chromatin. Biochim Biophys Acta 1677:64-73.

Wang T, Kobayashi T, Takimoto R, Denes AE, Snyder EL, el-Deiry WS, Brachmann RK (2001) hADA3 is required for p53 activity. EMBO J 20:6404-6413.

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