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Expression of Urokinase-type Plasminogen Activator, Its Receptor, and Its Inhibitor in Gastric Adenocarcinoma Tissues

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The plasminogen and plasmin system, which is mainly regulated by urokinase-type plasminogen activator(uPA), its receptor(uPAR) and its inhibitor(PAI-1), is generally believed to play a role in cancer invasion and metastasis. This study was conducted to investigate the role of uPA, uPAR and PAI-1 in the invasion and metastasis of gastric adenocarcinoma. The expression of mRNAs for uPA and PAI-1 was determined by Northern blot analysis in nine primary gastric cancer tissues, nine paired metastatic lymph nodes and normal gastric mucosa. The mRNA of uPA was not or faintly detected in normal mucosa, while the expression was increased in both primary gastric cancer tissues and metastatic lymph nodes to a similar degree. The mRNA expression for PAI-1 in the gastric cancer tissues was not different from that in the paired metastatic lymph nodes and normal mucosae. uPAR was determined by immunohistochemical staining, demonstrating that five(56 %) and six(67 %) out of nine primary gastric cancer tissues and nine paired metastatic lymph nodes were positive, respectively and the intensity was stronger in metastatic lymph nodes. The results support the concept that most gastric cancer cells may have an innately moderate level of uPA and uPAR, and that increase of uPAR expression can be considered to be closely associated with cancer invasion and metastasis.

Key Words : *Gastric adenocarcinoma, Metastatic lymph node, Urokinase-type plasminogen activator, Plasminogen activator receptor, Plasminogen activator inhibitor-1, Northern blot analysis, Immunohistochemistry*

INTRODUCTION

In recent years, much attention has been focused on the interactions occurring during metastatic cascades between cancer cells and adjacent normal cells, because the invasion and metastasis of cancer cells results in many clinically intractable problems.

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Studies have elucidated that the degradation of basement membrane and extracellular matrix by proteolytic enzymes is the critical step in cancer invasion and metastasis(Liotta et al., 1982 : Laiho and Keski-Oja, 1989 : Stetler-Stevenson et al., 1993).

A series of enzymes and their inhibitors, such as plasminogen activator(PA) and plasminogen activator inhibitor(PAI), have been found to be involved in the process of invasion and metastasis(Saksela, 1985 : Duffy, 1987 : Markus, 1988). PA is a serine protease which converts plasminogen to plasmin and is believed to play a key role in this process. Two types of PA, urokinase-type PA(uPA) and tissue-type PA(tPA), have been identified. uPA is known to play an important role in cell migration and tissue remodeling, while tPA is mainly associated with thrombolysis(Dan et al., 1985 : Sakasela and Rifkin, 1988). In addition to the direct activity of uPA, conversion of plasminogen to plasmin, uPA has its proteolytic activity indirectly by activating procollagenase to collagenase and degrades non-collagenous componants of extracellular matrix(Moscatelli and Rifkin, 1988).

uPA is known to express its biological activity effectively by binding to the specific receptor, uPA receptor(uPAR), on the surface of cancer cells(Nielsen et al., 1988 : Blasi, 1993). On the other hand, the proteolytic activity of uPA is inhibited by PAI, while the activity of plasmin is inhibited by alpha2-plasmin inhibiter(Sprengers and Kluft, 1987 : Andreasen et al., 1990). The inhibitory activity of PAI is more potent than alpha2-plasmin inhibitor. PAI is known to consist of three types, PAI-1, PAI-2 and PAI-3, among which PAI-1 is the main type present in plasma, platelet and endothelial cells(Collen and Lijnen, 1986 : Plow et al., 1986).

Gastric cancer still remains the leading cause of cancer death in Korea because of the rapidly invasive and metastatic progression of the cancer cells. However, the mechanism of this process in gastric cancer is still poorly understood. In this study, the expression levels of uPA, uPAR, and PAI-1 were compared among normal gastric mucosa, primary gastric cancer tissues and metastatic lymph nodes obtained at surgery from same patients in order to investigate the role of uPA, uPAR, and PAI-1 in the invasion and metastasis of human gastric cancer.

MATERIALS AND METHODS

Specimens

Specimens of primary cancer tissues, paired metastatic lymph nodes and normal mucosae were obtained from nine patients with gastric adenocarcinoma during operation at the Korea Cancer Center Hospital. The presence of metastasis in lymph nodes was confirmed histologically. The specimens were immediately frozen in liquid nitrogen and stored at -70℃ until experimentation.

Isolation of RNA and Northern blot analysis

After frozen tissues were pulverized, total cellular RNAs were extracted by the guanidine thiocyanate-phenol-chloroform extraction method. Twenty μg of RNA extracted were electrophoretically size-fractionated on 1 % agarose gel containing formaldehyde and transferred onto nylon membranes(Schleicher & Schuell, Germany) by capillary action in 10 × standard saline citrate(SSC) overnight. Before blotting, each gel was stained by ethidium bromide to visualize ribosomal RNA by ultraviolet(UV) -lighting. After washing the membranes in 2 × SSC and UV-cross-linked using UV Stratealinker 2400(Stratagene, USA), the membranes were prehybridized overnight at 42℃ in 50 % formamide, 1 × Denhardt's solution, 0.1 % sodium dodecyl sulfate(SDS), 100 μg/ml of salmon sperm DNA and 6 × SSC. The cDNA probes used for uPA and PAI-1 were obtained from ATCC. The membrane was hybridized at 42℃ with the probe labeled with [^{32}P]-dCTP by the random primer method. Hybridized filters were washed in 2 × SSC and 0.1 % SDS for 30 min. at room temperature and 0.1 × SSC and 0.1 % SDS at 65℃. Autoradiography was done at -70℃ with intensifying screens.

Immunohistochemistry

In nine paired samples, the expression of uPAR was determined by immunohistochemical staining using primary anti-uPAR rabbits antibody(American diagnostica inc. Greenwich, USA). The details have been described previously(Hong et al., 1994). In brief, sections were cut from the formalin-fixed and paraffin-embedded tumor block and mounted on silane-coated glass slides and deparaffinized with xylene. After quenching the endogenous peroxidase activity by the

incubation in methanol with 3 % hydrogen peroxide for 2 min. at 40℃, the slides were treated with 3 % normal goat serum for 2 min. at 40℃ to block the nonspecific binding.

The slides were incubated with 1 : 100 diluted anti-uPAR rabbits antibody in a moisture chamber for 2 hr at 40℃. Antibody binding was determined by immunohistochemical staining following the strep-ABC method using a Vectastain ABC kit(Vector Laboratories, CA) and diaminobenzidine substrate. As a negative control, phosphate buffered saline was used with the same method. Nuclear counterstaining was performed with Mayer's hematoxylin solution.

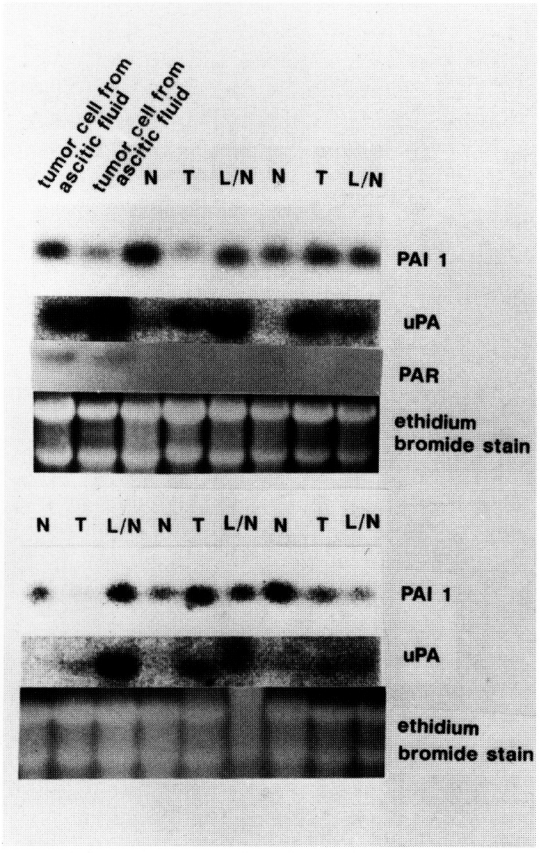

Fig. 1. Northern blot analysis of normal gastric mucosa (N), primarygastric cancer tissues (T) and metastatic lymph nodes(L/N). Blotswere hybridized with probes for urokinase-type plasminogenactivator (uPA), urokinase-type plasminogen activator receptor(uPAR), and plasminogen activator inhibitor-1 (PAI-1). Each lanes of N,T and L/N is originated from same patient.

RESULTS

Expression of mRNA for uPA, PAI-1, and uPAR in normal gastric mucosa, primary gastric cancer tissues and metastatic lymph nodes

The expression of the mRNA of uPA and PAI-1 was determined by Northern blot analysis in nine primary gastric cancer tissues, paired metastatic lymph nodes and paired normal gastric mucosa. The mRNA of uPA was not or even faintly detected in all normal mucosa, while expression was increased in all nine primary gastric cancer tissues and metastatic lymph nodes(Fig. 1). The expressions of the mRNA of uPA in metastatic lymph nodes were not significantly different from those in the primary gastric cancer tissues. The mRNA expressions for PAI-1 in the gastric cancer tissues were similar to those in the paired metastatic lymph nodes and normal mucosa.

Immunohistochemical detection of uPAR

uPAR was determined by immunohistochemical staining(Fig. 2), demonstrating that five(56 %) and six(67 %) out of nine primary cancer tissues and nine paired metastatic lymph nodes were positive, respectively. The intensity of stain was relatively strong in two(40 %) cases and weak in three cases(60 %) out of five cases of uPAR positive of primary cancer tissue. All(100 %) of the six metastatic lymph nodes expressing uPAR showed relatively strong reactivity of uPAR.

DISCUSSION

It is well known that cancer invasion and metastasis are closely associated with proteolytic activity which results from the final effects of the balance between the local concentrations of proteases and their inhibitors(Liotta et al., 1982 : Stetler-Stevenson et al., 1993). Ossowski and Reich(1983) reported that the proliferation, invasion and metastasis of cancer cells were correlated with uPA level and the metastasis was inhibited by specific antibodies against uPA in an animal model.

Levels of uPA and PAI-1 were also reported to be associated with prognosis in breast, colon, lung, brain cancer, etc., suggesting that uPA and PAI-1 might be possible prognostic factors in various human solid cancers(Tissot et al., 1984 : Sier et al., 1991 : Gr dahl-

Hansen et al., 1993 : Pederen et al., 1994 ; Yamamoto et al., 1994).

The expression of uPAR seems to increase the invasive potential by binding with uPA at the cell surface, which enables the plasmin concentration to be high only in surrounding cancer cells. Moreover, binding of uPA with uPAR was reported to increase in the rate of plasmin production at the cell surface(Vassalli et al., 1985). This indicates that uPAR is also important in the regulation of uPA-dependent proteolytic activity at cell to cell and cell to matrix interaction(Sprengers and Kluft, 1987 : P ll nen et al., 1991).

However, the prognostic importance of uPA, uPAR and PAI-1 in gastric cancer has not been fully examined, although several papers have been published on this point(Nishino et al., 1988 : Takai et al., 1991 : Nekarda et al., 1994).

In order to investigate the role of uPA, uPAR and PAI-1 in the carcinogensis, invasion and metastasis in gastric cancer, we examined the expression of uPA and PAI-1 by Northern blot anaylsis and uPAR by Northern blot annalysis and immunohistochemical staining in the paired three types of samples in gastric adenocarcinomas. The results have shown that PAI-1 expression was similar in three types, while uPA were expressed in all cancer tissues, regardless of the sites obtained, primary site or metastatic lymph nodes. uPAR was expressed in 56 % and 67 % in primary cancer and metastatic lymph nodes, respectively. In Northern blot analysis, the expression level of uPAR was faintly detected both in primary gastric cancer tissues and metastatic lymph nodes except cancer cells isolated from the malignant ascites(Fig. 1). Therefore, we investigated the expression of uPAR by using immunohistochemical staining in this study. Although we have not shown the data on malignant ascites, we evaluated the expression of mRNAs for uPA and uPAR by Northern blot analysis in cancer cells isolated from ascites of six patients with gastric adenocarcinoma. All these samples strongly expressed both uPA and uPAR(data not shown). The intensity of staining of uPAR in metastatic lymph nodes was stronger than that in primary gastric cancer tissues. From the results of this and previous studies, we think that uPA and uPAR may play an important role in the invasion and metastasis of gastric cancer. But the results of this study do not clearly show the existance of the relationship between the expression of uPA and /or uPAR and the invasive potentials in gastric cancer.

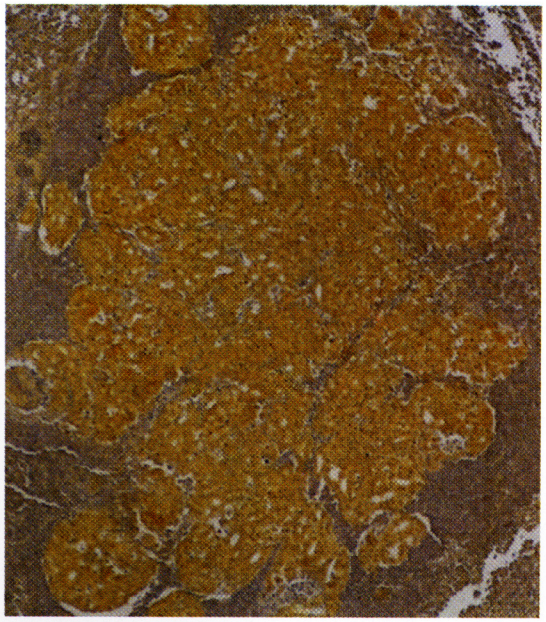

Fig. 2. Immunohistochemistry for plasminogen activator receptor (uPAR) of metatatic gastric cancer cell in lymph node.

One possible explanation for the no definitive results in this study is that we evaluated the expression of uPA, uPAR and PAI-1 qualitatively, not quantitively. To determine the precise role of uPA, uPAR and PAI-1 in metastasis of gastric cancer, further studies, including quantitative analysis at a large number of samples and prognostic valus, will be required.

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