Histidine Decarboxylase Expression in Human Melanoma

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Histamine has been implicated as one of the mediators involved in regulation of proliferation in both normal and neoplastic tissues. Histidine decarboxylase, the only enzyme that catalyzes the formation of histamine from L-histidine, is an essential regulator of histamine levels. In this study, we investigated the gene and protein expression of histidine decarboxylase in melanoma. Reverse transcriptase polymerase chain reaction and *in situ* hybridization studies of WM-35, WM-983/B, HT-168, and M1 human melanoma cell lines both resulted in positive signals for histidine decarboxylase messenger RNA. A polyclonal chicken antibody was developed against human histidine decarboxylase and protein expression was confirmed by western blot analysis of the cell lysates, revealing a predominant immunoreactive band at approximately 54 kDa corresponding to monomeric histidine decarboxylase. Protein expression of histidine decarboxylase was also shown by flow cytometric analysis and strong punctate cytoplasmic staining of melanoma cell lines. Moreover, both primary and metastatic human melanoma tissues were brightly stained for histidine decarboxylase. When compared with the very weak or no reactions on cultivated human melanocytes both western blot and immunohistochemical studies showed much stronger histidine decarboxylase expression in melanoma cells. These findings suggest that expression of histidine decarboxylase is elevated in human melanoma. ***Key words: antibody/histamine/histidine decarboxylase/melanoma. J Invest Dermatol 115:345–352, 2000***

Histamine has a pivotal role in a variety of *in vivo* reactions. In the immune system, histamine plays a central role as an effector molecule in hypersensitivity reactions following its release from mast cells and basophils (Pearce, 1991; Sitter *et al*, 1991). It also modulates other cellular responses of the immune system via specific histamine receptors expressed on immune competent cells (Beer and Rocklin, 1987; Nielsen, 1995). At physiologic concentrations, histamine can act as an immunostimulant, mediated primarily through the H1 receptor (Beer and Rocklin, 1987). At increased pathophysiologic concentrations it can lead to immunosuppression, as regulated predominantly through the H2 receptor (Dohlsten *et al*, 1987; Hellstand and Hermodsson, 1991). Others have correlated the rate of proliferation in wound repair, embryogenesis, hematopoiesis, and malignant growth with the level of histamine production (Kahlson and Rosengren, 1968; Tang and Xu, 1987). Even if the concentration of local histamine in proliferating tissues compared with that in immediate hypersensitivity (''allergic'') reactions is relatively low, its effectiveness acting on the autocrine and paracrine way is strongly implicated and a growth-factor-like activity of histamine has been described (Tilly *et al*, 1990).

Histamine levels in cells and tissues are regulated by histidine decarboxylase (HDC), the only enzyme that catalyzes the formation of histamine from L-histidine (Schneider *et al*, 1987; Imanishi *et al*, 1989; Mamune-Sato *et al*, 1990). Because of its unique function, HDC can serve as a specific marker for biosynthesis of histamine. High level expression of HDC mRNA precedes a sharp increase in the concentration of intracellular histamine (Brandes *et al*, 1991a,b). This observation implies that the binding of histamine to a putative intracellular binding site could be one of the key intracellular signal elements to initiate cellular proliferation. Because of the growth factor activity of histamine and its elevated levels in rapidly proliferating tumor cells, we have investigated the possibility that HDC might be an early indicator of neoplasia.

In this study, an antihuman HDC polyclonal antibody (pAb) was developed and used in conjunction with HDC molecular probes to evaluate HDC expression in human melanoma cell lines and primary tissues.

MATERIALS AND METHODS

RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) Cytoplasmic total RNA was prepared from human melanoma cell lines (WM-35, WM-983/B, EP, HT-168, and M1) and also from peripheral blood mononuclear cells of healthy donors. Total RNA was extracted according to the method of Chomczynski and Sacchi (1987) and was used for subsequent RT-PCR reactions. HDC

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Abbreviations: ABP, androgen binding protein; HDC, histidine decarboxylase; pAb, polyclonal antibody.

primers 5′-AATCTTCAAGCACATGTC-3′ and 5′-CTGGATAGTGG-CCGGGATGA-3′, designed to span exons 10 and 12, were used to amplify a 208 bp product (Mamune-Sato *et al*, 1990). The identity of the HDC PCR product was further confirmed by nucleotide sequencing. PCR products were separated by 2% wt/vol agarose gel electrophoresis and visualized by ethidium bromide staining.

***In situ* hybridization** WM-983/B melanoma cells were cultured on glass slides and then fixed with 4% (vol/vol) formaldehyde in phosphate-buffered saline (PBS), pH 7.4. The denaturation-hybridization process was performed in a Hybaid thermal cycler as described by Naoumov *et al* (1988), using a biotinylated HDC probe comprising the shorter sequence used for RT-PCR shown above. Hybrids were detected by the ABC-AP (Vector Laboratories, Burlingame, CA) method. Control cultures were processed in parallel without HDC probe.

Antibody development Peptide-derived antibodies corresponding to amino acid residues 3–14 (EPEEYRERGREM) and 318–325 (VKDKYKLQ) of the full-length human HDC protein were generated in white leghorn chickens. The two peptides were selected on the basis of hydrophilicity, surface probability, and uniqueness compared with known proteins in the GenBank database. Based on the database the protein with the closest sequence homology is the androgen binding protein (ABP); however, the sequences represented by these two peptides are not present in ABP. The corresponding oligonucleotides were inserted into a vector coding for carrier segment and the HDC fusion proteins were admixed with Freund's adjuvant prior to immunization of white leghorn hens. Following four subcutaneous 0.5 ml injections of 0.2 mg fusion eggs were collected from the immunized hens. Total IgY (the avian IgG homolog) was purified from the egg yolks of immunized hens by sequential precipitation (IgY Purification System, Promega, Madison, WI). For some experiments as indicated, the HDC318–325 pAb was affinity purified against the biotinylated peptide expressed using the PinPoint XA Purification System (Promega) bound to Tetralink Tetrameric Avidin resin (Promega). The pAb was eluted under acidic conditions and dialyzed overnight in tris(hydroxymethyl)-aminomethane (Tris)-buffered saline (pH 7.3) prior to use.

Western blot analysis Extracts of WM-983/B, WM-35, HT-168, M1, and EP melanoma cell lines, normal skin samples, cultivated human melanocyte cells, and primary and metastatic melanoma tissue biopsies were subjected to denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (Novex 4%–20% gels) and then transferred onto nitrocellulose membranes. In these experiments the lysis was performed in a lysis buffer (containing 10 mM 1 M Tris-HCl, pH = 8.0, 10 μg per ml leupeptine, 0.5 mM EGTA 0.5 M, 2% NaF, 1% Triton-X 100, 25 mM phenylmethylsulfonyl fluoride and 2% Na orthovanadate. Each gel lane was loaded with 10 μg of total extracted protein. The transfers were blocked with PBS containing 0.05% (vol/vol) Tween-20 and 0.1% (wt/vol) bovine

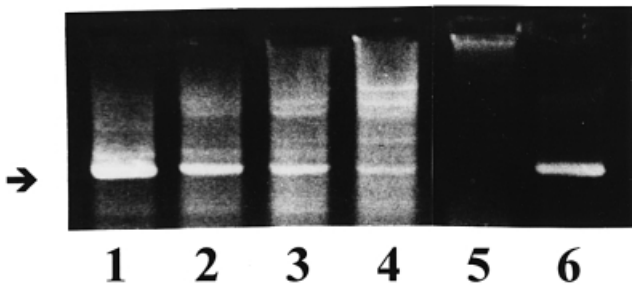

Figure 1. RT-PCR analysis of HDC mRNA expression in human melanoma cell lines. *Lane 1*, WM-983/B cell line cDNA; *lane 2*, WM-35 cell line cDNA; *lane 3*, HT-168 cell line cDNA; *lane 4*, M1 cell line cDNA; *lane 5*, unstimulated human PBL cDNA; *lane 6*, HDC control PCR products from pTN-2 plasmid containing full length cDNA of 2.4 kb coding for HDC. HDC cDNA band (208 bp) is indicated by the arrow.

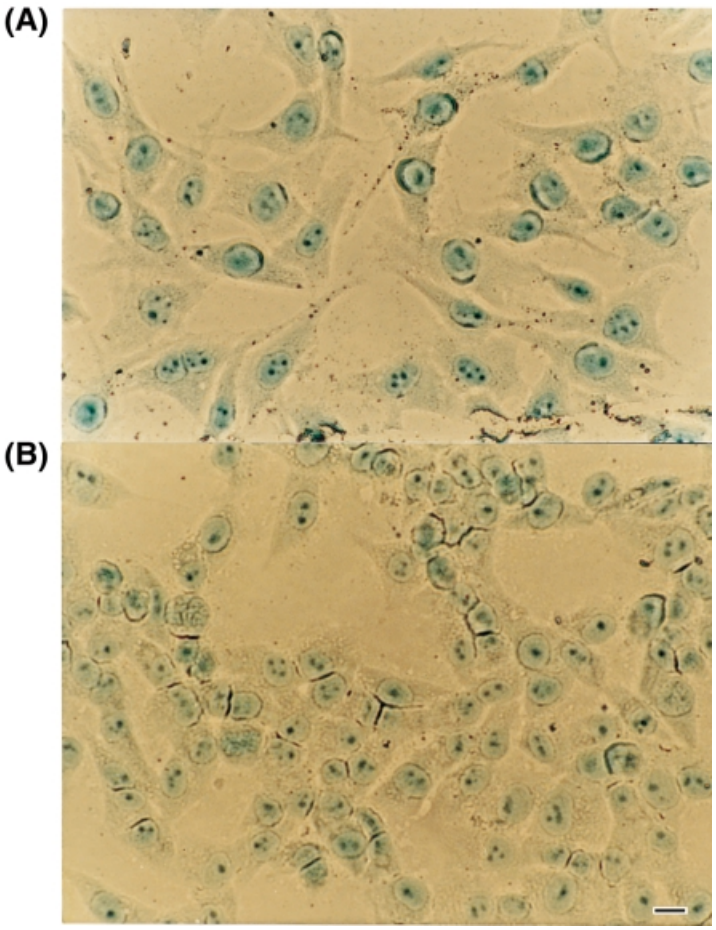

Figure 2. *In situ* hybridization for detection of HDC mRNA in WM-983/B human melanoma cells. WM-983/B human melanoma cells are shown to express HDC mRNA (*a*, dark spots in cytoplasm of the cells) when probed with a biotinylated DNA probe. Control cells, processed without probe, are shown in (*b*). *Scale bar*: 20 μm.

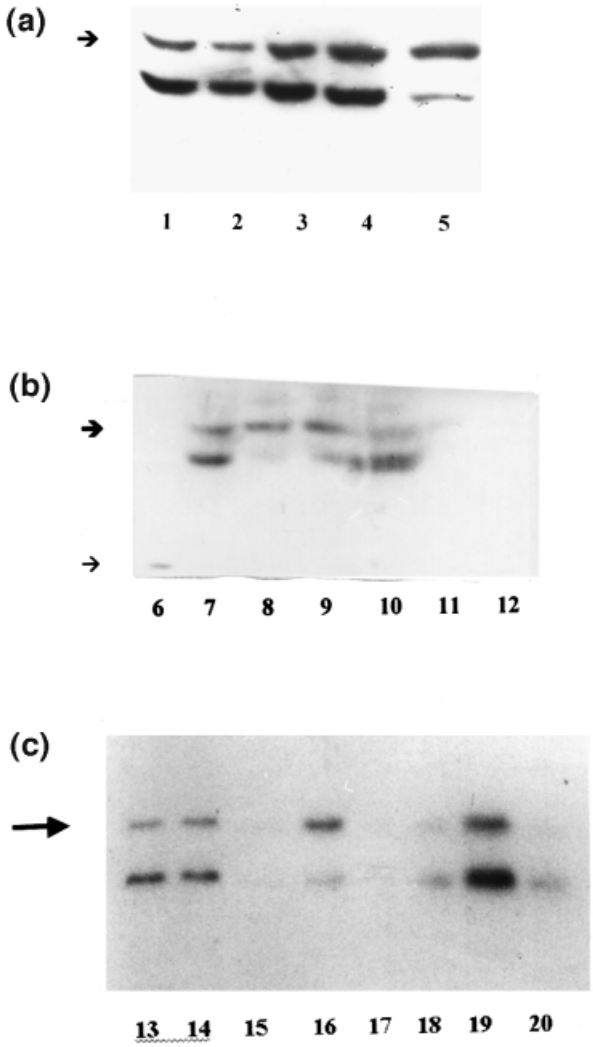

Figure 3. Detection of HDC protein by western blot analysis. (*a*) In human melanoma cell lines; (*b*) in melanoma tissue biopsies; (*c*) direct comparison of melanocytes with melanoma cells and melanoma tissues. Lysates (10 μg per lane) were from (*a*) WM-983/B (*1*), WM-35 (*2*), HT-168 (*3*), M1 (*4*), and EP (*5*); (*b*) fusion peptide immunogen (*6*), primary and metastatic melanoma biopsies (*7–10*), and normal human skin (*11*, *12*); (*c*) HT-168 melanoma cells (*13*, *14*), primary melanoma tissues (*16*, *19*), and melanocytes (*15*, *17*, *18*, and *20*). The samples were probed with 5 ng per ml affinity-purified HDC318–325 pAb. The position of the mature HDC 53–55 kDa protein and fusion peptide immunogen are indicated by the thick and thin arrows, respectively.

serum albumin (BSA), probed with 5 ng per ml affinity-purified HDC318–325 antibody, washed, incubated with 0.2 μg per ml AP conjugate, and developed with Western Blue (Promega).

Flow cytometry The WM-35 and WM-983/B human melanoma cell lines were evaluated for HDC by flow cytometric analysis. Cells, maintained in RPMI 1640 supplemented with 10% fetal bovine serum, were washed in PBS containing 1% (wt/vol) BSA and aliquoted at 5×10^6 cells per ml. The cells were fixed in 1% (vol/vol) paraformaldehyde and permeabilized using 1% (vol/vol) Triton x-100. The cells were subsequently stained for 30 min at 4°C with 180 μg per ml HDC3–14 and 18 μg per ml HDC318–325 primary antibodies. (The concentrations were optimized in previous experiments.) Preimmune chicken sera was used as a control. Following two PBS washes, cells were incubated 30 min with 1 μg per ml FITC-conjugated secondary antibody (Promega). Cells were then washed with PBS and resuspended in 0.5 ml 1% paraformaldehyde. Propidium iodide was added immediately before flow cytometric analysis to gate out the dead cells. Fluorescence was examined on an Elite flow cytometer (Coulter, Epics Elite, Hialeah, FL) using Elite 4.1 software.

Immunostaining Melanoma cell lines WM-35, WM-983/B, HT-168, and EP, human melanocytes, the basophilic leukemia CML, and human melanoma tissues, including primary and metastatic forms, were stained for HDC. Normal human melanocytes were obtained from a patient who underwent plastic surgery. Most of the dermal tissue was cut off before the skin was placed into dispase for overnight treatment at 4°C (Gibco Laboratories, Csertex, Hungary). After the dispase treatment the epidermis was peeled off from the remaining dermis. The epidermal sheet was then treated with 0.25% trypsin for 20 min at 37°C (Gibco). Cells were cultured in AIM V and KBM media (vol/vol) supplemented with 5% fetal bovine serum, 5 ng per ml epidermal growth factor, and 50 μg per ml bovine pituitary extract (all from Gibco). The cells were in third passage when used for the experiment. Cells were stained after the first passage with the monoclonal antibody Mel-5 (Signet Laboratories, Dedham, MA) and a pAb for S-100 (DAKO, Frank Diagnosztika, Hungary). After the first passage all cells in the culture showed positive staining for both Mel-5 and S-100, indicating 100% purity for melanocytes. For detection of HDC cultured normal melanocytes in third passage were briefly trypsinized (0.05% trypsin and 0.05% EDTA for 10 min at 37°C) and placed on slides. After removal of the dermis, epidermal cell suspensions were prepared by dispase treatment (Gibco) from skin samples of healthy persons who underwent plastic surgery. Melanocyte cells were cultivated in the presence of epidermal growth factor and bovine pituitary extract (Gibco). Cells were grown on slides and fixed in methanol for 10 min at –20°C followed by a cold acetone rinse. Slides then were blocked with 0.5% BSA for 30 min. The primary antibodies were applied to the slides at 30 or 90 μg per ml (HDC3–14 pAb) or 18 μg per ml (HDC318–325 pAb) in PBS and incubated in a humidified chamber for 1 h at room temperature. In some experiments, culture medium of isolated human melanocytes in second or third passage contained 10 ng per ml phorbol myristate acetate (TPA, Sigma) and the cells were subsequently stained with antibodies, either HDC318–325 pAb or Mel-5. Control chicken IgY (Promega) at an equivalent concentration was used to assess nonspecific background staining in all experiments. Following three PBS washes, the slides were incubated for 1 h at room temperature with 0.5 μg per ml rabbit antichicken IgY FITC conjugate. In some experiments the second antibody was labeled with biotin and for development streptavidine-peroxidase conjugate (Sigma, Hungary) and VIP kit (Vector) was applied.

RESULTS

HDC messenger RNA detection by RT-PCR and *in situ* hybridization Human melanoma cell lines WM-983/B, WM-35, HT-168, M1, and unstimulated peripheral blood mononuclear cells were tested for the presence of HDC messenger RNA by RT-PCR. The pTN-2 plasmid containing 2.4kb cDNA coding for HDC was used as the positive control. Using HDC primer pairs, a 208 bp product corresponding to a band of the predicted size was observed in the melanoma cell lysates (**Fig 1**). The proper identity of this band was further confirmed by sequencing the HDC PCR product (data not shown). *In situ* hybridization studies also revealed a clear signal for HDC in both WM-35 (data not shown) and WM-983/B melanoma cell lines (**Fig 2**), again confirming the presence of HDC mRNA in these cells.

Detection of 54 kDa HDC by western analysis Lysates from the human melanoma cell line WM-983/B, WM-35, HT-168, M1, and EP melanoma cell lines, human melanoma tissues, including primary and metastatic forms, and normal human skin were probed with affinity-purified HDC318–325 pAb (**Fig 3*a***, ***b***). The lysates from melanoma lines contained a prominent immunoreactive doublet (53–55 kDa and 46–47 kDa). The upper band (53–55 kDa) corresponds to the reported size of the monomeric form of human HDC. A very faint band in lanes 11 and 12 in **Fig 3(*b*)** corresponding to normal human skin is also seen. In contrast, lysate from unstimulated peripheral blood lymphocytes (PBL) showed no appreciable reactivity (not shown). A second band of approximately 46–47 kDa was prominent in all melanoma cell lines (**Fig 3*a***) in our studies. In melanoma tissue samples (**Fig 3*b***, *lanes 7–10*), the intensity of this 46–47 kDa band and the relative ratio to the 53–55 kDa band varies from sample to sample. The immunogen (fusion peptide) serves as a positive control, yielding an immunoreactive band at its predicted low molecular weight (**Fig 3*b***, *lane 6*). Previous adsorption of the antibody with

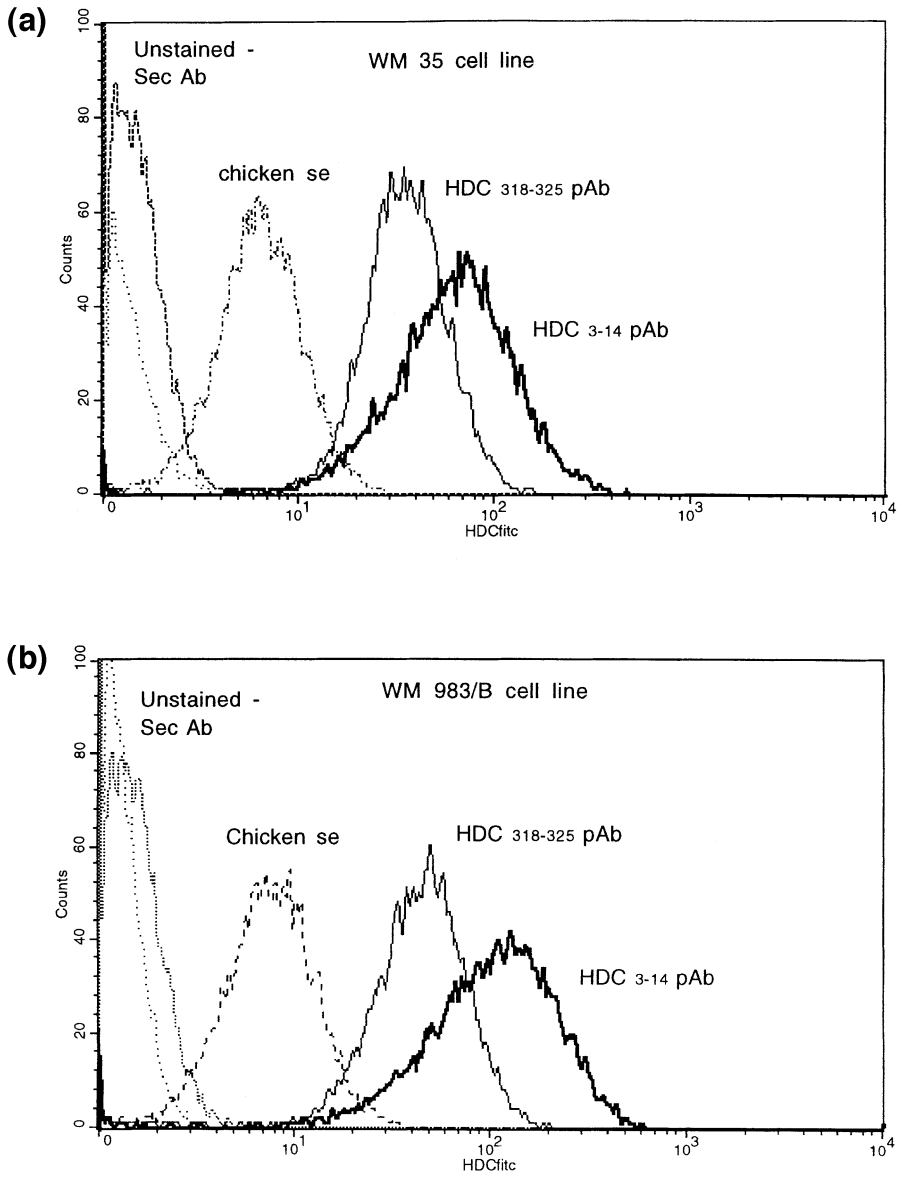

Figure 4. Flow cytometric analysis of WM-35 and WM-983/B human melanoma cell lines. WM-35 (*a*) and WM-983/B (*b*) cells were unstained (. . .); stained with 20 µl of secondary FITC-conjugated antibody at 10 µg per ml (……); stained with 40 µl chicken serum at a 1:2000 dilution (– – – –); stained with 35 µl HDC3–14 pAb at 180 µg per ml (____); stained with 40 µl HDC318–325 pAb at 18 µg per ml (——).

the immunogen peptide construct completely eliminated both bands (not shown).

Figure 3(*c*) shows a direct comparison of HDC expression in HT-168 melanoma cells (*lanes 13*, *14*), primary melanoma tissues (*lanes 16*, *19*), and melanocytes (*lanes 15*, *17*, *18*, and *20*).

HDC is detected in human melanoma cells by flow cytometry Both HDC3–14 and HDC318–325 peptide pAbs brightly stained the permeabilized human WM-35 (**Fig 4***a*) and WM-983/B (**Fig 4***b*) melanoma cells. There is an approximately 1.7-fold difference in the intensity of staining between **Fig 4**(*a*) and **Fig 4**(*b*), i.e., WM-983/B cells contain more immunoreactive HDC than WM-35. In separate experiments this staining was shown to be dose-dependent (data not shown).

HDC immunoreactivity in tumor cell lines and in both primary and metastatic human melanoma tissues Because of the growth factor activity of histamine, we speculated that HDC might be an early indicator of malignant cell proliferation. Immunocytochemistry employing CML basophilic leukemia cells, used as positive controls because they are known to contain high levels of histamine, showed strong punctate cytoplasmic staining with both HDC3–14 (**Fig 5***D*) and HDC318–325 (**Fig 5***E*) polyclonal chicken antibodies. Human melanoma cells showed similar bright, punctate staining in the cytoplasm with HDC3–14 (**Fig 5***A*, *G*) and HDC318–325 pAb (**Figs 5***B*, *F*, *H*, and *I*), thereby confirming that human melanoma cells contain significant amounts of HDC. Cells stained with control IgY showed minimal immunoreactivity (**Fig 5***C*). Using affinity-purified HDC318–325 antibody and peroxidase-labeled antichicken antibody we found no HDC immunostaining in normal human melanocytes (**Fig 6***A*), whereas strong positivity was detected in human melanoma cells (**Fig 6***C*). Non-immune chicken IgY gave no reaction (**Fig 6***B*, *D*). Similar findings were obtained with HDC3–14 antibody (not shown). TPA treatment increased the Mel-5 positivity of normal melanocytes (**Fig 7***A*, *B*) but did not induce visible HDC positivity (**Fig 7***C*, *D*). On testing WM-35 melanoma cells very strong Mel-5 positivity was found (**Fig 7***E*). TPA treatment did not further increase either the Mel-5 or HDC positivity of melanoma cells (not shown).

Human melanoma tissues from residual surgical samples, including primary and metastatic forms, were also stained with HDC3–14 and HDC318–325 pAb. Frozen sections from primary melanoma tissue were stained with 18 µg per ml HDC3–14 pAb

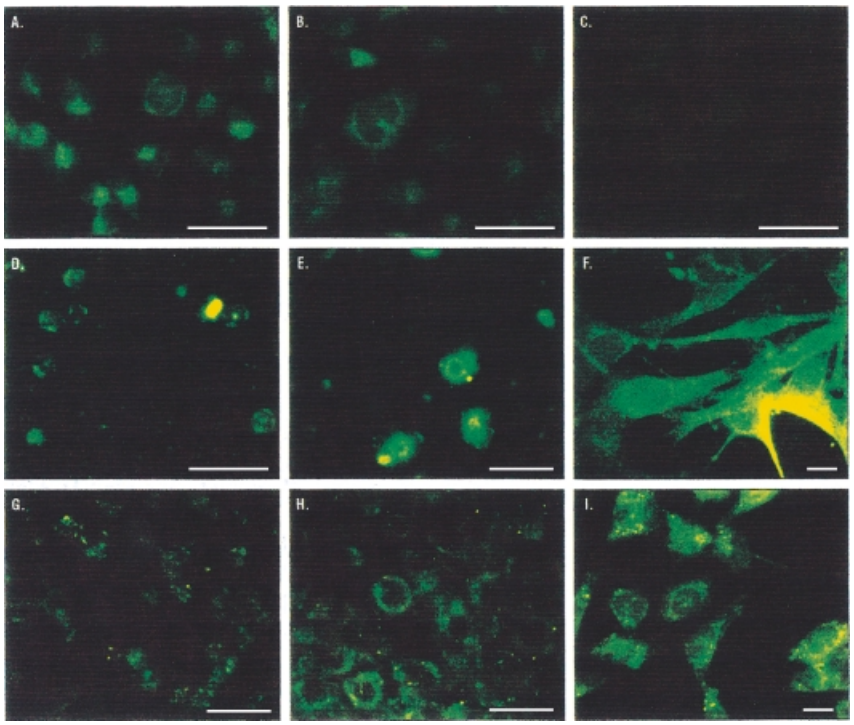

Figure 5. Bright staining of human basophilic leukemia and melanoma cell lines with HDC3–14 and HDC318–325 pAb. A distinctive punctate (granular) cytoplasmic staining pattern was observed in all melanoma and basophilic leukemia cells screened using HDC3–14 and HDC318–325 pAb. Human EP melanoma cells showed a similar cytoplasmic staining pattern when stained with HDC3–14 pAb (*A*) compared with HDC318–325 pAb (*B*). A typical negative control stained slide is shown in (*C*) (EP cells stained with control IgY and conjugate). CML basophilic leukemia cells demonstrated a striking punctate pattern with both HDC3–14 pAb (*D*) and HDC318–325 pAb (*E*), which was similar to that seen in HT-168 cells stained with HDC3–14 pAb (*G*) and HDC318–325 pAb (*H*). Other melanoma cell lines, WM-35 (*F*) and WM-983/B (*I*), stained with HDC318–325 pAb, also revealed a cytoplasmic punctate staining pattern. Antibodies were used at 18 μg per ml in all experiments. *Scale bars*: (*A–E*, *G*, *H*, *I*) 10 μm; (*F*) 20 μm.

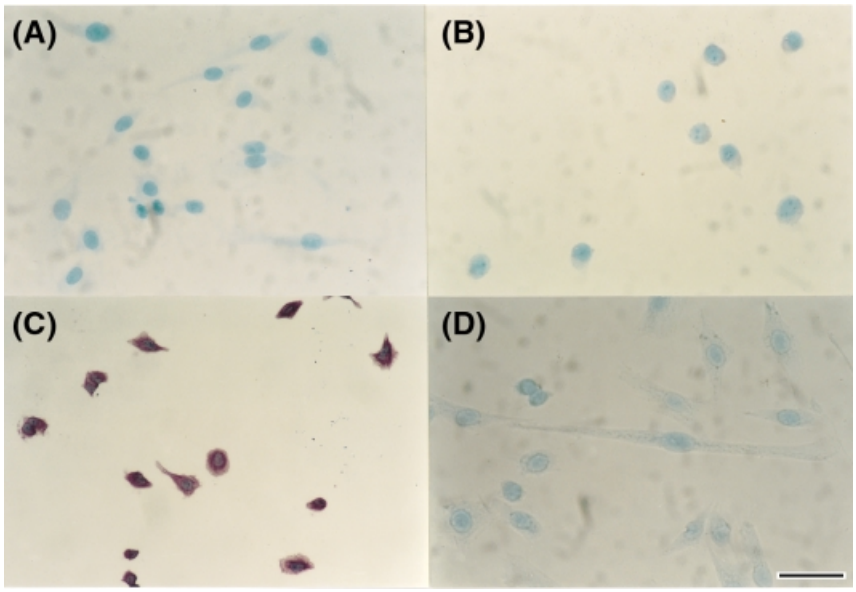

Figure 6. HDC immunostaining using peroxIdase-labeled second antibody of human melanocytes and HT-168 melanoma cell line. (*A*) human melanocytes; (*C*) HT-168 melanoma cell line. Parts (*B*) (melanocyte) and (*D*) (HT-168) show the immunostaining when nonimmune chicken IgY is applied.. The nuclei were stained with 0.5% methylgreen. *Scale bar*: 10 μm

(**Fig 8*A***) or HDC318–325 pAb (**Fig 8*B***). A strong cytoplasmic signal was observed. Frozen sections from a subcutaneous metastatic lesion also were stained with 18 μg per ml HDC3–14 (**Fig 8*C***), HDC318–325 (**Fig 8*D***), or control IgY (**Fig 8*E***). Non-tumorous skin tissue from the same patient was negative for HDC staining and also only very rarely positive for Mel-5 (not shown). Both HDC antibodies showed a more uneven distribution of cytoplasmic staining in the metastatic cells compared with the cells of the primary lesions, whereas no specific signal was seen with the control antibody.

DISCUSSION

Previous studies have shown that endogenous histamine plays an important role in regulating cell proliferation in normal and neoplastic cells. Highly increased histamine biosynthesis and content has been reported in different human and experimental neoplasias, such as in breast carcinomas and adenocarcinomas (Scolnik *et al*, 1984; Garcia-Caballero *et al*, 1989; Cricco *et al*, 1994). Both *in vitro* and *in vivo* experiments using histamine receptor antagonists (Van der Ven *et al*, 1993; Watson *et al*, 1993) have demonstrated that histamine acts through the specific histamine membrane receptors (H1, H2, and H3) and may regulate tumor growth and development (Cricco *et al*, 1993) in a paracrine or autocrine manner. Moreover, the most compelling evidence supporting a significant role for histamine in gastric and colorectal carcinomas are the results of clinical trials showing increased survival of gastric cancer patients after treatment with cimetidine, an H2 receptor antagonist (Burtin *et al*, 1988; Tonnesen *et al*, 1988). In addition to promoting the proliferation of tumor cells,

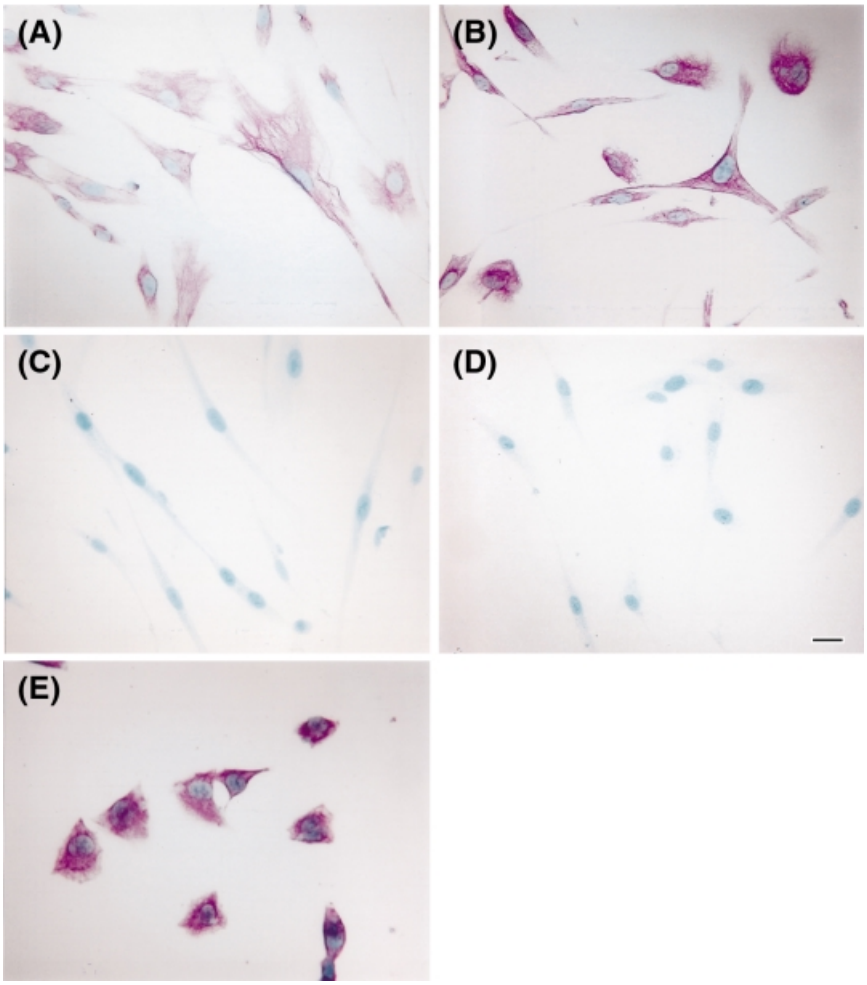

Figure 7. Mel-5 and HDC immunostaining of human melanocytes and melanoma cells. Mel-5 (*A*, *B*) and HDC (*A*, *D*) immunostaining of human melanocytes in the absence (*A*, *C*) or in the presence (*B*, *D*) of phorbol ester (10 ng per ml TPA). WM-35 melanoma cells (*E*) were stained with antibody specific to Mel-5.. The nuclei were stained with 0.5% methylgreen. *Scale bar*: 20 μm

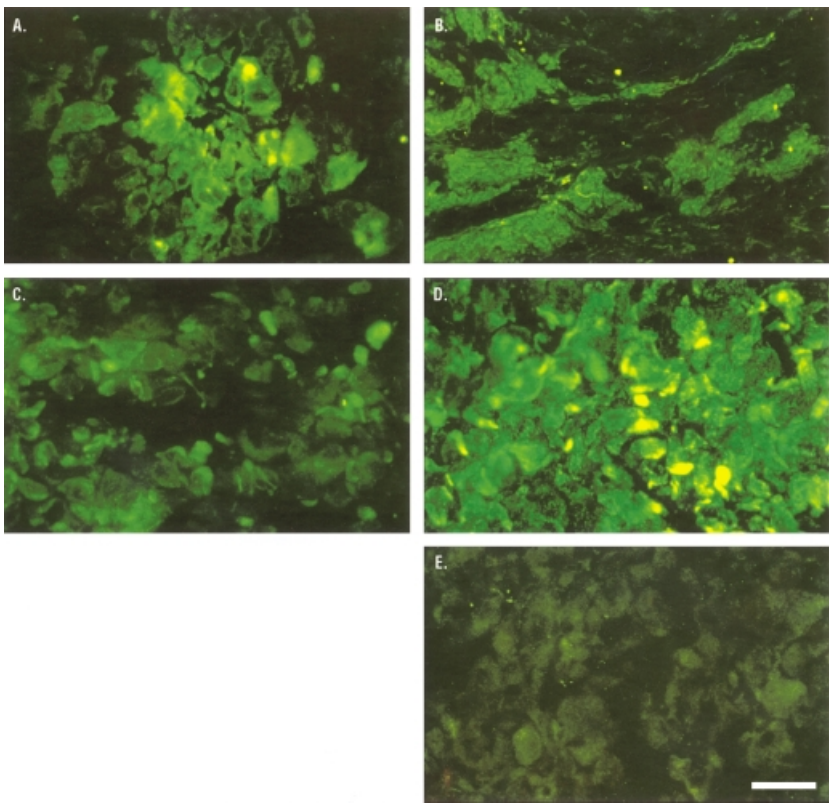

Figure 8. Cytoplasmic staining pattern of human primary and metastatic melanoma tissue. Frozen sections from primary melanoma tissue stained with HDC3–14 pAb (*A*) or HDC318–325 pAb (*B*) show strong cytoplasmic staining. Frozen sections from a subcutaneous metastatic lesion were stained with HDC3–14 (*C*), HDC318–325 (*D*), or control IgY (*E*). Both HDC antibodies show a more uneven distribution of cytoplasmic staining in the metastatic cells compared with the cells in the primary lesions. Antibodies were used at 18 μg per ml in all experiments. *Scale bars*: (*A*, *C–E*) 40 μm; (*B*) 10 μm.

increased histamine levels also have potent immunosuppressive effects that favor tumor cell growth by blunting natural killer activity (Hellstand and Hermodsson, 1991) and activating the suppressor function of T cells (Bartholeyns and Fozard, 1985). Histamine levels in cells and tissues are exclusively regulated by HDC; this enzyme catalyzes the formation of histamine from L-histidine (Schneider *et al*, 1987; Imanishi *et al*, 1989; Mamune-Sato *et al*, 1990). Expression of the HDC gene in melanoma cell lines is approximately one-tenth that of the basophilic cell line KU-812F as determined by competitive RT-PCR (manuscript in preparation). If the autocrine and the putative intracellular pathways are considered, however, this amount of histamine may also effectively interfere locally with various cellular activities such as proliferation and secretion of various growth factors.

Over the course of this study a specific pAb was developed that enabled us to test the expression of HDC by immunochemical analysis, western blot and cytofluorometry. As HDC acts as a specific marker for histamine, detection of HDC seems to be an early indicator of histamine-mediated proliferation and immune system suppression. Indeed, increased HDC activity has been measured in human colorectal tumor specimens (Garcia-Caballero *et al*, 1988) and the inhibitory effects of α-fluoromethyl-histidine, a suicide inhibitor of HDC (Watanabe *et al*, 1990), has been demonstrated in tumor models (Bartholeyns and Bouclier, 1984).

Much of the aforementioned clinical work linking high HDC and histamine levels to neoplasia was in gastric and colorectal cancer and various forms of leukemia. Recent studies, however, have shown that histamine levels, as detected by high performance liquid chromatography, are also elevated in human melanoma (Reynolds *et al*, 1996). Therefore, we have chosen to focus on melanoma cells and tissues for evaluating expression of HDC.

Results of this study demonstrate HDC immunoreactivity in all of the melanoma cell lines tested (**Figs 3–6**). This immunoreactivity corresponds to the presence of HDC messenger RNA, as assessed by RT-PCR (**Fig 1**) and nucleotide sequencing, as well as by *in situ* hybridization (**Fig 2**). The finding of paramount importance in this study is the demonstration that human primary and metastatic melanoma tissues from surgical samples are HDC immunoreactive. Although there is some sequence homology between HDC and ABP, the second band on western immunoblots probably does not correspond to ABP, as the two peptides in immunogen are not present in ABP. Specificity is suggested as adsorption with the peptide construct used for immunization completely removes the immunoreactivity of the antibody both in western immunoblot and in immunohistochemical studies. Further studies are in progress to elucidate the molecular nature of the minor band on immunoblots. The appearance of this 46–47 kDa band compared with that of monomeric HDC (53–55 kDa) shows a dissimilar pattern (i.e., there is relatively strong expression in melanoma cell lines and various manifestations in melanoma tissue specimens) that requires further analysis.

With regard to immunostaining, subtle differences were observed in the staining patterns between various melanoma samples. The primary lesions (**Fig 8*A*, *B***) showed a uniform cytoplasmic staining pattern whereas metastatic lesions (**Fig 8*C*, *D***) had a more irregular pattern of cytoplasmic staining. The differential staining pattern remains to be confirmed in a larger study, but it may have important implications for assessing neoplastic tissues. Subsequent on-going studies indicate that expression of HDC (both at mRNA and protein levels) correlates well with HDC enzyme activity (manuscript in preparation). The absence of expression of HDC in Mel-5 positive melanocytes isolated from skin samples of healthy persons suggests that level of HDC expression is strongly associated with malignancy in skin.

Further clinical data from benign naevi, dysplastic naevi, and primary and metastatic melanoma are required. This preliminary study, however, suggests that HDC immunoreactivity, indicating local (autocrine or paracrine rather than type I like ''allergic'') action of histamine, may be useful as a clinical correlate for melanoma staging.

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