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Histamine and histidine decarboxylase up-regulation in colorectal cancer: correlation with tumor stage

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Introduction

Histamine plays a pivotal role in a number of processes, including inflammation, allergic reaction, gastric acid secretion and neurotransmission. These actions are mediated by the activation of specific histamine receptors, namely H_1, H_2, H_3 and H_4. Histamine levels in cells and in tissues are regulated by the activity of histidine decarboxylase (HDC), that is the only enzyme responsible for the generation of histamine from L-histidine [1]. Therefore, HDC can serve as a specific marker for the biosynthesis of histamine. It has been hypothesized that histamine could regulate tumor cell growth; in fact the activation of H_2 receptors leads to cell proliferation through the transcription of the protooncogene *c-fos*. Histamine stimulates the in vitro and in vivo growth of gastrointestinal cancer cells and treatment with cimetidine, an H_2 antagonist, can reverse this effect [2]. Tumor invasion and metastases are clinically more relevant than tumor growth and histamine has been reported to act as an angiogenic factor, inducing vascular endothelial growth factor production in granulation tissue [3]. Moreover, histamine seems to be also involved in inhibition of the local immune response against cancer.

In the present study, we determined histamine content and HDC activity in human colorectal cancer and correlated them with tumor stages and intratumor microvessel density (MVD).

Materials and methods

Patients and tissue collection

Tissue samples were obtained from 27 patients (13 males, 14 females; median age 64 years) who had consecutively undergone surgical resections for colorectal adenocarcinomas at the General Surgery Unit, University of Florence, Italy. All the patients were informed about the aims of the study and gave written consent for the investigation. Cancers were classified into four stages according to the American Joint Commette on Cancer staging system. Tissue samples from the edge of the tumor and normal mucosa 10 cm far from the tumor were excised from each patient. The samples were either washed in PBS and frozen at –80 °C for use in the HDC and histamine assay or fixed in 4% formaldehyde and embedded in paraffin for immunostaining.

Biochemical determinations, immunostaining and microvessel counting

Histidine decarboxylase activity was assayed by measuring $^{14}CO_2$ evolved from L-[carboxyl ^{14}C]-histidine (46 mCi/mmol, Amersham, England) using a previously reported method [4]. Histamine content was determined after a buthanol/heptane extraction from the tissue, using a fluorometric method as previously reported [5]. Sections were cut from the formaldehyde-fixed and paraffin-embedded tissue blocks and processed as previously described [6]. Immunohistochemical staining was performed using the streptavidin-biotin peroxidase method. Staining for CD31, an endothelial antigen, was used to highlight the endothelial cells and determine MVD [5].

Statistical analysis

MVD values, HDC activity and histamine content were expressed as means ± SEM. The relationship between MVD, HDC activity and histamine content was evaluated using the Spearman correlation cefficient (r_s). P values less than 0.05 were considered statistically significant.

Results and discussion

HDC and histamine content were significantly higher in the tumor specimens than in the corresponding normal mucosa (42.3 ± 2.2 vs 14.8 ± 1.3 nmol histamine/µg protein/h, $p < 0.001$ and 26.3 ± 2.6 vs 0.6 ± 0.3 ng/µg protein, $p < 0.001$, respectively). These parameters were also significantly higher in tumors with lymph node and/or hematogenous metastases (stages III–IV) than in those without any metastases (stages I–II; Table 1). Histamine content was higher in tu-

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Table 1. Histamine (H) content and histidine decarboxylase (HDC) activity in tumor samples and in normal mucosa (taken 10 cm from the tumor) from colonic cancer patients.

	Normal mucosa	Tumor stage I–II	Tumor stage III–IV
H content (ng/µg protein)	0.6 ± 0.3	21.4 ± 1.8*	35.2 ± 3.1**
HDC activity (nmoles H/µg protein per h)	14.8 ± 1.0	34.6 ± 2.9*	50.45 ± 3.6**

*$p < 0.001$ vs normal mucosa; **$p < 0.001$ vs normal mucosa and tumor stage I–II.

mors with a higher immunostaining for MVD, although HDC activity did not correlate with MVD. When all the patients were considered, the mean value of MVD was 26.5 ± 2.3 microvessels per field.

The hypothesis that histamine could be involved in carcinogenesis and tumor proliferation was proposed in the 1960s, but the question is poorly defined until now and in these latter years, accumulated evidence has pointed to the direct relationship between HDC activity and tumor growth. Our results demonstrated that both HDC activity and histamine content were significantly higher in tumor specimens than in the corresponding normal mucosa, and showed for the first time that high level of HDC activity and histamine content correlated with the presence of lymph and/or distant metastases ($r_s = 0.42$, $p < 0.01$ and $r_s = 0.30$, $p < 0.05$ respectively). These data strongly suggest a role of histamine in the acquisition of an invasive and metastatic phenotype for colorectal cancer cells. The specific mechanism by which HDC and histamine are involved in cancer progression is poorly understood. Histamine has been reported to be a potent angiogenic factor in some inflammatory models, but our results seem not to support the hypothesis of a direct involvement of histamine in inducing tumor angiogenesis, in fact intratumoral HDC activity and histamine content did not correlate with MVD. Histamine has been reported to have an immunosuppressive effect and our findings also support the hypothesis that the histamine high content of tumors could represent one of the underlying causes of local decreased immune response against colonic cancer.

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References

[1] Hough LB. Genomics meets histamine receptors: new subtypes, new receptors. Mol Pharmacol 2001; 59: 415–9.
[2] Wang LD, Hoeltzel M, Butler K, Hare B, Todisco A, Wang M et al. Activation of the human histamine H_2 receptor is linked to cell proliferation and c-fos gene transcription. Am J Physiol 1997; 273: C2037–45.
[3] Ghosh AK, Hirasawa N, Ohuchi K. Enhancement by histamine of vascular endothelial growth factor production in granulation tissue via H(2) receptors. Br J Pharmacol 2001; 134: 1419–28.
[4] Masini E, Di Bello MG, Raspanti S, Fomusi NJ, Baronti R, Cappugi P et al. The role of histamine in platelet aggregation by physiological and immunological stimuli. Inflamm Res 1998; 47: 211–20.
[5] Bechi P, Amorosi A, Mazzanti R, Dei R, Bianchi S, Mugnai L et al. Reflux-related gastric mucosal injury is associated with increased mucosal histamine content in humans. Gastroenterology 1993; 104: 1057–63
[6] Cianchi F, Cortesini C, Bechi P, Fantappie O, Messerini L, Vannacci A et al. Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. Gastroenterology 2001; 121: 1339–47