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Inflamm. res. 55, *Supplement 1* (2006) S81–S82
1023-3830/06/01S81-02
DOI 10.1007/s00011-005-0053-x

Inflammation Research

Prostaglandin E_2 correlates with histamine production in human colorectal cancer

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Introduction

Histamine plays a pivotal role in a number of processes, including inflammation, allergic reaction, gastric acid secretion and neurotransmission. Expression of histidine decarboxylase (HDC), the key enzyme in the synthesis of histamine, has been shown to be increased in colorectal carcinoma [1] and other types of human tumors, suggesting that histamine may be directly involved in tumor development and progression. Histamine has been shown to stimulate the in vitro and in vivo growth of gastrointestinal cancer cells while treatment with cimetidine, an H_2 receptor antagonist, can reverse this effect [2]. Histamine also seems to be involved in the inhibition of local immune response against cancer. At increased pathophysiological concentrations histamine can exert potent immunosuppressive effects by activating T cell suppressor function and inhibiting lymphocyte proliferation [3, 4].
In the present study, we addressed the hypothesis that activation of the COX-2 pathway mediates the possible link between histamine and colorectal cancer.

Materials and methods

Patients and tissue collection

Tissue samples were obtained from 33 patients (15 males, 18 females, median age 65 years) who had consecutively undergone surgical resections for primary sporadic colorectal adenocarcinomas at the Department of General Surgery, University of Florence, Italy. The patients had not undergone any chemotherapy or other chronic pharmacological treatment. All patients were informed about the aims of the study and gave written consent for the investigation in accordance with the ethical guidelines of our University. Tumors were classified into four stages according to the AJCC staging system. Tissues from the edge of the tumor and normal mucosa 10 cm away from the tumor were excised from each patient. The samples were washed in PBS and frozen at –80°C until use; other samples were fixed in 4 % formaldehyde and embedded in paraffin for immunostaining as previously described [5].

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Biochemical determinations

Histamine content was determined after butanol/heptan tissue extraction, using a previously reported fluorimetric method [6]. PGE_2 production was measured using 100 µl of the supernatant of tissue homogenate using a competitive enzyme immunoassay kit (Cayman Chemical Co, Ann Arbor, MI, USA) according to a previously described method [7]. Cyclic AMP (cAMP) levels were measured in the aqueous phase of tissue homogenates extracted from 10 % trichloroacetic acid with 0.5 mol/L tri-N-octylamine dissolved in 1,1,2-trichlorofluoroethane. cAMP was measured with commercially available radioimmunoassay.

Statistical analysis

Histamine, PGE_2 and cAMP content were expressed as mean values +/–standard error of mean (SEM). The extent of tumor infiltration by lymphocytes was classified as absent/little, moderate and diffuse. The relationships among histamine content, PGE_2 production and lymphocyte infiltrate were evaluated using the Spearmann correlation coefficient (r_s). P values less than .05 were considered statistically significant.

Results and discussion

Histamine content was significantly higher in the tumor specimens than in the corresponding normal mucosa and was also higher in tumors with lymph node and/or distant metastases (stage III–IV) than in those without any metastases (stage I–II) as we have previously reported [8]. Our present results evidentiated a significant correlation between histamine content and PGE_2 production (Fig.1A). We also found a significant correlation between histamine content and cAMP production ($r_s = 0.3.5$, $p = 0.02$) in tumor samples. Our results showed also that the extent of intratumor lymphocyte infiltrate increased as histamine content decreased (Fig. 1B).

Our study shows that high levels of histamine content are correlated with the presence of lymph node and/or distal metastases in colorectal cancer. Moreover we found a signifi-

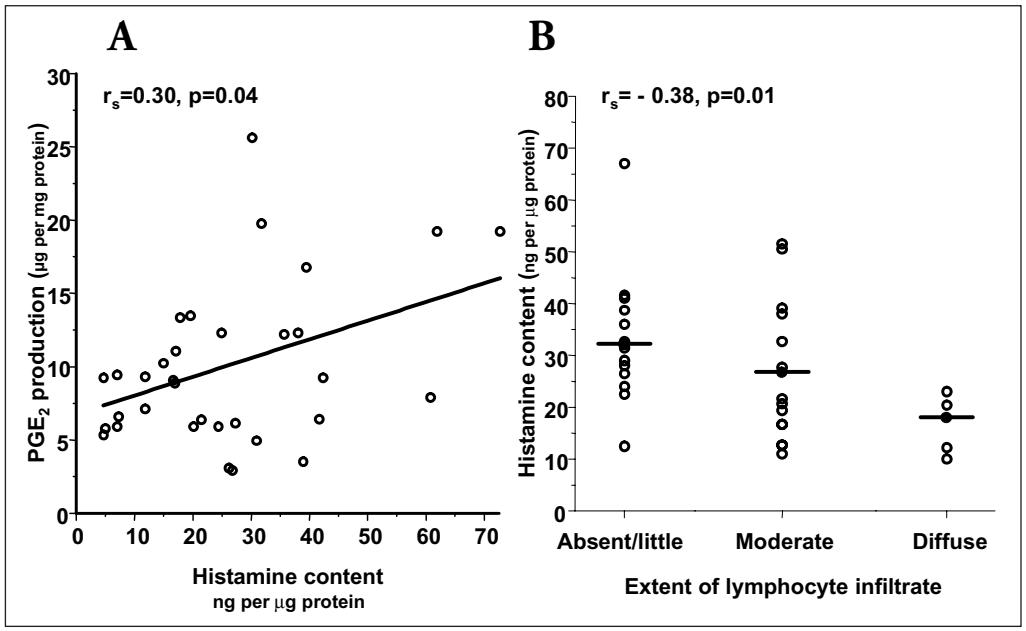

Fig. 1. Histamine content significantly correlated with PGE_2 production (A) and decreased as the extent of intratumor lymphocyte infiltrate increased (B). Histamine was expressed as ng per µg protein; PGE_2 production was expressed as µg per mg protein.

cant correlation between these parameters and PGE_2 levels as well as between histamine and tumor production of cAMP that is one of the most important intracellular mediators of PGE_2.

Much experimental evidence demonstrates the close involvement of COX-2 activity and thus, PGE_2 production, in tumor proliferation and progression [5]. Histamine has been reported to have an immunosuppressive effect through negative regulation of helper T-cell functions [3, 4]. The presence of tumor infiltrating lymphocytes within colorectal tumor tissues is considered to be a marker of good prognosis; histamine content is inversely correlated with the extent of intratumor lymphocyte infiltrate.

Although we did not address the mechanisms by which histamine may modulate immune response, our findings support the hypothesis that histamine may negatively regulate lymphocyte proliferation and chemotaxis in the tumor microenvironment.

In conclusion, our findings supported the hypothesis that the high histamine content of tumor may represent one of the underlying causes of local decreased immune surveillance against colon cancer.

Acknowledgements: This work was supported by a grant from MURST 40%. We thank Mrs Mary Forrest for English editing.

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