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Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine

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Abstract

Production of histamine in colon tumours has been described earlier. Histamine-mediated signals have been shown to be implicated in tumour growth, and the effects of histamine are largely determined locally by the histamine receptor expression pattern. We analysed histamine receptor expression in human colorectal cancer, adenoma and normal mucosa by quantitative reverse transcription-polymerase chain reaction (RT-PCR), Western blot analysis and immunostaining. Real-time RT-PCR results revealed significantly decreased ($p<0.001$) H1R and H4R mRNA levels in tumours compared to normal colonic mucosa, without any significant change in H2R mRNA expression. H3R was absent in most samples; it was detected at low levels in 7.9% of the cases. Protein analysis showed a similar decrease in histamine receptor expression in carcinoma and adenoma compared to normal mucosa controls. Based on these results, we performed further Western blot analysis on Dukes-classified and -selected tumour samples. We found significantly decreased H4R levels in neoplastic samples compared to normal colonic tissue, but there was no significant correlation between histamine receptor expression profile and the Dukes stage of tumours. Immunohistochemical staining revealed expression patterns of H1R, H2R and H4R similar to those suggested by the mRNA and Western blot results. In the present study, we demonstrate that H1R, H2R and H4R are expressed in colon carcinoma and the adjacent normal mucosa. The results suggest a dramatic alteration in the distribution of histamine receptors in colon cancer. These findings raise the perspective of targeted pharmacological studies with selective histamine receptor antagonists or agonists in the therapy of colorectal tumours.

Keywords: Histamine; Histamine receptors; Colorectal tumour; Colon adenoma; Colon mucosa

Introduction

Histamine is a potent bioamine with multiple activities in various physiological and pathological conditions. There are data supporting a potential role of histamine in tumour development and progression.

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Histamine levels in cells and tissues are regulated by the activity of histidine decarboxylase (HDC) which is the only enzyme responsible for the generation of histamine from L-histidine. It has been shown that histidine decarboxylase protein expression and enzymatic activity are significantly increased in both experimental and human tumours, such as melanoma (Darvas et al., 2003; Falus et al., 2001), small cell lung carcinoma (Graff et al., 2002), breast carcinoma (Reynolds et al., 1998; Sieja et al., 2005) and colorectal carcinoma (Boer et al., 2003; Masini et al., 2005). The role of histamine in colorectal carcinogenesis has been reported (Igaz and Falus, 2004). Elevation of histamine concentration in colorectal tumours is attributable to an increase in HDC and a decrease in diamine oxidase (DAO) activity (Chanda and Ganguly, 1987). Histamine has been shown to stimulate the in vitro and in vivo growth of both melanoma and gastrointestinal cancer cells. These processes can be reversed by H2 receptor antagonists (Tomita et al., 2005). The different biological effects of histamine are mediated through the activation of specific histamine membrane receptor types (i.e. H1R, H2R, H3R and H4R). The diverse effects of histamine are due to differential expression of these receptors and their distinct intracellular signals (Hegyesi, 2004). H1R is mainly expressed in the brain, endothelial cells and smooth muscle cells, and is thought to play an important role in allergy. The main effect of H2R is the regulation of gastric acid secretion. Moreover, there are data supporting the role of both H1R and H2R in melanoma cell proliferation. H3R is located mainly in the central nervous system as a presynaptic autoreceptor (Drutel et al., 2001). Finally, H4R is predominantly expressed in leucocytes, but, unlike to other histamine receptors, its functional relevance is not well understood yet (Ling et al., 2004).

Colorectal cancer is a very serious public health problem. Polyps of the colon, especially tubular and villous adenomas, may undergo malignant transformation. When localised to the bowel, colorectal cancer is highly treatable and often curable. Recurrence following radical surgery is a major problem, up to 50% of patients operated on for colorectal cancer will develop locally recurrent or distant metastatic disease (Bleiberg, 2005; Huang et al., 2005).

Based on experimental data, histamine has a dual activity in melanoma and colon tumours. In these malignancies, histamine acts as a growth factor, and also impairs the local immune response through Th2 polarization. From the clinical point of view, invasion and spreading of a malignant tumour are even more relevant processes than tumour growth itself. Several reports support the direct role of histamine in carcinogenesis and tumour progression, mainly via H1R and H2R (Kobayashi et al., 2000; Tomita et al., 2003). Histamine also influences the activity of cytotoxic T lymphocytes and NK cells, and modifies the cytokine production of other types of immune cells as well (Kubota et al., 2002; Takahashi et al., 2001; Tomita et al., 2005). Recently, Sander et al. (2006) have reported the histamine receptor distribution in the human intestinal tract. However, only limited data are available regarding the histamine receptor expression in colorectal tumours (Kapoor et al., 2005; Nielsen et al., 2002). Results of clinical trials have shown that administration of H2R antagonists (cimetidine or ranitidine) improves survival of patients with colorectal cancer (Nielsen et al., 2002).

Therefore, in this study we have analysed the histamine receptor expression pattern in colon tumours and benign polyps. We examined matched human colon tumour samples including adenomas and carcinomas and compared them with normal colonic mucosa using real-time RT-PCR, Western blot analysis and immunohistochemical staining methods.

Materials and methods

Patients and tissue collection

Biopsy samples were obtained from 40 patients of the Department of Gastroenterology, St. Margit Hospital, Budapest. Patients underwent colonoscopy for diverse reasons, e.g. surveillance, blood in stool, anaemia. Endoscopic biopsies of polyps ($n = 20$) and macroscopically malignant lesions ($n = 20$) were obtained from the tumour tissue, omitting necrotic parts of the tumours. Normal colonic mucosa samples served as controls, and were taken at the same time in each case. Adjacent normal mucosa samples located at least 2 cm far from the macroscopically unaffected margins of the tumour (polyp or carcinoma) were defined as normal controls. Eighteen tumours were adenocarcinomas and two mucinous carcinomas (when $>50\%$ of the tumour volume was composed of mucin). Adenocarcinomas were classified as well differentiated (Grade I), moderately differentiated (Grade II) and poorly differentiated (Grade III). Tumours were staged according to the Dukes classification system: Dukes A (T_1–T_2, N_0, and M_0; $n = 10$), Dukes B (T_3–T_4, N_0, and M_0; $n = 10$), Dukes C (any T, N_{1-2}, M_0; $n = 10$) and Dukes D (any T and any N and M_1; $n = 10$). Tissue samples (carcinoma = 20 and adenoma = 20) for Western blot analysis were put immediately in lysis buffer and frozen at $-20\,^{\circ}C$. Matched samples of colon carcinomas ($n = 40$) and normal colonic mucosa ($n = 40$) were subjected to real-time RT-PCR and further Western blot analysis. Samples were collected from patients undergoing bowel resection due to colon cancer, and stored in liquid nitrogen (Biobank tissue sample collection of