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Original Contribution

Involvement of hydrogen peroxide in histamine-induced modulation of WM35 human malignant melanoma cell proliferation

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ABSTRACT

Histamine is a recognized growth factor in melanoma, and exogenous histamine produces a dual effect on proliferation. We have previously reported that histamine at micromolar concentrations reduces the proliferation of melanoma cell lines. To investigate the mechanism by which histamine inhibits proliferation of WM35 human melanoma cells, we have studied the involvement of histamine in reactive oxygen species production and antioxidant enzyme regulation in these cells. Results indicate that histamine treatment (10 μM) significantly increased hydrogen peroxide levels, whereas it slightly decreased superoxide levels associated with an enhancement of superoxide dismutase and a reduction in catalase activity. Additionally, catalase treatment reversed the inhibitory effect of histamine on proliferation, and various treatments that reduce hydrogen peroxide formation increased proliferation of these cells. Furthermore, we demonstrate that the inhibition of proliferation produced by histamine was mediated at least in part by an induction of cell senescence. We conclude that hydrogen peroxide is involved in histamine-mediated modulation of proliferation in malignant melanoma cells.

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Malignant melanoma is an aggressive, therapy-resistant malignancy of melanocytes. The incidence of melanoma has been steadily growing worldwide, resulting in an increasing public health problem [1]. In its early stages malignant melanoma can be cured by surgical resection, but once it has progressed to the metastatic stage it is extremely difficult to treat and does not respond to current therapies [2]. The immunoactivating cytokine interleukin 2 (IL-2)[1] is employed in the treatment of stage IV melanoma in many European countries and in the United States. IL-2 therapy, which aims at expanding and activating tumoricidal lymphocytes, induces complete regression of melanoma metastases in 3–5% of treated patients, but the toxicity of many IL-2 regimens limits its use. Clinical trials are being performed with IL-2 alone or combined with histamine dihydrochloride, which has been shown to protect tumoricidal lymphocytes from dysfunction and apoptosis induced by mononuclear phagocytes. Data suggest that administration of IL-2 and histamine dihydrochloride may specifically prolong the survival of melanoma patients with liver metastases [3–5]. Therefore, it is essential to improve knowledge regarding the role of histamine in melanoma cell growth.

Melanoma cells constitutively produce various cytokines as well as growth factors and express their corresponding receptors [6,7]. Melanoma cells, but not normal melanocytes, express the histamine-synthesizing enzyme L-histidine decarboxylase (EC 4.1.1.22) and contain large amounts of histamine [8,9]. Moreover, H1, H2, and H3 histamine receptors have been described in diverse melanoma cells and both endogenous and exogenous histamine have the ability to regulate melanoma cell growth [10–16].

We have previously described that histamine exerts a dual effect on proliferation of the human primary melanoma cell line WM35, increasing proliferation at low concentrations through the H2 receptor while decreasing it at higher concentrations via the H1 receptor [13,14]. Recently, we have reported that histamine modulates the proliferation of MDA-MB-231 breast cancer cells in a dose-dependent manner, producing a significant decrease at 10 μM concentration that was associated with an increase in hydrogen peroxide levels [17].

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical are highly reactive and can exert deleterious effects on cell function and viability, depending on cellular antioxidant defenses and ability to repair oxidative damage. ROS levels are normally controlled by the antioxidant defense system including the antioxidant enzymes manganese-containing superoxide dismutase (MnSOD) and

Abbreviations: ROS, reactive oxygen species; MnSOD, manganese-containing superoxide dismutase; CuZnSOD, copper–zinc-containing superoxide dismutase; GPX, glutathione peroxidase; PBS, phosphate-buffered saline; DCFH-DA, dichlorodihydrofluorescein diacetate; HE, dihydroethidium; NBT, nitroblue tetrazolium; IL-2, interleukin 2.

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copper–zinc-containing superoxide dismutase (CuZnSOD), which catalyze the dismutation of the superoxide radical into hydrogen peroxide, and catalase and glutathione peroxidase (GPX), which further degrade hydrogen peroxide [18–20].

To better understand the mechanism by which histamine inhibits proliferation of WM35 human melanoma cells, we have investigated the involvement of histamine in ROS production and antioxidant enzyme modulation in these cells.

Materials and methods

Cell culture

The human melanoma cell line WM35 was kindly provided by Professor A. Falus (Budapest, Hungary). Cells were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, 0.3 g/L glutamine, and 0.04 g/L gentamicin (Gibco BRL, Grand Island, NY, USA). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cell growth assays

For clonogenic assay, cells were seeded in six-well plates (1×10^3 cells/well) and incubated for 8 days. The cells were treated with 0.01 to 10 μM histamine, 10 μM forskolin (direct activator of adenylyl cyclase), 125 units/ml catalase (Sigma Chemical Co., St. Louis, MO, USA), 10 μM Dimaprit (H2 receptor agonist; Tocris, UK), or 1–5 μM hydrogen peroxide (Merck, Argentina). Cells were fixed with 10% (v/v) formaldehyde in phosphate-buffered saline (PBS) and stained with 1% (w/v) toluidine blue in 70% (v/v) ethanol. The clonogenic proliferation was evaluated by counting the colonies containing 50 cells or more and was expressed as a percentage of the untreated wells.

Cells were also incubated overnight in six-well plates (8×10^4 cells/well) and then incubated with or without treatment for up to 72 h. Cells were trypsinized at various periods of time and counted using a hemacytometer.

Senescence-associated β-galactosidase staining

Senescence-associated β-galactosidase-positive cells were detected using the method described by Dimri et al. [21]. Cells were seeded into 12-well plates in culture medium. After 24 h drugs were added and the cells were incubated for 48 h. The cells were washed twice and fixed for 5 min in 4% (v/v) formaldehyde in PBS. They were then washed and incubated at 37 °C for 6 h with 1 mg/ml 5-bromo-4-chloro-indolyl-β-galactoside (USB Corp., Cleveland, OH, USA) dissolved in a solution containing 40 mM citric acid (pH 6), 5 mM potassium ferrocyanide, 150 mM NaCl, and 2 mM $MgCl_2$. After incubation, cells were washed twice with PBS and counterstained with hematoxylin and the percentage of β-galactosidase-positive cells was assessed under light microscopy (Axiolab Karl Zeiss, Göttingen, Germany). At least 500 cells were scored for each determination. All photographs were taken at 630× magnification using a Canon PowerShot G5 camera (Tokyo, Japan).

Measurement of intracellular ROS production

Cells were incubated with either 5 μM DCFH-DA (dichlorodihydrofluorescein diacetate) or HE (dihydroethidium), a peroxide- or superoxide-sensitive fluorescent probe, respectively (Sigma Chemical Co.), for 30 min at 37°C. Cells were then washed, detached by trypsinization, and suspended in PBS. Levels of intracellular ROS were measured immediately by flow cytometry (FACSCalibur; Becton–Dickinson, Rutherford, NJ, USA) and data analysis was performed using WinMDI 2.8 software (Scripps Institute, La Jolla, CA, USA).

Cell homogenization and protein determination

Cells that were untreated or treated with 10 μM histamine were washed, scraped, and collected in phosphate buffer (KH_2PO_4/K_2HPO_4 50 mM, pH 7.8). This was followed by sonic disruption, and the suspensions were centrifuged at 10,000*g* for 10 min at 4°C. Protein concentration was determined by Bradford assay [22].

Measurement of catalase activity

Catalase activity was measured spectrophotometrically by monitoring the disappearance of hydrogen peroxide at 240 nm, as we have previously described [17].

Measurement of SOD activity

Enzymatic activity of SOD was assayed by competitive inhibition performed using xanthine–xanthine oxidase-generated superoxide to reduce the nitroblue tetrazolium (NBT) to blue diformazan at a constant rate (0.015 to 0.025 absorbance units/min). The rate of NBT reduction was monitored spectrophotometrically at 560 nm. The amount of protein that inhibits the NBT reduction to give 50% of maximum was defined as 1 unit (U) of SOD activity [23].

Measurement of GPX activity

GPX activity was examined indirectly by spectrophotometrically monitoring the oxidation of NADPH at 340 nm as previously described by Flohé and Gunzler [24]. The coupled assay system contained glutathione, glutathione reductase, and *t*-butyl hydroperoxide as the substrate. One unit of enzyme was defined as the oxidation of 1 nmol of NADPH per minute ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$) [23].

Western blot analysis

Samples were processed as previously described [17]. Equal amounts of protein (50 μg) were fractionated on SDS–polyacrylamide gels (12%) and transferred electrophoretically onto nitrocellulose membranes (Sigma Chemical Co.). Membranes were blocked and probed overnight with primary mouse anti-catalase, mouse anti-β-actin (1:1000; Sigma Chemical Co.), sheep anti-MnSOD, sheep anti-CuZnSOD, and mouse anti-GPX (1:1000; Calbiochem, San Diego, CA, USA) antibodies, as stated. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse or anti-sheep IgG, as appropriate, and visualized by enhanced chemiluminescence (Amersham Biosciences, Arlington Heights, IL, USA). Densitometric analyses were performed using the software ImageJ 1.32J (NIH, Bethesda, MD, USA).

Statistical analysis

All determinations were performed in triplicate. Representative results are presented as means with standard error of mean (SEM). Statistical evaluations were made by Student *t* test and analysis of variance, which was followed by Newman–Keuls multiple comparison test.

All statistical analyses were performed with GraphPad Prism version 5.00 software (San Diego, CA, USA).

Results

Histamine modulates formation of intracellular ROS and regulates antioxidant enzyme activity in WM35 melanoma cells

The intracellular levels of ROS were evaluated by flow cytometry using specific fluorescent dyes. Histamine (10 μM)

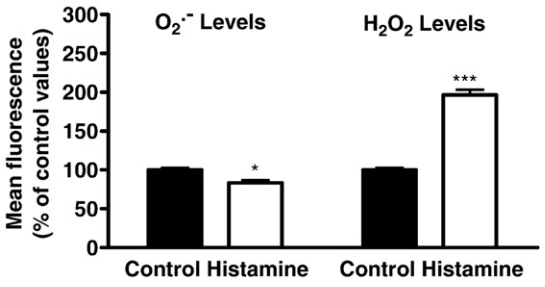

Fig. 1. Effect of histamine on ROS production. Intracellular hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$) levels were determined by flow cytometry using DCFH-DA and HE fluorescent probes, respectively. WM35 cells were treated with histamine (10 μM) for 24 h or were left untreated (control). Data represent the mean percentage fluorescence intensity with respect to control values. Error bars represent the means ± SEM (*$p<0.05$, ***$p<0.001$ versus control).

treatment increased hydrogen peroxide levels in WM35 cells to 196 ± 7%, whereas it slightly decreased superoxide levels to 83 ± 4% (Fig. 1). Accordingly, histamine significantly enhanced the activity of SOD and diminished that of catalase (Figs. 2A and C). Although not significant, a slight decrease in GPX activity was observed (Fig. 2E). These effects did not seem to be mediated by a modification of protein expression of those enzymes (Figs. 2B, D, and F).

Histamine inhibition of proliferation is associated with an increase in the hydrogen peroxide levels

We next investigated the involvement of hydrogen peroxide levels in WM35 cell growth. Addition of catalase completely reversed the decrease in colony formation and cell number exerted by histamine at 10 μM concentration. Furthermore, treatment with hydrogen peroxide at doses higher than 1 μM remarkably inhibited proliferation (Fig. 3A). The reduction of proliferation produced by histamine and hydrogen peroxide was mediated at least in part by an induction of premature or accelerated cell senescence, a biological program of terminal growth arrest characterized by disruption of lysosomal function through enhanced activity of senescence-associated β-galactosidase, which is highly associated with ROS levels [19,25–28].

In agreement with our previous reports [13,14], histamine at low concentrations as well as H2 receptor agonist and forskolin augmented

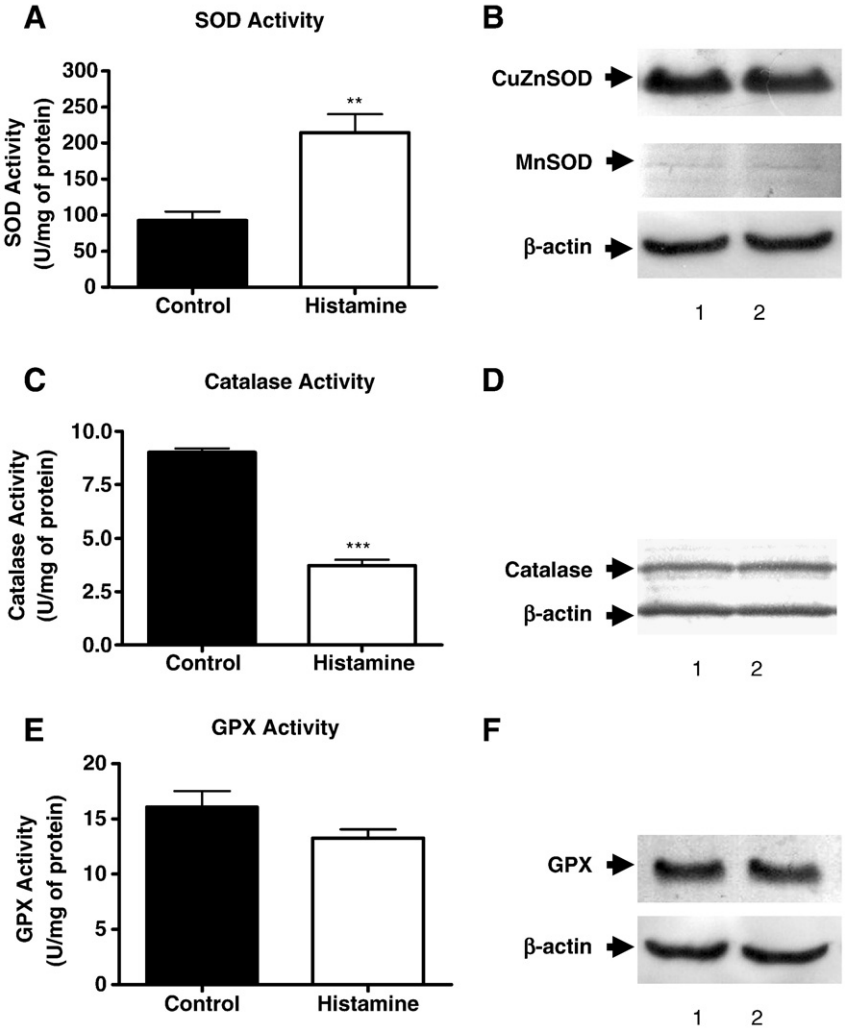

Fig. 2. Effects of histamine on the activity and expression of SOD, catalase, and GPX. (A) SOD, (C) catalase, and (E) GPX activities were determined in WM35 cells that were untreated or treated with 10 μM histamine for 24 h. Error bars represent the means ± SEM (**$p<0.01$, ***$p<0.001$ versus control). (B) CuZnSOD (16 kDa) and MnSOD (25 kDa), (D) catalase (60 kDa), and (F) GPX (22 kDa) protein expression was evaluated by Western blot. Lanes: 1, untreated cells; 2, histamine-treated cells. Western blots are representative of three independent experiments. β-Actin (42 kDa) was used as loading control.

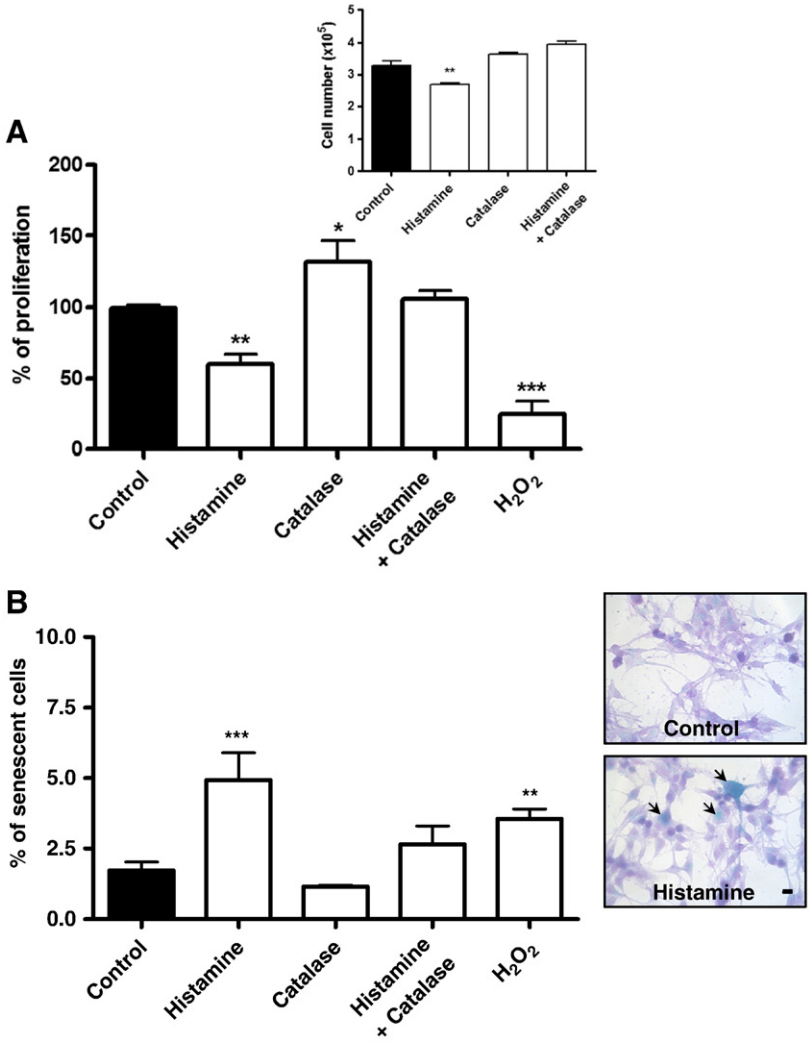

Fig. 3. Involvement of hydrogen peroxide in cell proliferation and senescence. WM35 cells were treated with histamine (10 μM), catalase (125 units/ml), histamine plus catalase, or H_2O_2 (5 μM) or were left untreated. (A) Proliferation was evaluated by the clonogenic assay. Inset: WM35 cells were treated or left untreated, harvested, and counted at 48 h after treatment. (B) Senescence induction was measured by senescence-associated β-galactosidase staining after 48 h of treatment. Arrows indicate senescent cells. Pictures were taken at a 630× original magnification. Scale bar, 20 μm. Error bars represent the means ± SEM (*$p<0.05$, **$p<0.01$, ***$p<0.001$ versus control). Results are representative of three independent experiments.

proliferation of WM35 cells. Furthermore, these treatments, which increased proliferation, significantly decreased hydrogen peroxide levels (Table 1).

Discussion

ROS are generated by all aerobic organisms and their production seems to be needed for signal-transduction pathways that regulate multiple physiological processes [19,20,29]. A variety of cancer cells have altered levels of antioxidant enzymes and often express elevated levels of ROS-responsive proteins compared with their normal counterparts. Consequently, it is suggested that the alteration in ROS control may sensitize tumor cells for a better response to selective treatments [29–31].

It is well documented that histamine modulates the proliferation of numerous malignant cells [14–17,32,33]. Additionally, we have previously determined that histamine decreased MDA-MB-231 breast cancer cells' proliferation and this effect was related to an increase in hydrogen peroxide levels [17].

In this study, we showed that exogenous histamine modulated the activity of the antioxidant enzymes, increasing SOD while decreasing catalase activity. Accordingly, histamine treatment markedly augmented the levels of hydrogen peroxide and diminished those of superoxide anion.

Recent data support the notion that increasing the cellular levels of hydrogen peroxide by using hydrogen-peroxide-generating drugs may be an efficient way of killing cancer cells [29,34]. Several studies have demonstrated that the overexpression of SOD can reduce tumor cell growth, metastasis, and other malignant features of cancer cells. Because this enzyme catalyzes the conversion of superoxide anion to

Table 1
H_2O_2 intracellular levels are inversely correlated with proliferation

	% of H_2O_2 levels[a]	% of proliferation[b]
Histamine 0.01 μM	57.8 ± 8.2**	135.0 ± 5.0*
Forskolin	42.3 ± 9.7**	200.0 ± 22.7**
Dimaprit	59.8 ± 5.8**	138.2 ± 7.8*

WM35 cells were treated with 0.01 μM histamine, 10 μM forskolin, or 10 μM Dimaprit or were left untreated.

[a] Data represent the means ± SEM of the mean percentage fluorescence intensity with respect to control values.

[b] Data represent the means ± SEM of the percentage of proliferation with respect to control values.

* $p<0.05$ versus control.

** $p<0.01$ versus control.

hydrogen peroxide, the anticancer effects induced by SOD overexpression may be mediated by a decrease in the cellular levels of superoxide anion or by an increase in the cellular concentration of hydrogen peroxide. Experimental data suggest that these anticancer effects can be reversed by overexpression of catalase and glutathione peroxidase. The antitumoral effect of augmented hydrogen peroxide levels may be attributed not only to changes in the cellular redox status but also to the formation of noxious hydroxyl radicals produced in the Fenton reaction [29,31,35,36].

To determine whether histamine-induced inhibition of proliferation could be related to the increase in hydrogen peroxide levels, we investigated the effects of histamine and catalase treatment on WM35 melanoma cell proliferation. Scavenging of hydrogen peroxide with exogenously added catalase slightly increased the proliferation of melanoma cells and completely reversed the inhibitory effect on cell growth exerted by histamine. Additionally, we observed that treatment with histamine at nanomolar concentration, or with forskolin or an H2 receptor agonist, increased proliferation while reducing hydrogen peroxide formation. These results provided evidence to suggest that hydrogen peroxide levels are inversely correlated with proliferation in WM35 cells, as we have previously determined for breast cancer cells [17].

There is a large body of experimental evidence that a rise in intracellular ROS contributes to senescence that is a biological process associated with permanent growth arrest and lack of proliferative activity and, therefore, may influence the overall tumor response to anticancer therapy [25,27,28]. It was reported that Ras proteins regulate oxidant production and that a rise in intracellular hydrogen peroxide represents a critical signal mediating replicative senescence in fibroblast [26]. As a result, we evaluated whether the histamine-induced reduction in proliferation was associated with an enhancement of cellular senescence. In this study we provide for the first time evidence that histamine is able to induce cellular senescence in melanoma cells. This effect was reversed by catalase addition and mimicked by hydrogen peroxide, indicating the participation of hydrogen peroxide in this response. In agreement, we have also determined that histamine enhances senescence in breast cancer cells (data not shown).

We hypothesize that the histamine-induced imbalance of antioxidant enzymes resulting in an elevation of intracellular hydrogen peroxide levels leads to inhibition of cell proliferation. This is in line with reports indicating that the anticancer effect of various chemotherapeutic agents currently used in the clinic (e.g., paclitaxel, cisplatin, arsenic trioxide, etoposide, doxorubicin) is mediated, at least in part, by an increase in the cellular levels of hydrogen peroxide. It has also been proposed that the identification of compounds that trigger a significant increase in intracellular peroxide levels and their use in conjunction with chemotherapy agents could be an attractive strategy to enhance the sensitivity of tumor cells to drug therapy [29,37].

On the basis of the present results, we conclude that histamine stimulates the production of hydrogen peroxide, suggesting the involvement of oxidative stress in the mechanism by which this biogenic amine induces growth suppression of WM35 melanoma cells.

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References

[1] Markovic, S. N.; Erickson, L. A.; Rao, R. D.; Weenig, R. H.; Pockaj, B. A.; Bardia, A.; Vachon, C. M.; Schild, S. E.; McWilliams, R. R.; Hand, J. L.; Laman, S. D.; Kottschade, L. A.; Maples, W. J.; Pittelkow, M. R.; Pulido, J. S.; Cameron, J. D.; Creagan, E. T. Malignant melanoma in the 21st century. Part 1. Epidemiology, risk factors, screening, prevention, and diagnosis. *Mayo Clin. Proc.* **82:**364–380; 2007.

[2] Gray-Schopfer, V.; Wellbrock, C.; Marais, R. Melanoma biology and new targeted therapy. *Nature* **445:**851–857; 2007.

[3] Agarwala, S. S.; Hellstrand, K.; Gehlsen, K.; Naredi, P. Immunotherapy with histamine and interleukin 2 in malignant melanoma with liver metastasis. *Cancer Immunol. Immunother.* **53:**840–841; 2004.

[4] Galmarini, C. M. Histamine dihydrochloride (subcutaneous) Maxim. *Curr. Opin. Invest. Drugs* **5:**1298–1310; 2004.

[5] Mitchell, M. S. Immunotherapy as part of combinations for the treatment of cancer. *Int. Immunopharmacol.* **3:**1051–1059; 2003.

[6] Molnár, E. L.; Hegyesi, H.; Tóth, S.; Darvas, Z.; Laszló, V.; Szalai, C.; Falus, A. Biosynthesis of interleukin-6, an autocrine growth factor for melanoma, is regulated by melanoma-derived histamine. *Semin. Cancer Biol.* **10:**25–28; 2000.

[7] Molnár, E. L.; Hegyesi, H.; Tóth, S.; Falus, A. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine* **12:**547–554; 2000.

[8] Haak-Frendscho, M.; Darvas, A.; Hegyesi, H.; Karpati, S.; Hoffman, R.; Laszló, V.; Bencsath, M.; Szalai, C.; Fürész, J.; Timár, J.; Bata-Csörgo, Z.; Szabad, G.; Pivarcsi, A.; Pállinger, É.; Kemény, L.; Horváth, A.; Dovozy, A.; Falus, A. Histidine decarboxylase expression in human melanoma. *J. Invest. Dermatol.* **115:**345–352; 2000.

[9] Darvas, Z.; Sakurai, E.; Schwelberger, H. G.; Hegyesi, H.; Rivera, E.; Othsu, H.; Watanabe, T.; Pállinger, E.; Falus, A. Autonomous histamine metabolism in human melanoma cells. *Melanoma Res.* **13:**239–246; 2003.

[10] Hegyesi, H.; Horváth, B.; Pállinger, E.; Pós, Z.; Mólnar, E. L.; Falus, A. Histamine elevates the expression of Ets-1, a protooncogene in human melanoma cell lines through H2 receptor. *FEBS Lett.* **579:**2469–2475; 2005.

[11] Tilly, B. C.; Tertoolen, L. G. J.; Remorie, R.; Ladoux, A.; Verlaan, I.; De Laat, S. W.; Moolenaar, W. H. Histamine as a growth factor and chemoattractant for human carcinoma and melanoma cells: action through Ca^{2+}-mobilizing H1 receptors. *J. Cell Biol.* **110:**1211–1215; 1990.

[12] Hegyesi, H.; Somlai, B.; Varga, V. L.; Toth, G.; Kovacs, P.; Mólnar, E. L.; Laszlo, V.; Karpati, S.; Rivera, E.; Falus, A.; Darvas, Z. Suppression of melanoma cell proliferation by histidine decarboxylase specific antisense oligonucleotides. *J. Invest. Dermatol.* **117:**151–153; 2001.

[13] Molnár, E. L.; Cricco, G.; Martin, G.; Darvas, Z.; Hegyesi, H.; Fitzsimons, C.; Bergoc, R.; Falus, A.; Rivera, E. Histamine as a potential autocrine regulator of melanoma. *Inflammation Res.* **50:**S102–S103; 2001.

[14] Molnár, E. L.; Hegyesi, H.; Pállinger, E.; Kovács, P.; Tóth, S.; Fitzsimons, C.; Cricco, G.; Martin, G.; Bergoc, R.; Darvas, Z.; Rivera, E. S.; Falus, A. Inhibition of human primary melanoma cell proliferation by histamine is enhanced by interleukin-6. *Eur. J. Clin. Invest.* **32:**743–749; 2002.

[15] Reynolds, J. L.; Akhter, J.; Morris, D. L. In vitro effect of histamine and histamine H1 and H2 receptor antagonists on cellular proliferation of human malignant melanoma cell lines. *Melanoma Res.* **6:**95–99; 1996.

[16] Pós, Z.; Hegyesi, H.; Rivera, E. S. Histamine and cell proliferation. In: Falus, A. (Ed.), Histamine: Biology and Medical Aspects. SpringMed Publ., Budapest,pp. 199–217; 2004.

[17] Medina, V.; Cricco, G.; Nuñez, M.; Martín, G.; Mohamad, N.; Correa-Fiz, F.; Sanchez-Jimenez, F.; Bergoc, R.; Rivera, E. Histamine-mediated signaling processes in human malignant mammary cells. *Cancer Biol. Ther.* **5:**1462–1471; 2006.

[18] Pani, G.; Bedogni, B.; Anzevino, R.; Colavitti, R.; Palazzotti, B.; Borello, S.; Galeotti, T. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p-53-deficient cells. *Cancer Res.* **60:**4654–4660; 2000.

[19] Dröge, W. Free radicals in the physiological control of cell function. *Physiol. Rev.* **82:** 47–95; 2002.

[20] Karihtala, P.; Soini, Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* **115:**81–103; 2007.

[21] Dimri, G. P.; Lee, X.; Basile, G.; Acosta, M.; Scout, G.; Roskelley, C.; Medrano, E.; Linskens, M.; Rubeli, I.; Pereira-Smith, O.; Peacocke, M. A biomarker that identifies senescent human cells in culture and aging skin in vivo. *Proc. Natl. Acad. Sci. USA* **92:**9363–9367; 1995.

[22] Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:**248–254; 1976.

[23] Policastro, L.; Molinari, B.; Larcher, F.; Blanco, P.; Podhajcer, O. L.; Costa, C. S.; Rojas, P.; Durán, H. Imbalance of antioxidant enzymes in tumor cells and inhibition of proliferation and malignant features by scavenging hydrogen peroxide. *Mol. Carcinog.* **39:**103–113; 2004.

[24] Flohé, L.; Gunzler, W. A. Assays of glutathione peroxidase. *Methods Enzymol.* **105:** 114–121; 1984.

[25] Kim, R.; Emi, M.; Tanabe, K. Role of apoptosis in cancer cell survival and therapeutic outcome. *Cancer Biol. Ther.* **52:**1424–1429; 2006.

[26] Lee, A. C.; Fenster, B. E.; Ito, H.; Takeda, K.; Bae, N. S.; Hirai, T.; Yu, Z. X.; Ferrans, V. J.; Howard, B. H.; Finkel, T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J. Biol. Chem.* **274:**7936–7940; 1999.

[27] Roninson, I. B. Tumor cell senescence in cancer treatment. *Cancer Res.* **63:** 2705–2715; 2003.

[28] Lu, T.; Finkel, T. Free radicals and senescence. *Exp. Cell Res.* **314:**1918–1922; 2008.

[29] López-Lázaro, M. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. *Cancer Lett.* **252:**1–8; 2007.

[30] Adler, V.; Yin, Z.; Tew, K. D.; Ronai, Z. Role of redox potential and reactive oxygen species in stress signalling. *Oncogene* **18:**6104–6111; 1999.

[31] Li, S.; Yan, T.; Yang, J. Q.; Oberley, T. D.; Oberly, L. W. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res.* **60:**3927–3939; 2000.

[32] Cricco, G.; Martin, G.; Lombarda, F.; Cocca, C.; Bergoc, R.; Rivera, E. Human pancreatic carcinoma cell line Panc-I and the role of histamine in growth regulation. *Inflammation Res.* **49:**68–69; 2000.
[33] Adams, W. J.; Lawson, J. A.; Morris, D. L. Cimetidine inhibits in vivo growth of human colon cancer and reverses histamine stimulated in vitro and in vivo growth. *Gut* **35:**1632–1636; 1994.
[34] Gorman, A.; McGowan, A.; Cotte, T. G. Role of peroxide and superoxide anion during tumour cell apoptosis. *FEBS Lett.* **404:**27–33; 1997.
[35] Church, S. L.; Grant, J. W.; Ridnour, L. A.; Oberley, L. W.; Swanson, P. E.; Meltzer, P. S.; Trent, J. M. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc. Natl. Acad. Sci. USA* **90:** 3113–3117; 1993.
[36] Kinnula, V. L.; Crapo, J. D. Superoxide dismutases in malignant cells and human tumors. *Free Radic. Biol. Med.* **36:**718–744; 2004.
[37] Agostinelli, E.; Seiler, N. Lysosomotropic compounds and spermine enzymatic oxidation products in cancer therapy. *Int. J. Oncol.* **31:**473–484; 2007.