PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(18):2750-2752
World Journal of Gastroenterology
 ISSN 1007-9327

• BRIEF REPORTS •

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

Shu-Qiang Yue, Yan-Ling Yang, Jing-Shi Zhou, Kai-Zong Li, Ke-Feng Dou

Shu-Qiang Yue, Yan-Ling Yang, Jing-Shi Zhou, Kai-Zong Li, Ke-Feng Dou, Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Correspondence to: Ke-Feng Dou, Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China. gdwk@fmmu.edu.cn
Telephone: +86-29-3375259 **Fax:** +86-29-3375561
Received: 2003-12-10 **Accepted:** 2004-01-15

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.

Yue SQ, Yang YL, Zhou JS, Li KZ, Dou KF. Relationship between urokinase-type plasminogen activator receptor and vascular endothelial growth factor expression and metastasis of gallbladder cancer. *World J Gastroenterol* 2004; 10(18): 2750-2752
http://www.wjgnet.com/1007-9327/10/2750.asp

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 μm 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 χ^2-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[b]	87.1[b]
Negative	37	12	32.4

[b]*P*<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	15	55.6
III	20	8	40.0
Metastasis			
Positive	31	24[b]	77.4[b]
Negative	37	11	29.7

[b]*P*<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 ($\gamma = 0.671$).

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 hydrolyzing[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 intensively promotes splitting and chemotaxis by: (1) increasing microvessel permeability, leading to plasm fibrous protein exosmose, 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.

REFERENCES

1 **Braun S**, Harbeck N. Molecular markers of metastasis in breast cancer: current understanding and prospects for novel diagnosis and prevention. *Expert Rev Mol Med* 2001; **6**: 1-14

2 **Liu LX**, Zhang WH, Jiang HC. Current treatment for liver metastases from colorectal cancer. *World J Gastroenterol* 2003; **9**: 193-200

3 **Quaranta V**, Giannelli G. Cancer invasion: watch your neighbourhood. *Tumori* 2003; **89**: 343-348

4 **Townson JL**, Naumov GN, Chambers AF. The role of apoptosis in tumor progression and metastasis. *Curr Mol Med* 2003; **3**: 631-642

5 **Reuning U**, Sperl S, Kopitz C, Kessler H, Kruger A, Schmitt M, Magdolen V. Urokinase-type plasminogen activator (uPA) and its receptor (uPAR): development of antagonists of uPA/uPAR interaction and their effects *in vitro* and *in vivo*. *Curr Pharm Des* 2003; **9**: 1529-1543

6 **Cunningham O**, Andolfo A, Santovito ML, Iuzzolino L, Blasi F, Sidenius N. Dimerization controls the lipid raft partitioning of uPAR/CD87 and regulates its biological functions. *EMBO J* 2003; **22**: 5994-6003

7 **Nakamura Y**, Yasuoka H, Tsujimoto M, Yang Q, Imabun S, Nakahara M, Nakao K, Nakamura M, Mori I, Kakudo K. Flt-4-positive vessel density correlates with vascular endothelial growth factor-d expression, nodal status, and prognosis in breast cancer. *Clin Cancer Res* 2003; **9**: 5313-5317

8 **Jiang CQ**, Liu ZS, Qian Q, He YM, Yuan YF, Ai ZL. Relationship of Hypoxia-Inducible Factor 1 Alpha (HIF-1alpha) Gene Expression with Vascular Endothelial Growth Factor (VEGF) and Microvessel Density (MVD) in Human Colorectal Adenoma and Adenocarcinoma. *Aizheng* 2003; **22**: 1170-1174

9 **Zhu Z**, Bohlen P, Witte L. Clinical development of angiogenesis inhibitors to vascular endothelial growth factor and its receptors as cancer therapeutics. *Curr Cancer Drug Targets* 2002; **2**: 135-156

10 **Viereck V**, Grundker C, Blaschke S, Niederkleine B, Siggelkow H, Frosch KH, Raddatz D, Emons G, Hofbauer LC. Raloxifene concurrently stimulates osteoprotegerin and inhibits interleukin-6 production by human trabecular osteoblasts. *J Clin Endocrinol Metab* 2003; **88**: 4206-4213

11 **Hudelist G**, Kostler WJ, Attems J, Czerwenka K, Muller R, Manavi M, Steger GG, Kubista E, Zielinski CC, Singer CF. Her-2/neu-triggered intracellular tyrosine kinase activation: *in vivo* relevance of ligand-independent activation mechanisms and impact upon the efficacy of trastuzumab-based treatment. *Br J Cancer* 2003; **89**: 983-991

12 **Michael-Robinson JM**, Pandeya N, Cummings MC, Walsh MD, Young JP, Leggett BA, Purdie DM, Jass JR, Radford-Smith GL. Fas ligand and tumour counter-attack in colorectal cancer stratified according to microsatellite instability status. *J Pathol* 2003; **201**: 46-54

13 **Machner A**, Baier A, Wille A, Drynda S, Pap G, Drynda A, Mawrin C, Buhling F, Gay S, Neumann W, Pap T. Higher susceptibility to Fas ligand induced apoptosis and altered modulation of cell death by tumor necrosis factor-alpha in periarticular tenocytes from patients with knee joint osteoarthritis. *Arthritis Res Ther* 2003; **5**: 253-261

14 **Andreasen PA**, Egelund R, Petersen HH. The plasminogen activation system in tumor growth, invasion, and metastasis. *Cell Mol Life Sci* 2000; **57**: 25-40

15 **Hemsen A**, Riethdorf L, Brunner N, Berger J, Ebel S, Thomssen C, Janicke F, Pantel K. Comparative evaluation of urokinase-type plasminogen activator receptor expression in primary breast carcinomas and on metastatic tumor cells. *Int J Cancer* 2003; **107**: 903-909

16 **Korte W.** Changes of the coagulation and fibrinolysis system in malignancy: their possible impact on future diagnostic and therapeutic procedures. *Clin Chem Lab Med* 2000; **38**: 679-692

17 **Van Hensbergen Y**, Broxterman HJ, Peters E, Rana S, Elderkamp YW, Van Hinsbergh VW, Koolwijk P. Aminopeptidase inhibitor bestatin stimulates microvascular endothelial cell invasion in a fibrin matrix. *Thromb Haemost* 2003; **90**: 921-929

18 **Wang Y**. The role and regulation of urokinase-type plasminogen activator receptor gene expression in cancer invasion and metastasis. *Med Res Rev* 2001; **21**: 146-170

19 **Ramont L**, Pasco S, Hornebeck W, Maquart FX, Monboisse JC. Transforming growth factor-beta1 inhibits tumor growth in a mouse melanoma model by down-regulating the plasminogen activation system. *Exp Cell Res* 2003; **291**: 1-10

20 **De Bock CE**, Wang Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. *Med Res Rev* 2004; **24**: 13-39

21 **Le Gat L**, Gogat K, Bouquet C, Saint-Geniez M, Darland D, Van Den Berghe L, Marchant D, Provost A, Perricaudet M, Menasche M, Abitbol M. *In vivo* adenovirus-mediated delivery of a uPA/uPAR antagonist reduces retinal neovascularization in a mouse model of retinopathy. *Gene Ther* 2003; **10**: 2098-2103

22 **Harvey SR**, Hurd TC, Markus G, Martinick MI, Penetrante RM, Tan D, Venkataraman P, DeSouza N, Sait SN, Driscoll DL, Gibbs JF. Evaluation of urinary plasminogen activator, its receptor, matrix metalloproteinase-9, and von Willebrand factor in pancreatic cancer. *Clin Cancer Res* 2003; **9**: 4935-4943

23 **Rabbani SA**, Mazar AP. The role of the plasminogen activation system in angiogenesis and metastasis. *Surg Oncol Clin N Am* 2001; **10**: 393-415

24 **Mabrouk RA**, Ali-Labib R. Detection of urokinase plasminogen activator receptor and c-erbB-2 in sera of patients with breast and ovarian carcinoma. *Clin Biochem* 2003; **36**: 537-543

25 **Hamdi HK**, Castellon R. ACE inhibition actively promotes cell survival by altering gene expression. *Biochem Biophys Res Commun* 2003; **310**: 1227-1235

26 **Shin YC**, Folk WR. Formation of polyomavirus-like particles with different VP1 molecules that bind the urokinase plasminogen activator receptor. *J Virol* 2003; **77**: 11491-11498

27 **Zhang F**, Tom CC, Kugler MC, Ching TT, Kreidberg JA, Wei Y, Chapman HA. Distinct ligand binding sites in integrin alpha3beta1 regulate matrix adhesion and cell-cell contact. *J Cell Biol* 2003; **163**: 177-188

28 **Leung YK**, Ng TB, Ho JW. Transcriptional regulation of fosl-1 by licorice in rat Clone 9 cells. *Life Sci* 2003; **73**: 3109-3121

29 **Lloyd FP Jr**, Slivova V, Valachovicova T, Sliva D. Aspirin inhibits highly invasive prostate cancer cells. *Int J Oncol* 2003; **23**: 1277-1283

30 **Roelofs JJ**, Rowshani AT, van den Berg JG, Claessen N, Aten J, ten Berge IJ, Weening JJ, Florquin S. Expression of urokinase plasminogen activator and its receptor during acute renal allograft rejection. *Kidney Int* 2003; **64**: 1845-1853

31 **Baker EA**, Bergin FG, Leaper DJ. Plasminogen activator system, vascular endothelial growth factor, and colorectal cancer progression. *Mol Pathol* 2000; **53**: 307-312

32 **Konno H**, Abe J, Kaneko T, Baba M, Shoji A, Sunayama K, Kamiya K, Tanaka T, Suzuki S, Nakamura S, Urano T. Urokinase receptor and vascular endothelial growth factor are synergistically associated with the liver metastasis of colorectal cancer. *Jpn J Cancer Res* 2001; **92**: 516-523

33 **Poulaki V**, Mitsiades CS, McMullan C, Sykoutri D, Fanourakis G, Kotoula V, Tseleni-Balafouta S, Koutras DA, Mitsiades N. Regulation of vascular endothelial growth factor expression by insulin-like growth factor I in thyroid carcinomas. *J Clin Endocrinol Metab* 2003; **88**: 5392-5398

34 **Koide N**, Nishio A, Kono T, Hiraguri M, Watanabe H, Igarashi J, Hanazaki K, Adachi W, Amano J. Histochemical study of angiogenesis in basaloid squamous carcinoma of the esophagus. *Dis Esophagus* 2000; **13**: 142-147

35 **Koshida K**, Konaka H, Kato H, Miyagi T, Egawa M, Uchibayashi T, Namiki M. Correlation between expression of metastasis-related genes and lymph node metastasis in testicular cancer. *Hinyokika Kiyo* 2000; **46**: 775-781

36 **Oh CW**, Hoover-Plow J, Plow E. The role of plasminogen in angiogenesis *in vivo*. *J Thromb Haemost* 2003; **1**: 1683-1687

37 **Dazzi C**, Cariello A, Maioli P, Magi S, Rosti G, Giovanis P, Giovannini G, Lanzanova G, Marangolo M. A high cytosol value of urokinase-type plasminogen activator (uPA) may be predictive of early relapse in primary breast cancer. *Cancer Invest* 2003; **21**: 208-216

38 **Kaneko T**, Konno H, Baba M, Tanaka T, Nakamura S. Urokinase-type plasminogen activator expression correlates with tumor angiogenesis and poor outcome in gastric cancer. *Cancer Sci* 2003; **94**: 43-49

39 **Gruss CJ**, Satyamoorthy K, Berking C, Lininger J, Nesbit M, Schaider H, Liu ZJ, Oka M, Hsu MY, Shirakawa T, Li G, Bogenrieder T, Carmeliet P, El-Deiry WS, Eck SL, Rao JS, Baker AH, Bennet JT, Crombleholme TM, Velazquez O, Karmacharya J, Margolis DJ, Wilson JM, Detmar M, Skobe M, Robbins PD, Buck C, Herlyn M. Stroma formation and angiogenesis by overexpression of growth factors, cytokines, and proteolytic enzymes in human skin grafted to SCID mice. *J Invest Dermatol* 2003; **120**: 683-692

40 **Behzadian MA**, Windsor LJ, Ghaly N, Liou G, Tsai NT, Caldwell RB. VEGF-induced paracellular permeability in cultured endothelial cells involves urokinase and its receptor. *FASEB J* 2003; **17**: 752-754

Edited by Chen WW and Zhu LH **Proofread by** Xu FM