Immunohistochemical demonstration of pancreatic secretory trypsin inhibitor in normal and neoplastic colonic mucosa

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Abstract
Specimens of normal and neoplastic colonic mucosa from 52 patients were analysed by immunohistochemistry using a monospecific polyclonal antiserum against human pancreatic secretory trypsin inhibitor (PSTI). In normal colonic mucosa PSTI was found in the goblet cells in the basal parts of the crypts. In adenomas of tubular, villous, and tubulo-villous types PSTI was also found in the upper parts of the polyps, usually occurring in the regeneration zone. There was a more intense staining reaction in polyps with increased atypia. Carcinomas of different types and of various grades of differentiation and of in situ type did not contain PSTI.

These findings indicate that PSTI could be a marker for adenomatous rather than carcinomatous epithelium in the colon. Furthermore, the absence of the inhibitor in malignant cells might facilitate tissue invasion by malignant cells because of deficient protease inhibition.

Pancreatic secretory trypsin inhibitor (PSTI) was first described by Kazal as a product of the acinar cells of the pancreas.1 The main function of PSTI is believed to be that of preventing the autodigestion of the pancreatic gland by inhibiting preactivated trypsin. An increasing body of evidence has accumulated which indicates that PSTI may be produced beyond the pancreas.2,3 The presence of immunoreactive PSTI (irPSTI) has been shown in and isolated from the normal gastrointestinal mucosa.4-7 In the stomach irPSTI is found in the foveolar cells of the gastric pits; in gastric metaplastic mucosa with intestinal metaplasia irPSTI is also found in Paneth cells5; in the small intestine and duodenum irPSTI is found in Paneth cells6 and goblet cells7; in the colon irPSTI is located in goblet cells.8 IrPSTI has also been shown in colonic adenomas.9 The aim of this study was to analyse normal and neoplastic colorectal mucosa for the presence and localisation of irPSTI.

Methods
Specimens of surgically resected colonic mucosa were obtained from 52 patients undergoing surgery because of colonic carcinoma or adenoma. The specimens were fixed in buffered 10% formalin and embedded in paraffin wax. They were then analysed using the peroxidase-antiperoxidase method (PAP) described by Sternberger et al,9 with some modifications.10 The antiserum used was a monospecific polyclonal rabbit antiserum against human PSTI.11 The antiserum was used in serial dilution: 1 in 2000 gave the best staining reaction. Control staining was performed by using the antiserum previously absorbed by PSTI and by using non-immune rabbit serum instead of the specific antiserum. The following types of mucosa were analysed: colonic mucosa of the normal type (n=10), colonic adenomas of the tubular type (n=8), of the villous type (n=6), of the tubulo-villous type (n=6), colonic carcinomas of the in situ type or of intramucosal type (n=10), well differentiated colonic carcinomas (n=6), and poorly differentiated colonic carcinomas (n=6).

The terms carcinoma in situ (basement membrane unbroken) and intramucosal carcinoma (intramucosally infiltrating carcinoma) have been used to delineate those tumours in early carcinomatous change without signs of infiltration through the muscularis mucosae, but with pronounced cellular atypia and cribriform structures in the tumour.

Swine anti-rabbit IgG, peroxidase-antiperoxidase (PAP), and normal swine serum were obtained from Dako Immunoglobulins, Copenhagen, Denmark. Human pancreatic secretory trypsin inhibitor was purified from human pancreatic juice.11

The staining reaction was completely absent when the antiserum was absorbed by PSTI at higher concentrations than 1 µg/ml (of diluted antiserum 1 in 2000).

Results
Normal colonic mucosa contained irPSTI in the goblet cells in the basal parts of the crypts (fig 1). In adenomas irPSTI was also shown in the upper part of the polyps corresponding to the reversed mode of epithelial regeneration in adenomas. The staining reaction was often related to the grade of atypia, with a more intense staining reaction in cases with more pronounced atypia (fig 2).
Colonic carcinomas of the in situ or intramucosal types did not contain irPSTI (figs 3 and 4). Poorly differentiated, infiltrating colonic carcinomatous did not contain irPSTI (fig 5). In two of the six specimens of well differentiated colonic carcinomas there were small areas with a few cells with positive staining for irPSTI.

**Discussion**

The findings of this study show that irPSTI is found not only in the goblet cells of normal colonic mucosa but also in colonic adenomas. Negative controls using non-immune rabbit serum or antiserum previously absorbed by PSTI showed the specificity of the staining reaction.

In normal colonic mucosa the cells containing irPSTI were found predominantly in the basal parts of the crypts. In the adenomas they were also found in the upper parts of the adenomas. Thus the goblet cells containing irPSTI seemed to occur at the zone of re-
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Figure 3  Part of an adenomatous polyp with a focus of intramucosal carcinoma. The adenomatous part (lower) has PSTI staining while the carcinomatous part (upper) does not express PSTI.

generation. In normal colonic mucosa the epithelium regenerates from the basal parts of the tubular crypts while in adenomas there is a reversed mode of epithelial regeneration localised in the luminal areas. The staining reaction also seemed to be associated with the degree of epithelial atypia, with an increased staining reaction in polyps with more pronounced atypia. On the other hand, cells containing irPSTI were not found in in situ carcinoma, intramucosal carcinoma, or in invasive carcinomas, except for in a few cells in small areas in well differentiated carcinomas. This shift from an intense staining reaction in adenomas with a more pronounced atypia to an almost entire lack of irPSTI in carcinomas is difficult to explain. The apparent absence of the inhibitor could, however, facilitate tissue infiltration by cancer cells—that is, the proteases produced by the cancer cells can more easily degrade surrounding tissue and thus facilitate invasion. More studies, however, will be required to establish the exact function of PSTI.

Figure 4  Detail of figure 3. The borderline zone of the adenomatous polyp with a focus of early intramucosal carcinomatous change without penetration of the muscularis mucosae. There is a distinct borderline (arrow) between benign adenomatous epithelium with a high content of PSTI and the carcinomatous epithelium (upper) in which there is no PSTI expression. (Immunohistochemical staining with an antiserum against human PSTI.)
The nomenclature of early carcinomatous changes in colorectal adenomas corresponding to the stages of carcinoma in situ and intramucosally infiltrating carcinoma varies. Some judge the intraepithelial (in situ) and the intramucosal stages to be carcinomatous changes, but others classify them as severely dysplastic. Our present findings would rather support the opinion that these early stages are true carcinomatous changes.

The pronounced shift from adenomatous epithelium with a high PSTI content to carcinomatous epithelium without PSTI, already progressed to carcinoma in situ, is of great interest both from a theoretical and a practical point of view. In the future PSTI might be a marker for the adenoma-carcinoma sequence in the epithelium of the large bowel.

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