Relationship between urokinase-type plasminogen activator receptor and vascular endothelial growth factor expression and metastasis of gallbladder cancer

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Abstract

AIM: To investigate the relationship of urokinase type plasminogen activator receptor (uPAR) and vascular endothelial growth factor (VEGF) expression with clinical and pathological characteristics of human gallbladder cancer.

METHODS: uPAR and VEGF expressions in 68 gallbladder cancer tissues were detected with anti-receptor immunohistochemical stain.

RESULTS: Expression rate of uPAR was 57.4% (39/68), and VEGF 51.5% (35/68) in gallbladder cancer tissues. Expression of both uPAR and VEGF was significantly related to metastasis, but not significantly correlated with differentiation stage and size of gallbladder cancer.

CONCLUSION: Expression of uPAR and VEGF may be an invasive phenotype of gallbladder cancer and indicator for predicting prognoses, and uPAR expression is significantly correlated with the expression of VEGF.

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INTRODUCTION

The main lethal cause of patients with malignant tumor is the invasion and metastasis of tumor cells. Tumor blood vessels can not only get rich nutrient from hosts, but also spread many malignant cells to hosts. And all these result in unceasing tumor growth and metastasis(1-4). Urokinase-type plasminogen activator (uPA) is a kind of serine protease, which can activate plasminogen to fibrinolysin, the latter can degrade most kinds of extracellular matrix, which then form extracellular local lysis region, thus constructing the path for metastasis. The role of uPA in vivo is dependent on the expression of corresponding receptor (uPAR) in cell membrane. The conjugation of uPA and uPAR can reinforce tumor cells’ infiltration ability(5,6). Formation of tumor blood vessels is a very complicated process. Vascular endothelial growth factor (VEGF) has an important role in the formation of tumor blood vessels, as well as in growth and metastasis of tumor, because it can adjust angiogenesis and is specific(7-9). Prognosis of operation of gallbladder cancer is poor, and its relapse rate is very high. So investigation of metastasis and recurrence of gallbladder cancer has great clinical significance. We made use of immunohistochemistry to detect the expression of uPAR and VEGF in gallbladder cancer, and investigated the relationship between the expression of uPAR and VEGF and pathologic characteristics, invasion and metastasis of gallbladder cancer. Image analyses were also used to quantitatively analyze the relationship between them.

MATERIALS AND METHODS

Materials

Sixty-eight specimens of gallbladder cancer resected or biopsied from 1990 to 2001 were pathologically diagnosed. Retrospective analyses were performed on these routine paraffin embedded sections of 4 um thick. According to WHO classification standard of gallbladder cancer differentiation, 21 cases were in grade I, 27 cases grade II, 20 cases grade III. Twenty-six cases had a diameter of tumor </=2 cm, 23 cases 2-4 cm, 19 cases >/=4 cm. Metastasis was found in 31 cases by clinic examination and in surgical operation. Murine anti-uPA, -VEGF and -SABC monoclonal antibodies were purchased from Wuhan Bosted Co, and uPA from Guangdong Tipus Co.

Methods

Anti-ligand antibodies were used to determine uPAR(10-13), routine de-waxing, trypsin digestion, non-specific antigen blocking. A 1 mg/L of urokinase was used to saturate receptors; anti-uPA monoclonal antibody was added; the rest procedures were according to routine SABC. The procedures of VEGF mAb staining was according to instruction of SABC test kit. There were blank, substitute and normal controls. Positive cells were defined as cytoplasm and/or cell membrane stained clearly buffy or brown. Samples were analyzed by image analyzer. Firstly, strong positive expression regions were selected under low power visual field, then, 10 high power visual fields (400 times) were randomly selected, their grey scales were detected, the average value was used as average expression intensity of the sample.

Statistics

Analyses were performed by χ2-test, t-test and correlation-test. P<0.05 was considered significant.

RESULTS

Results of immunohistochemistry

Positivity rates of uPAR and VEGF expressions in tissue of gallbladder cancer were 57.4% (39/68) and 51.5% (35/68), respectively. uPAR and VEGF were negatively stained in corresponding noncancerous tissues, including relatively normal liver tissue and normal mucosa tissue of gallbladder.

Expression of uPAR and VEGF and clinical pathological stages

Analysis of the relationship between expression rate and
intensity of uPAR and VEGF and clinical features revealed that expression of uPAR and VEGF was closely correlated to metastasis of gallbladder cancer, but not significantly correlated to the differentiation stage and size of gallbladder cancer (Tables 1, 2).

**Table 1** Relationship between uPAR expression and clinical pathological stages of gallbladder cancer

| Tumor | Patients (n) | uPAR-positive patients (n) | Positive rate (%) |
|-------|--------------|----------------------------|-------------------|
| Diameter of tumor | | | |
| <2 cm | 26 | 15 | 57.7 |
| 2-4 cm | 23 | 11 | 47.8 |
| >=4 cm | 19 | 13 | 68.4 |
| Pathological stage | | | |
| I | 21 | 12 | 57.1 |
| II | 27 | 16 | 59.3 |
| III | 20 | 11 | 55.0 |
| Metastasis | | | |
| Positive | 31 | 27 | 87.1 |
| Negative | 37 | 12 | 32.4 |

bP<0.01 vs others.

**Table 2** Relationship between VEGF expression and clinical pathological stage of gallbladder cancer

| Tumor | Patients (n) | uPAR-positive patients (n) | Positive rate (%) |
|-------|--------------|----------------------------|-------------------|
| Diameter of tumor | | | |
| <2 cm | 26 | 14 | 53.8 |
| 2-4 cm | 23 | 12 | 52.2 |
| >=4 cm | 19 | 9 | 47.4 |
| Pathological stage | | | |
| I | 21 | 12 | 57.1 |
| II | 27 | 16 | 59.3 |
| III | 20 | 8 | 40.0 |
| Metastasis | | | |
| Positive | 31 | 24 | 77.4 |
| Negative | 37 | 11 | 29.7 |

bP<0.01 vs others.

**Image analysis of expression of uPAR and VEGF**

Grey scales for positively expressed uPAR and VEGF were 238.4±6.2 and 231.2±4.1, respectively, that for negative expression were 32.1±4.3 and 36.2±3.7, respectively. Correlation analysis showed that the expression intensity of uPAR was significantly positively correlated to that of VEGF (γ = 0.671).

**DISCUSSION**

Human uPAR is composed of 313 amino acid residues. The binding site for uPAR and its ligand-uPA is domain I which is close to N-terminal. Amino acid residues involved in the interaction with ligand are mediated by hydrophobic interaction[14-17]. uPAR and its ligand-uPA’s binding is highly specific. Moreover, this kind of highly effective binding (Kd = 0.1-1.0 nmol/L) makes uPA strongly gather on cell surface, thus activating plasminogen to fibrinolysin locally, leading to extracellular matrix hydrolysis[18-21]. On the other hand, uPAR also has high avidity to pro-uPA. After pro-uPA binding to its membrane receptor, pro-uPA is easily activated into uPA by fibrinolysin around, then pre-fibrinolysin is activated into fibrinolysin by uPA, forming positive feedback enlargement effect. In addition, fibrinolysin on cell membrane is not easily hydrolyzed to inactive form by its inhibitor-α2 anti-fibrinolysin[22-25]. Furthermore, uPAR also activates pre-fibrinolysin by taking part in complex formation of pro-uPA and pre-fibrinolysin on cell surface. Therefore, expression of uPAR in tumor cells has an important localizing role in process of local extracellular matrix hydrolysis, and closely correlates to metastasis[26-30].

VEGF is a kind of specific vascular endothelial cell stimulating factor. It high-effectively and specifically acts on vascular endothelial cells, and intensely promotes splitting and chemotaxis by: (1) increasing microvessel permeability, leading to plasm fibrous protein exosome, thus providing a fiber network for cell migration during the process of vascularization[31-34]; (1) directly stimulating endothelial cell proliferation by acting on two special receptors flt and flk (kdk) of endothelial cell, and producing plasminogen activator (tissue-type and urokinase-type) and collagenase[35-37]. It not only promotes endothelial cell movement, which is in favor of vascularization, but also benefits cancer cells shedding and entrance to blood vessel or infiltrating to neighboring fibrous protein and connective tissue matrix. This specificity provides conditions for tumor invasion and metastasis.

Our results display that expression of uPAR and VEGF is closely correlated to invasion and metastasis of gallbladder cancer, but not significantly correlated to the differentiation stage and size of gallbladder cancer. uPAR and VEGF can be regarded as an invasive phenotype of gallbladder cancer and used for predicting the prognoses, and as evaluation marker for therapeutic efficacy as well. The results also revealed the correlation between the incidence of gallbladder cancer and expression of uPAR and VEGF. On the one hand, extracellular matrix hydrolysis by uPAR provides advantages over vascularization; on the other hand, plasminogen activator induced by VEGF stimulates endothelial cell growth and increases microvascular permeability by interacting with uPAR, herein, extracellular matrix hydrolysis is reinforced by uPAR[38-40]. The regulatory mechanism between uPAR and VEGF, and effective gene therapy methods need further investigation.

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