Vasoactive intestinal peptide and its relationship to tumor stage in colorectal carcinoma: an immunohistochemical study

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Introduction

Vasoactive intestinal peptide (VIP) is a 28-amino acid peptide that has a wide range of biological activity.1 It is abundant in the gastrointestinal tract, and regulates motility, modulates inflammatory reactions, and stimulates secretion. VIP binds to VIP receptors on the cell surface, is internalized in heterogeneous endosomes,2,3 and then activates adenylate cyclase in the cells.4,5 The activated adenylate cyclase increases cyclic adenosine monophosphate (cAMP) and enhances proliferation.6,7

Although recent studies8–11 found VIP receptors in most colorectal carcinomas, it is controversial whether VIP is trophic or inhibitory for the development and progression of carcinoma.12–18 Moreover, most previous studies have been in-vitro ones, and reports of clinical research using surgically resected specimens, are very few.19

Colorectal carcinoma is shown to arise as a result of the accumulation of genetic changes.20 However, changes in morphology, histochemistry,21,22 and cell proliferation21–25 in tumor-neighboring mucosa (TM) have drawn attention, making the hypothesis of field defect26 prior to or along with the development of colorectal carcinoma still viable. In addition, because TM is a site of tumor-host interaction, the characteristics of TM may affect the progression of colorectal carcinoma, or, conversely, they may be influenced by the biological nature of the carcinoma. VIP in TM, therefore, is possibly related to the development and progression of carcinoma, and may differ from that in remote normal mucosa (RM).

In the present study, we studied VIP immunoreactivity in TM and RM in patients with colorectal adenocarcinoma, and its relationship with the clinical characteristics of the patients and the pathological findings of the tumors.
Methods

Surgically resected specimens of 55 out of 68 consecutive lesions of colorectal carcinoma obtained between January 1998 and July 1998 at the Department of Surgery, Koshigaya Hospital, Dokkyo University School of Medicine, were studied. The median age of the patients was 65.3 years, with a range of 49 to 84 years. Thirty-five patients were men and 20 were female. The site of the tumor was the right colon in 11 patients, the left colon in 21 patients, and the rectum in 23 patients. Patients who received preoperative radiotherapy and those with extensive diverticular disease were excluded from the study, because these conditions are known to cause alterations in VIP.27,28

Fresh full-thickness colonic samples, 1 × 3 cm in size, were collected immediately after resection from TM within 2 cm from the lateral invasive front of the tumor and from RM at 10 cm both oral and anal to the tumor. However, in cecal carcinomas, a sample of the remote site was obtained only from 10 cm distal to the tumor, whereas, in rectal carcinomas, it was obtained only from 10 cm oral to the tumor. The clinical and routine pathological characteristics, including tumor stage according to the TNM classification,29 of the 55 lesions are summarized in Table 1.

The fresh samples were immediately fixed in Hollande’s solution (shown to be the best fixative for VIP immunohistochemical staining by Kubota et al.30), for 4 h. They were then routinely processed, embedded in paraffin blocks, and used for the immunohistochemical staining of VIP.

VIP immunohistochemistry

VIP immunohistochemical staining was carried out with the avidin-biotin peroxidase complex (ABC) technique. Serial 4-µm-thick sections were cut, deparaffinized, and immersed for 30 min in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidases. The slides were then washed in three changes of phosphate-buffered saline (PBS) over 5 min. After nonspecific binding sites were saturated with nonimmune goat serum (Dako Protein Block; Dako, Carpinteria, CA, USA) for 30 min, primary rabbit anti-VIP antibody (1:500, B34; Euro-Diagnostica, Malmo, Sweden) was applied and the slides were incubated in a humidified chamber at room temperature for 30 min. Parallel sections were incubated with the nonimmune rabbit serum (negative control; Fig. 1). After the slides were washed with three changes of PBS over 5 min, biotinylated, affinity-purified goat anti-rabbit IgG (1:200, Vector Laboratories, Burlingame, CA, USA) with 1% human serum was applied to the tissue sections, and the slides were incubated at room temperature for 30 min. The slides were again washed with three changes of PBS over 5 min, and then incubated with freshly preformed ABC (avidin DH-biotinylated peroxidase; Vector Laboratories) for 30 min. After they were washed with three changes of PBS over 5 min, the color reaction product was developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB, 1.6%; Wako, Osaka, Japan) for 90 s. The slides were washed in PBS and counterstained in hematoxylin for 10 s, followed by a washing in tap water for 10 min. Finally, the slides were covered and mounted.

The specificity of the primary rabbit anti-VIP antibody was examined with an absorption test for ten samples of TM. It was confirmed that preincubation of the primary rabbit anti-VIP antibody with purified VIP (Sigma Chemical, St. Louis, MO, USA) at 4°C for 24 h abolished staining (Fig. 1).