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Immunohistochemical Detection of Pancreatic Secretory Trypsin Inhibitor (PSTI) in Urothelial Cancer

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Summary. **Pancreatic secretory trypsin inhibitor (PSTI), a specific trypsin inhibitor secreted by acinar cells of pancreas, also acts as a growth factor. PSTI in various cancers has been reported to be associated with tumor invasiveness and pathological malignancy; however, such studies in urological cancers have not yet been performed. We therefore examined the expression of PSTI in fresh frozen specimens of urothelial cancers immunohistochemically, using a polyclonal antibody. In 59 tumors (30 urinary bladder cancers, 16 ureteral cancers, and 13 renal pelvic cancers), 28 (47%) showed positive staining. In 44 transitional cell carcinomas (TCC), immunopositive tumor cells were observed in 16 (36%). However, there were no obvious differences in the incidence of PSTI expression among stages and grades. In 12 tumors with squamous cell cancer (SCC) cells, 10 tumors (83%) showed intense positive staining in SCC cells. These results suggest that the degree of expression of PSTI might be used for prognostic purposes since the prognosis of urothelial SCC patients has been reported to be significantly poorer than that of TCC ones.**

INTRODUCTION

Pancreatic secretory trypsin inhibitor (PSTI) is a specific trypsin inhibitor secreted by acinar cells of the pancreas.[1] Increased serum PSTI levels are often observed in acute pancreatitis,[2] after operation,[3] or with some types of malignant diseases.[4] Although the presence of PSTI or its mRNA in normal and neoplastic tissues has been examined by immunohistochemistry and northern blots,[5-7] urothelial cancer has not been studied. The present study examines the expression of PSTI in urothelial cancer by immunohistochemical methods.

MATERIALS AND METHODS

Specimens were obtained from 59 patients (30 urinary bladder cancers, 16 ureteral cancers, and 13 renal pelvic cancers), 45 males and 14 females, the median age being 69 years (range 39-82). None of the patients had received chemotherapeutic, immunomodulatory agents, or irradiation for 3 months before operation. Of the 59 tumors, 44 cases were histologically evaluated as transitional cell cancer (TCC), 7 as TCC+squamous cell cancer (SCC), 3 as SCC, and each of the other 5 tumors as adenocarcinoma(AC), TCC+SCC+AC, TCC+squamous metaplasia, TCC+undifferentiated carcinoma, and small cell carcinoma. Normal urothelia of the bladder, ureter and pelvis were obtained from patients undergoing surgery of renal cell cancer or transurethral resection of the prostate for benign prostatic hypertrophy. Normal pancreas was obtained from patients undergoing a pancreatico-duodenectomy. All the tissue samples were embedded in OCT compound (Miles Laboratories, Naperville, IL, USA) after being rinsed in PBS, and then snap-frozen in isopentane which was precooled in dry ice and acetone. These blocks were stored at −80℃ untill 5 μm frozen sections were cut in a cryostat.

Histological examination was performed on hematoxyline and eosin (H & E)-stained tissue sections according to WHO staging and grading. Immunohistochemical study was performed according to the streptavidin-biotin bridge technique which has been previously described.[8] Briefly, sections were air dried for 30 min and fixed in cold acetone for 10 min. After incubation in 20% normal donkey serum, endogenous biotin was blocked (Endogenous biotin blocking kit: Vector Laboratories, Burlingame, CA, USA). The sections were then incubated with rabbit

polyclonal anti-PSTI serum (Shionogi Co., Osaka, Japan; kindly provided by Prof. Ogawa, 2nd Department of Surgery, Kumamoto University School of Medicine, Japan) diluted 1 : 400 for 60 min followed by incubation with biotinylated donkey anti-rabbit serum (Amersham International, Buckinghamshire, UK) diluted 1 : 100 containing 20% human AB serum for 30 min. After adding streptavidin peroxydase (Amersham) diluted 1 : 100 for 45 min, the sections were immersed in 0.05% diamino-benzidine (Sigma Chemical, St. Louis, MO, USA) and 0.01% H_2O_2 in 0.05 M Tris-HCl buffer for 3-5 min to visualize the reaction products. All incubation was performed at room temperature (20℃). As a positive control for PSTI, normal pancreas was stained. As a negative control for PSTI, serial sections of tumor tissue were stained with the rabbit polyclonal anti-IL-6 antibody (Genzyme, Boston, MA, USA).

RESULTS

Specimens of 15 normal urothelia (6 bladder, 5 ureter, 4 pelvis) were not stained by PSTI, excepting the cytoplasm of some umbrella cells (i.e., superficial or covering cells, which are cells larger than those of the deeper layers and cover them like an umbrella). The staining patterns of PSTI in the tumors were almost intracytoplasmic (Fig. 1A). In some tumors, the cell membrane was stained more strongly than the cytoplasm (Fig. 1B). Heterogenous staining in the sections and a variety of staining intensities from cell to cell were noted (Fig. 1B). Thus, when the cytoplasm of any tumor cell was observed to be stained, the tumor was defined as 'positive,' regardless of its proportion in the section.

According to this criterion, 28 fo 59 tumors (47%) appeared immunopositive. Positive staining was observed in 36% (16 cases) of TCCs (44 cases); however, no correlation was noted between the incidence of PSTI expression and pathological stage or grade (Table 1). Of the 16 PSTI-positive TCCs, 14 cases had no more than 40% PSTI-stained cells in the tumor sections. In such cases, PSTI-stained tumor cells often existed in basal layers. In SCC (3 cases), TCC+SCC (7 cases), TCC+squamous metaplasia (1 case), or TCC+SCC+AC (1 case), 10 tumors (83%) stained positively (Table 1, Fig. 1). Therefore, SCC cells expressed PSTI more frequently than TCC cells. Moreover, in 6 of 10 tumors with PSTI-positive SCC cells, PSTI was intensely and widely (more than 80% of all tumor cells in the specimen) expressed (Fig. 1A). In some tumors, the expression was marked at the basal layer or at invading zone.

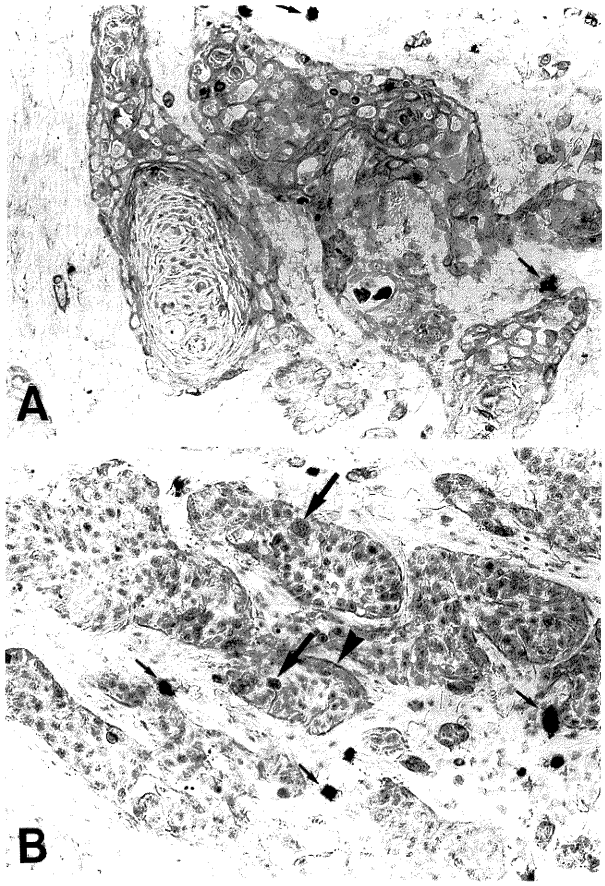

Fig. 1. Immunohistochemical staining of PSTI. **A.** squamous cell cancer. **B.** grade 3, invasive transitional cell cancer. Note that the cytoplasm of all tumor cells is clearly stained (**A, B**). A variety of staining intensity from cell to cell is observed (**B.** Strongly immunopositive cells are indicated by large arrows). In one part, the membrane of the tumor cells is marked (**B.** indicated by arrowhead). Neutrophils, indicated by small arrows, show nonspecific staining because of their endogenous peroxydase. (Streptavidin-biotin complex immunostaining, counterstained with Mayer's hematoxylin; magnifications ×130)

DISCUSSION

PSTI was first described by Kazal[1] as a product of the acinar cells of the pancreas, and has been considered to prevent autodigestion of pancreatic gland from preactivated trypsin.[1] PSTI also exists in several normal tissues including foveolar cells in the stomach,[5] Paneth's cells in the small intestine,[9] and transitional epithelia of the urinary tract.[5] However, in our study, normal adult urothelia do not express

Table 1. PSTI expression of pure TCC in relation to tumor stage and grade and that of cancer with squamous cell cancer cells (SCC(+))

PSTI expression		−	+
pure TCC	grade 1	3(1, 2, 0)	2(0, 1, 1)
	2	14(4, 5, 5)	8(2, 2, 4)
	3	11(8, 3, 0)	6(5, 1, 0)
	stage-T1	7(2, 3, 2)	10(4, 2, 4)
	T2+	21(11, 7, 3)	6(3, 2, 1)
	total	28(13,10, 5)	16(7, 4, 5)
SCC(+)	total	2(2, 0, 0)	10(5, 2, 3)

Figures in parentheses are the number of cases with carcinoma of the urinary bladder, ureter and renal pelvis, respectively. The tumor grade and stage are not correlated with the expression of PSTI. SCC(+) significantly expressed PSTI more frequently than pure TCC ($\chi^2 = 8.363$, $p < 0.01$)

PSTI except for umbrella cells. Therefore PSTI, which might be secreted in the urine, would affect PSTI expression in umbrella cells always contacting urine, and would not affect the expression in tumor cells existing in basal layer or invading to muscular layer.

Gastric cancers,[6] colorectal cancers,[7,10] ovarian cancers[11] and lung cancers[12] have been reported to express PSTI. In intestinal-types of gastric cancer, a higher frequency of PSTI expression was observed in high stage and/or high grade tumors. Furthermore, in early diffuse-type gastric cancer, PSTI positive cells were localized only at the invading zone.[6] In colorectal cancer, no difference have been noted in the incidence of PSTI expression among tumor stages; however, low grade and/or large sized tumor expressed PSTI more frequently.[7] In our recent study of urothelial cancer, no correlation was noted between the incidence of PSTI expression and clinical stage or grade.

PSTI in malignant tumors is considered to perform two roles. First, it may act as a protease inhibitor for self-defense against some proteolytic activities produced by itself.[13] Secondly, PSTI may act as a growth factor.[14] This idea is supported by similarities of the primary amino acid sequence between PSTI and the epidermal growth factor (EGF),[14] mRNA homology (46%) between human PSTI and rat EGF,[15] and stimulation of the growth of rat pancreatic carcinoma cells by PSTI.[16] In the present study, several tumors intensely expressed PSTI at the invading zone. Furthermore, frequent and intense PSTI expression was observed in SCC which progresses more rapidly than TCC and whose prognosis is reported to be significantly poorer than that of TCC.[17] These results suggest that PSTI might act such roles in urothelial tumors as well, and that PSTI might be a useful marker of the malignant potential for urothelial cancer.

In conclusion, it was observed that PSTI in urothelial cancer is expressed more frequently in tumors with squamous cell cancer cells than in pure transitional cell cancers.

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