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HISTAMINE, PART OF THE *METABOLOME**

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Histamine, a decarboxylated amino acid with a molecular mass of 112 daltons reveals multicoloured functional activities. Its role in allergy and inflammation is abundantly characterized. Moreover histamine is one of the neurotransmitters, has a role in gastric acid production and in maintenance of blood-brain barrier.

In the last decade, many data were collected suggesting an important function of histamine in events of immune response and also in both benign and malignant cell proliferation. Our group collected data on the relevance of histamine as an autocrine factor in human melanoma. The outcome of the action seems to be closely related to the local and actual balance of histamine receptors (H1R, H2R, H3R and H4R) on tumor cells.

Recently, using a gene targeted mouse strain (lacking an enzyme, histidine decarboxylase, the only one responsible for histamine production) many phenotypes of the histamine-free mice were demonstrated. Our data suggest, that histamine, as part of the poorly characterized metabolome of the mammalian cells plays significant role in many physiological and pathological processes.

Keywords: DNA – forensic medicine – genotyping – VNTR

INTRODUCTION

Histamine, first described almost hundred years ago [1] is generated by decarboxylation of histidine and appears as one of the most general mediators. The most important ones are: the contraction of smooth muscle, dilation of capillaries, secretion of gastric acid, influence on inflammation and immune response and the neurotransmitter function [2]. The effectiveness of histamine on cell proliferation (embryonal development, tissue regeneration, tumors, etc.) though it has been observed in 1968 [10], its thorough study started only about ten years ago.

Highest amount of histamine is found in mast cells and basophil granulocytes. Its generation is catalyzed by histidine decarboxylase (HDC), an enzyme using pyridoxal-phosphate as coenzyme. This phylogenetically highly conserved enzyme is the

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only enzyme capable for the synthesis of histamine. HDC is expressed not only in mast cells and basophils but in many other cells, including lymphocytes and macrophages as well as in all benign and malignant proliferating cells. Uncovering of the tertiary structure of HDC has just been started by our group. Catabolism of histamine is performed by two enzymes diaminooxidase and histamine-N-methyl transferase [16].

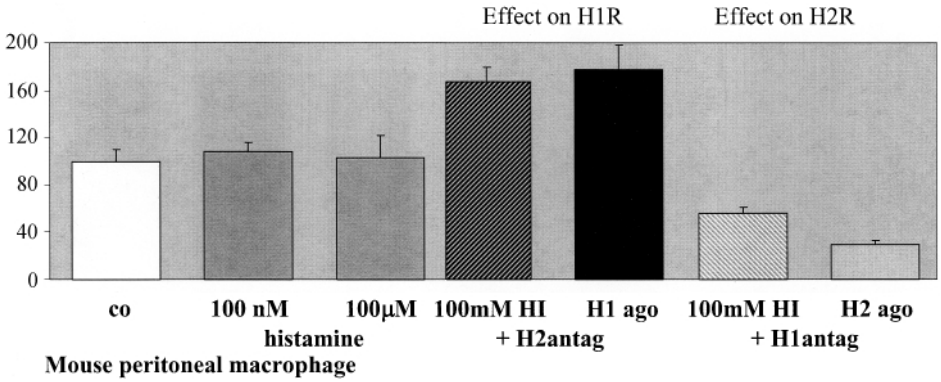

Fig. 1. Histamine modifies expression of C3 gene expression, it increases through histamine receptor 1 (H1R)-, and decreases it through H2R. Co: control, ago: agonist, antag: antagonist

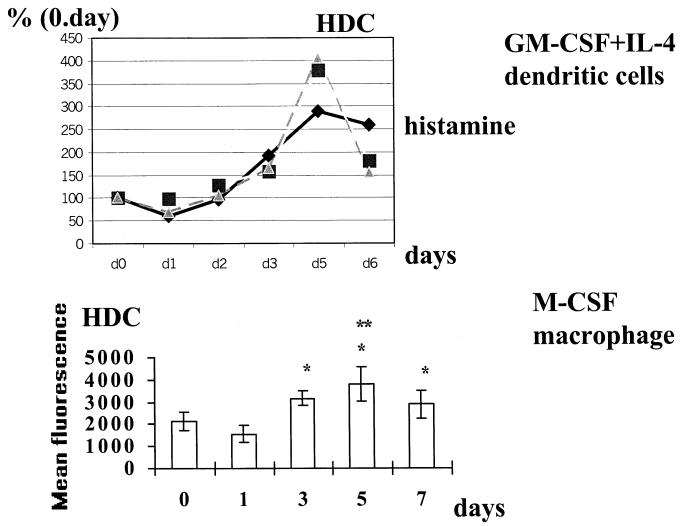

Fig. 2. Dendritic cells and monocytes contain histidine decarboxylase (HDC), it increases during differentiation. GM-CSF: granulocyte-monocyte colony stimulating factor, IL-4: interleukin-4, M-CSF: monocyte colony stimulating factor

Histamine and immune response

We started our studies in the early eighties with Katalin Merétey on the effect of histamine on the mitogen response of T cell [13]. It has been revealed that histamine inhibits the growth and activation of T cells. Then, we discovered first that histamine influences the gene expression and production of C3 in the peritoneal macrophages of mice, it inhibits through histamine-2 receptors (H2R) and upregulates acting on H1R [3] (Fig. 1). Later we found that histamine affects the effectiveness of interleukin (IL)-1 and interferon on the biosynthesis of complement proteins [4].

"Non-conventional" histamine production in macrophages and dendritic cells

Among *in vitro* conditions in the presence of human colony stimulating factors (M-CSF) human monocytes develop to macrophages, while IL-4 and granulocyte-monocyte CSF support their differentiation toward dendritic cell lineage [11, 17] (Fig. 2). During this differentiation expression of HDC and the amount of histamine increases. The autocrine effect of histamine is confirmed by the effectivity of histamine receptor antagonists on the differentiation markers and maturation of these cells.

Histamine in melanoma

One of the most malignant human cancer, melanoma contains unexpectedly large amount of HDC and histamine [7] (Fig. 3). As the functional relevance of this observation it is suggested that HDC specific antisense oligonucleotides may decrease the proliferation rate of melanoma cells (Fig. 4). Soon, it has been revealed that histamine is a "double-edge sword" since through H2R histamine rather elevates, while acting on H1R it inhibits the proliferation of melanoma cells [12]. Similar conclusions can be drawn if growing characteristics of human melanoma cells xenotransplantanted *in vivo* into immunodeficient SCID mice was studied. The strong antitumor effects of H2R antagonists prolong the survival of the mice with human melanoma [18]. Besides, the endogenously produced histamine locally inhibits the interferon production, however elevates the synthesis of IL-6, reflecting a local shift of immune balance toward Th2 polarization [12]. In other words, melanoma impairs the cell-mediated antitumor strength of the neighboring immune cells (Fig. 5).

In vivo *model: the HDC "knock-out" mice*

The significance of histamine has been studied on a murine model in which, using homologous recombination, the intact HDC gene has been replaced by an inactive mutant lacking the exons responsible for coenzyme binding [14] (Fig. 6). The

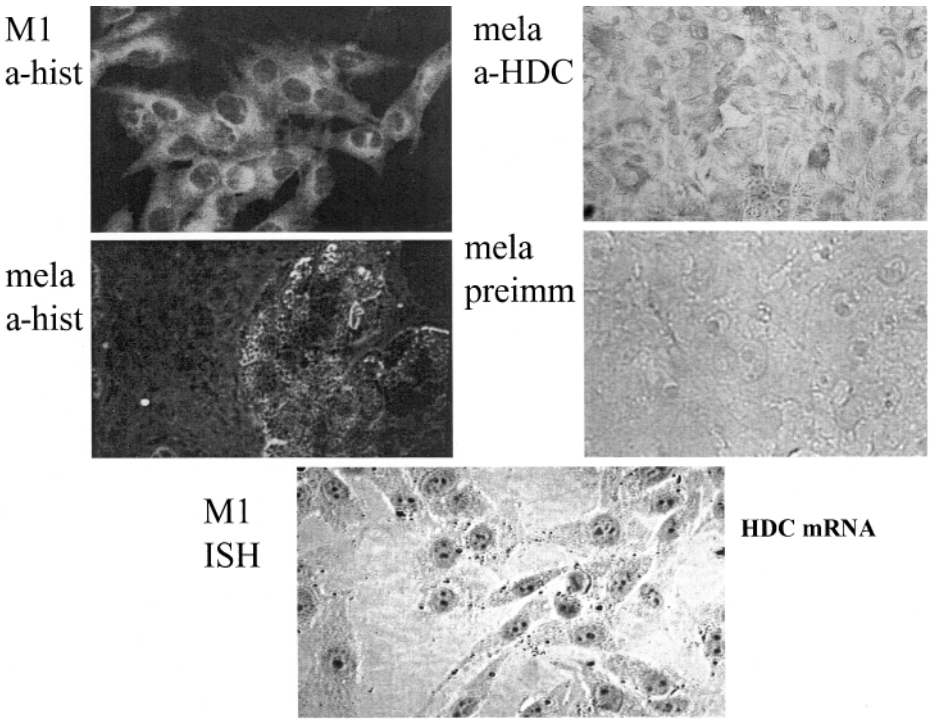

Fig. 3. Melanoma cell lines (e.g. M1) and melanoma tissue contain HDC and histamine a-hist: anti-histamine, ISH: *in situ* hybridization

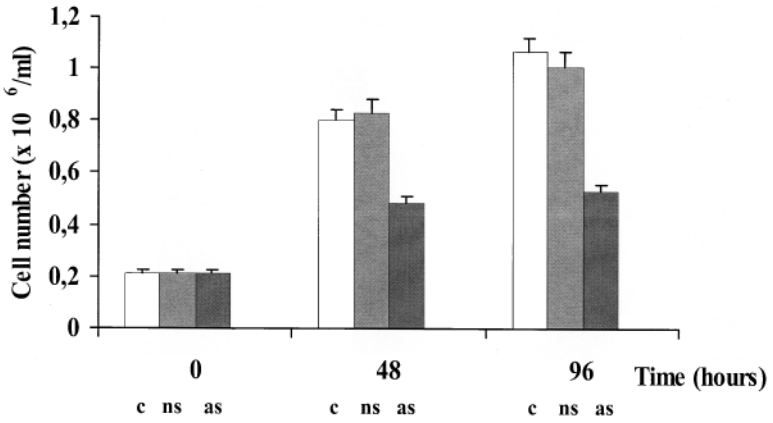

Fig. 4. HDC specific antisense oligonucleotides decrease melanoma proliferation.
c: control, ns: nonsense, as: antisense

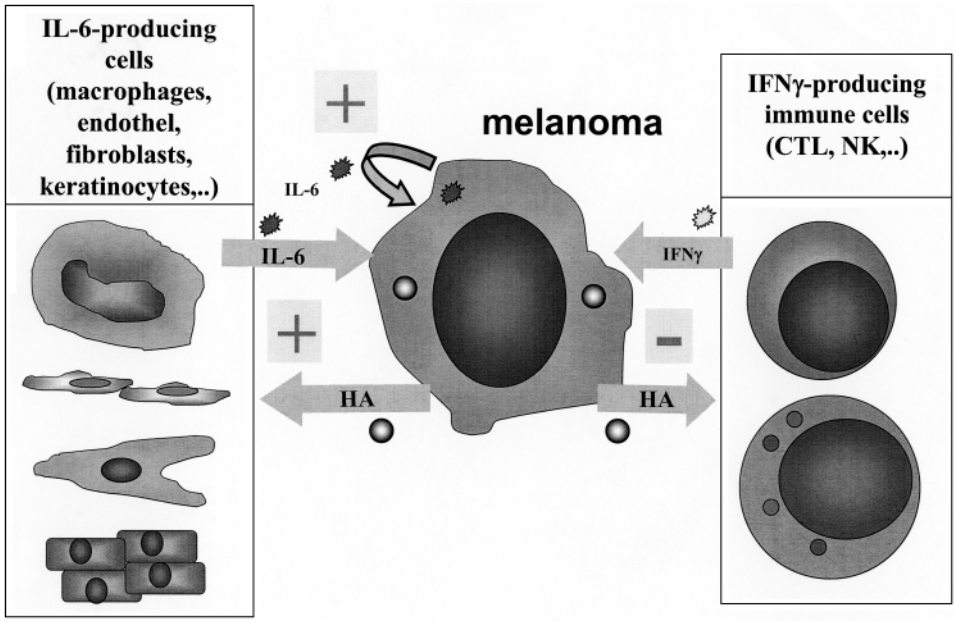

Fig. 5. Melanoma-derived histamine (HA) and interleukin (IL)-6 modify the Th1/2 immune balance in the vicinity of melanoma cells. IFN: interferon. CTL: cytotoxic T cells, NK: natural killer cell

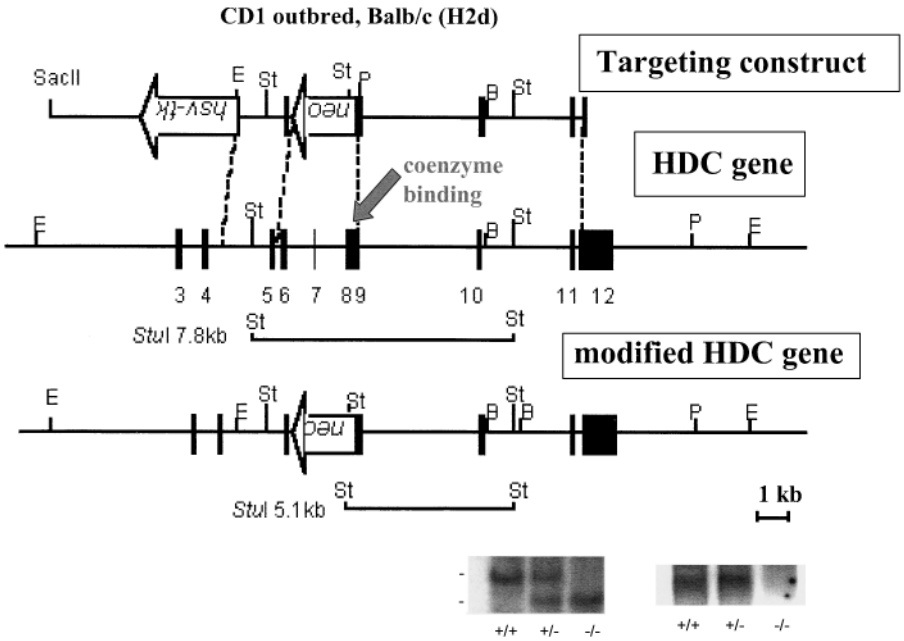

Fig. 6. The HDC "knock-out" model

$HDC^{-/-}$ mice has no histamine, however the histamine taken up by the food is present. Furthermore, using this "exogenous" source of histamine some phenotypic changes are abolished. This fact means that the phenotype(s) of genetic deficiency of histamine can be conditionally induced just by withdrawing this low molecular weight substance-histamine (112 daltons) from the food.

Phenotype changes of the histamine deficiency in the $HDC^{-/-}$ mice (compared to the $HDC^{+/+}$ mice) are as follows:

• if pregnant mothers are kept on histamine-free diet the F2 segregation is "non-Mendelian", much less homozygous $HDC^{-/-}$ are born as expected;

• H2R expression is decreased. H1R does not change if kept on histamine free food [5];

• complete absence of "allergic" skin signs [14];

• basic reduction in the number, granulation and enzyme content of mast cells [20];

• regeneration of bone marrow after sublethal irradiation is slower;

• decrease of CD4+ and CD8+ cells in thymus is found; apoptotic rate of double positive (CD4+CD8+) cells decreases;

• reduction of peripheral T cell proliferation;

• elevation of many autoantibodies in the blood;

• lower acute phase proteins, decreased IL-6, elevated IL-6 receptor expression on histamine-free diet [8, 9];

• higher protection against *Chlamydia* and *E. coli* infections;

• elevated cortical bone density and resistance against ovariectomy (i.e. low estrogen)-induced osteoporosis;

• highly elevated leptin level (probably due to the lack of histamine in the hypothalamic tuberomammilar cells, targets of leptin), leptin resistance [6];

• elevated activity (c-fos expression) in tanycytes and ependymal cells in the brain after pain-shock [15];

• converted night/day cycle, decreased night motility

Expression profile analysis by microarray (cDNA chip)

The highly colored changes in phenotype attract further studies. We used three independent microarray systems (Atlas, Incyte and HAS Biological Center of Szeged) and studied the expression of 588 (Atlas), 3200 (HAS Szeged) or 9888 (Incyte) murine genes of total embryos of HDC knock-out and wild type mice. At the beginning of the annotation and evaluation, it is seen that approximately 3–8% of the genes the expression is changed markedly, about half of them are up- or downregulated. There are many ESTs showing major changes in histamine deficient mice are to be annotated, yet.

Histamine, as a part of "metabolome"

Metabolome is a collective term for low molecular weight metabolite pools (amino acids, amines-such as histamine), monosaccharides, lipid moieties, etc.) present as consequences of normal metabolism. In the last 1–2 years, mainly in bacteriological systems reproducible changes of metabolome were detected in various bacterial cultures with dissimilar densities [19].

The totality of genes (genome), mRNAs (transcriptome) and proteins (proteins) are closely related to the elements of metabolome. This low molecular weight pools serve as "background" or "medium" for macromolecular events. Methodologically, the metabolome research today is comparable that of nucleic acid studies in the early 1940s years.

Biological significance of histamine provide a good reason to believe, that hierarchic regulation (DNA-mRNA-protein) is combined with an other, however closely related other system in the living cells.

The recognition of the role of metabolome is one of the challenges of the postgenomic "era".

Our histamine experiments may contribute to this field in the future.

Our plans involve three levels:

1. Using histamine deficient mice functional models (allergy, oncology, infection, autoimmunity) should be characterized;
2. The microarray data have to be further analyzed using bioinformatics and international data banks;
3. In the frame of merging metabolome studies the role of biological amines should be further characterized in various biological systems.

REFERENCES

1. Dale, H. H., Laidlaw, P. P. (1910) The physiological action of β-iminazolyl-ethylamine. *J. Physiol. (Lond.) 41,* 318–344.
2. Falus, A. (1994) *Histamine and Inflammation.* Landes, Austin, Texas.
3. Falus, A., Merétey, K. (1987) Effect of histamine on the gene expression and biosynthesis of complement components C2, factor B and C3 in mouse peritoneal macrophages. *Immunology 60,* 547–551.
4. Falus, A., Rokita E., Walcz, E., Brozik, M., Hidvégi, T., Merétey, K. (1990) Hormonal regulation of complement biosynthesis in human cell lines-II. Upregulation of the biosynthesis of complement components C3, factor B and C1 inhibitor by interleukin-6 and interleukin-1 in human hepatoma cell line. *Molecular Immunology 27,* 197–201.
5. Fitzsimons, C. P., Lazar-Molnar, E., Tomoskozi, Z., Buzas, E., Rivera, E. S., Falus, A. (2001) Histamine deficiency induces tissue-specific down-regulation of histamine H2 receptor expression in histidine decarboxylase knockout mice. *FEBS Lett 508,* 245–248.
6. Fülöp, A. K., Buzas, E., Hegyi, K., Miklos, I. H., Nagy, A., Falus, A., Kovacs, K. J. (2002) Hyperleptinemia and visceral adiposity in histidine decarboxylase (HDC) knockout mice (submitted for publication).

7. Haak-Frendscho, M., Darvas, Zs., Hegyesi, H., Kárpáti, S., Randall, L., Hoffmann, László, V., Bencsáth, M., Szalai, Cs., Fürész, J., Timár, J., Bata-Csörgő, Zs., Szabad, G., Pivarcsi, A., Pállinger, É., Kemény, L., Horváth, A., Dobozy, A., Falus, A. (2000) Histidine decarboxylase expression in human melanoma. *J. Invest. Dermatol. 115,* 345–352.
8. Hegyi, K., Fülöp, A. K., Tóth, S., Buzás, E., Watanabe, T., Ohtsu, H., Ichikawa, A., Nagy, A, Falus, A. (2001) Histamine deficiency suppresses murine haptoglobin production and modifies hepatic protein tyrosine phosphorylation. *Cell. Mol. Life Sci. 58,* 850–854.
9. Horváth, B. V., Falus, A., Tóth, S., Szalai, C., Lázár-Molnár, E., Holub, M. C., Buzás, E., Nagy, A., Fülöp, A. K. (2002) Inverse regulation of interleukin-6 (IL-6) and IL-6 receptor in histamine deficient histidine decarboxylase-knock-out mice. *Immunol. Lett. 80,* 51–54.
10. Kahlson, G., Rosengren, E. (1968) New approaches to the physiology of histamine. *Physiol. Rev. 48,* 155–196.
11. László, V., Rothe, G., Hegyesi, H., Szeberényi, J. B., Orsó, E., Schmitz, G., Falus, A. (2001) Increased histidine decarboxylase expression during *in vitro* monocyte maturation; a possible role of endogenously synthesised histamine in monocyte/macrophage differentiation. *Inflamm. Res. 50,* 428–434.
12. Lázár-Molnár, E., Hegyesi, H., Tóth, S., Darvas, Zs., László, V., Szalai, Cs., Falus, A. (2000) Biosynthesis of interleukin-6, and autocrine growth factor for melanoma, is regulated by melanoma-derived histamine. *Seminars in Cancer Biology 10,* 25–28.
13. Merétey, K., Fekete, M. I. K., Bohm, U., Falus, A. (1985) Effect of H1 and H2 agonists on the chemiluminescence of human blood mononuclear cells induced by phytohaemagglutinin. *Immunopharm. 9,* 175–180.
14. Ohtsu, H., Tanaka, S., Terui, T., Hori, Y., Makabe-Kobayashi, Y., Pejler, G., Tchougounova, E., Hellman, L., Gertsenstein, M., Hirasawa, N., Sakurai, E., Buzás, E., Kovács, P., Csaba, G., Kittel, A., Okada, M., Hara, M., Mar, L., Numayama-Tsuruta, K., Ishigaki-Suzuki, S., Ohuchi, K., Ichikawa, A., Falus, A., Watanabe, T., Nagy, A. (2001) Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett. 27,* 53–56.
15. Palkovits, M., Gallatz, K., Tóth, Z. E., Buzás, E., Falus, A. (2002) Activation of c-*fos* expression in circumventricular organs and tanycytes of l-histidine decarboxylase deficient mice in response to acute formalin stress. Neurosciences (in press).
16. Schwelberger, H. G. (1999) Molecular cloning of mammalian diamine oxidase genes and cDNAs. *Inflamm. Res. 48,* Suppl 1: S79–80.
17. Szeberényi, J., Pállinger, E., Zsinkó, M., Pós, Z., Rothe, G., Orsó, E., Szeberényi, Sz., Schmitz, G., Falus, A., László, V. (2001) Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. *Immunol. Lett. 76,* 175–182.
18. Szincsák, N., Hegyesi, H., Hunyadi, J., Martin, G., Lázár-Molnár, E., Kovács, P., Rivera, E., Falus, A., Juhász, I. (2002) Cimetidine and a tamoxifene derivate reduce tumour formation in SCID mice xenotransplanted with a human melanoma cell line. *Melanoma Res.* 12, 231–240.
19. ter Kuile, B. H., Westerhoff, H. V. (2001) Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. *FEBS Lett. 500,* 169–171.
20. Wiener, Z., Andrásfalvy, M., Pállinger, E., Kovács, P., Szalai, C., Erdei, A., Tóth, S., Nagy, A., Falus, A. (2002) Bone marrow derived mast cell differentiation is strongly reduced in histidine decarboxylase knock-out, histamine-free mice. *Intern. Immun.* 14, 381–387.