

THE EXPRESSION OF RECEPTORS FOR VASOACTIVE INTESTINAL PEPTIDE AND SECRETIN IN COLON NEOPLASMS

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ABSTRACT

Objective: To investigate the expression of the receptors for vasoactive intestinal peptide (VIP) and secretin in colon cancer. **Methods:** This study visualized and characterized the receptors for VIP and secretin in the sequence of human tumor-free colon, adenoma, carcinoma, liver metastasis using storage phosphor autoradiography. **Results:** Receptors for VIP and secretin were demonstrated in tumor-free colon and colon tumors. A decrease in affinity of VIP receptors was shown in the colonic liver metastasis ($K_d = 3.30$ nmol) when compared with tumor-free colon ($K_d = 0.82$ nmol). An up-regulation of receptors for secretin was found in colonic liver metastases. **Conclusions:** VIP and secretin were both expressed on normal colon tissues. Binding of VIP decreased while secretin increased in colonic liver metastasis. A down-regulation of receptors for VIP in colonic liver metastases may helpful to understand the migration of colon cancer.

Key words: Storage phosphor autoradiography, Gut peptide receptor

Rapid proliferation of knowledge about the molecular and biologic characteristics of colorectal cancer has

provided useful insights into the pathogenesis of the tumors^[1-2]. Besides the advances in the molecular mechanism underlying colorectal tumor pathogenesis, there is increasing evidence that colorectal tumors are, at least in part, hormone dependent^[3]. The human colon cancer cell line, HT 29 ever exhibited an increase in response to VIP^[4]. It was also observed that VIP inhibited growth of certain colonic adenocarcinoma-derived cell lines *in vitro*^[5]. Interestingly, VIP could inhibit invasion and migration of colon 26-L5 carcinoma cells through partial suppression of their motility^[6]. Receptors for vasoactive intestinal peptide (VIP) have been demonstrated in colorectal carcinoma cell lines^[7-9]. In addition, ¹²³I-VIP receptor scintigraphy revealed a high density of ¹²³I-VIP binding in colorectal cancers and their metastatic liver lesions^[10].

Whether this observation points to an up-regulation of VIP receptors in colonic carcinoma and its metastasis compared to tumor-free colon has not yet been studied. Furthermore, there is no information on the expression of the receptors for secretin, a member of the VIP receptor family, in the colorectal cancer sequence i.e. normal colon-adenoma- cancer- metastasis.

In the present study, the receptors for VIP and secretin were visualized and characterized in the sequence of human tumor-free colon, adenoma, carcinoma, liver metastasis using storage phosphor autoradiography.

MATERIALS AND METHODS

Tissue samples from 10-14 individuals were obtained at surgery or polypectomy in each of following groups: tumor-free colon, colonic adenoma, colonic adenocarcinoma, colonic liver

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metastasis and tumor-free liver (Table 1).

Table 1. Patient data of the tissue samples used in this study

Group	n	male	female	Age (yr)
Tumor-free colon	14	7	7	53.34
Colonic adenoma	11	6	5	46.25
Colonic carcinoma	13	6	7	56.25
Colonic liver metastasis	10	4	6	46.67
Tumor-free liver	13	7	6	43.75

Immediately after removal of the tissue, one part was used for histopathological verification, while the other part was rapidly frozen at -70°C until sectioned. Tumor-free colon and liver were defined as normal on histopathological examination. Tissue sections ($12\text{ }\mu\text{m}$) were cut at -20°C using a cryostat microtome, mounted onto gelatin-coated slides and dried overnight at -80°C .

Tyr-B-ala-secretin and VIP-28 were iodinated using the Chloramine-T oxidation method^[11-12] which yielded a specific activity of 2200 Ci/mmol for ^{125}I -Tyr-B-ala-secretin and 4000 Ci/mmol for ^{125}I -VIP-28.

Binding of radioligands to tissue sections was done according to the method described by our group^[13]. The concentration of each labeled peptide was 100 pmol. Alternate slides were incubated with the addition of 1 μmol of the corresponding nonradioactive peptides to determine the extent of nonspecific binding. Specific binding was calculated by subtracting non-specific binding from total binding. The dried tissue sections and one slide with two dried drops of 10 μL 100 pmol labeled peptide for standardization were placed in a storage phosphor cassette for 24 h at 22°C . The latent image stored in the storage phosphor screen was scanned by the PhosphorImager® and the data were processed with ImageQuant® software (Molecular Dynamics, Sunnyvale, CA, USA). Receptor binding data were normalized to fmol of radioactive peptides bound per mg of protein^[14]. Binding parameters [Kd (dissociation constant) and Bmax (maximum binding capacity)] were determined for each binding site by using a nonlinear least-squares curve-fitting program^[15]. To determine the significance of differences of the affinity or binding capacity of receptors for each peptide in colon, adenoma, carcinoma and liver metastasis, an analysis of multiple means, ANOVA or Newman-Keuls test was used.

RESULTS

VIP receptors were visualized in all tissue sections of the colon cancer sequence (Figure 1). The binding of VIP in tumor-free colonic mucosa and liver was found to be higher than that in colonic tumors in different stages (Figure 2). The affinity (Kd) of VIP receptors in liver metastasis was significantly lower than that in tumor-free colon ($P<0.05$) (Table 2). The binding capacities (Bmax) of VIP receptors in colonic mucosa-adenoma-carcinoma-metastasis sequence displayed no marked differences ($P>0.05$) (Table 2). With the data from these tissue sections, a one-site model shows the best fit for the dose-inhibition curves of VIP.

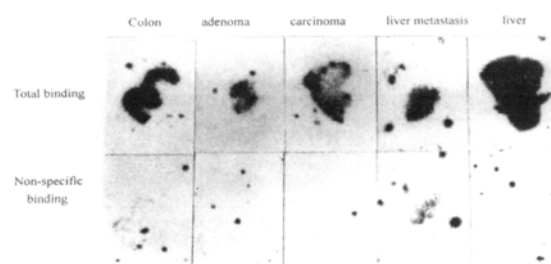


Fig. 1. Autoradiographs of tissue sections of tumor-free colon, colonic adenoma, colonic carcinoma, colonic liver metastasis and tumor-free liver showing expression of receptors for VIP. Magnification $3\times$.

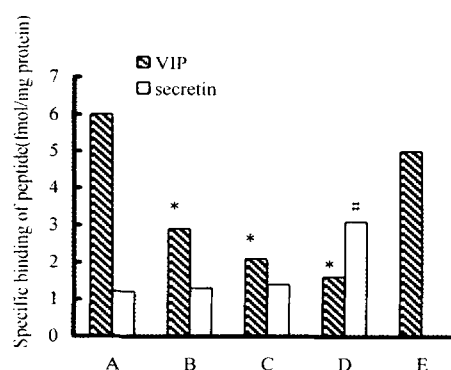


Fig. 2. Quantification of specific binding for ^{125}I -VIP-28 or ^{125}I -secretin in tissue sections

Specific binding of ^{125}I -secretin was shown on the tissue sections of tumor-free colonic mucosa, colonic adenoma, colonic carcinoma and its liver metastasis (Figure 3). Specific binding of ^{125}I -secretin in the colonic metastases of liver is significantly higher than that in tumor-free colon and primary lesions ($P<0.05$) (Figure 2). The binding