IMMUNOHISTOCHEMICAL LOCALIZATION OF u-PA AND PAI-1 IN COLON CARCINOMAS

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INTRODUCTION

Urokinase-type plasminogen activator(u-PA) is one of the serine protease, and it is thought to be produced by tumor cell, then related to tissue degradation and metastasis^{1,2)}. On the other hand, Plasminogen activator inhibitor-1(PAI-1) is the inhibitor of u-PA. Now it is not known exactly the localization and the role of u-PA and PAI-1 in the tumor tissue. We report here the localization of u-PA and PAI-1 in human colon carcinomas.

MATERIALS AND METHODS

13 cases of colon carcinomas were examined. Tumors were obtained at the surgical procedure and immediately fixed in 4% Paraformaldehyde for 4 hours. Then Paraffin-embedded specimens were prepared. Using anti-u-PA and anti-PAI-1 polyclonal antibodies, tissue sections (4 μ m thick) were stained by SAB method. The process of immunohistochemical staining was following. Endogenous peroxidase activity was quenched by 3% H₂O₂ in absolute methanol for 10 minutes. To unmask tissue antigens, sections were incubated at room temperature with 0.3% (wt/vol) pepsin in 0.01N HCl for 15 minutes, then exposed for 30 minutes to 10%

normal serum. Without washing, the first antibodies diluted 10 μ g/ml were incubated for over night at 4°C. Biotinylated IgG was added for 30 minutes, sections were then incubated with streptavidin-peroxidase conjugate for 30 minutes. After these treatments, visualization of peroxide was achieved by 3-amino-9-ethylcarbazole. Between steps, sections were washed three times in phosphate-buffered saline.

RESULT

In 8 of 13 cases(61.5%), the staining for u-PA were present in the cytoplasm of tumor cell, but not in the stroma (Table 1, Fig 1). Intensity of immunohistochemical staining is higher in the marginal, especially invasive part of the tumor than that in the central part. Lymph node metastasis can be seen more frequently in u-PA positive cases(5/8 62.5%) than negative cases(1/5 20.0%) (Table 2). On the other hand, the staining of PAI-1 was positive in the cytoplasm of fibroblast in the stroma adjacent to the invasive foci in 11 of 13 cases (84.6%) (Table 1, Fig 2). But there is no staining in fibroblast that located in the

case NO	u-PA(tumor cell)	PAI-1(fibroblast)
1	+	+
2	-	+
3	+	+
4	-	+
5	+	+
6	+	+
7	+	+
8	-	+
9	-	-
10	+	+
11	+	+
12	-	-
13	+	+
8/13(61.5%)		11/13(84.6%)

Table.1 Result of immunohistochemical staining for u-PA and PAI-1 in 13 cases of colon carcinomas.

Fig.1 Immunohistochemical staining for u-PA. U-PA is positive in the cytoplasm of tumor cells.

	lymph node metastasis(+)	lymph node metastasis(-)	
u-PA positive	5(62.5%)	3	8
u-PA negative	1(20.0%)	4	5

Table.2 Relationship between u-PA expression and lymph node metastasis in colon carcinomas.

Fig.2 Immunohistochemical staining for PAI-1. PAI-1 is positive in the cytoplasm of fibroblast adjacent to the invasive foci.

distant part of tumor. These results suggested that the expression of u-PA in colon carcinomas may induce the metastatic potential, especially lymph node metastasis, resulting in poor prognosis, and fibroblasts in the stroma produce PAI-1 in order to protect the tissue degradation and invasion of tumor.

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