Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-1323-2
June 2014, Vol. 13, No. 6, P283–P287

Expression of Hpa and CD222 in bladder carcinoma and analysis of clinico-pathologic correlation

Shuhong Shi, Hui Zhang

Department of Blood and Oncology, Shaoyang Central Hospital, Shaoyang 422000, China

Received: 25 April 2014 / Revised: 3 May 2014 / Accepted: 15 May 2014

Abstract ***Objective:*** Our study aimed to investigate the relationships between the clinico-pathologic features and the heparanase (Hpa) and CD222 expressions in bladder carcinoma. ***Methods:*** The expressions of Hpa and CD222 in 95 bladder carcinoma specimens and 20 paraneoplastic bladder tissues (controls) were assessed using the immunohistochemical staining method. ***Results:*** The positive expression rates of Hpa and CD222 in bladder carcinoma were 68.42% and 61.05%, respectively. The positive rate of Hpa was significantly higher in the carcinoma specimens than in the control specimens ($P < 0.01$). Similarly, the Hpa expression in the invasive bladder carcinoma was significantly higher than that in the non-invasive bladder carcinoma ($P < 0.01$). A positive correlation was observed between the expressions of Hpa and CD222 ($P < 0.05$). The expressions of Hpa and CD222 were significantly correlated with lymphatic invasion and TNM staging ($P < 0.05$). The 5-year survival rate was significantly higher in negative expression of the Hpa group than that in the positive expression group ($P < 0.05$). Compared with the non-co-positive expression group, the 5-year survival rate in the co-positive expression of Hpa and CD222 group was significantly lower ($P < 0.05$). ***Conclusion:*** High Hpa and CD222 expressions in tumor tissues were associated with the occurrence and development of bladder carcinoma. Our results provide helpful information for the further diagnosis and therapy of bladder carcinoma.

Key words bladder carcinoma; heparanase; CD222

Bladder carcinoma is the most common malignant tumors of genitourinary system in China. The incidence and mortality of bladder carcinoma both rank first in tumors of urinary system. After the first diagnosis, 70%–85% of patients are superficial bladder cancer, 50%–70% of patients after first bladder preserving operation relapse. Although the grade and staging of majority of tumors's recurrence and that of primary tumor are similar, 10%–15% of superficial bladder cancers eventually evolve highly malignant invasive tumors [1]. Bladder perfusion chemotherapy and immunotherapy after postoperative bladder cancer can reduce bladder cancer's recurrence, but can not completely prevent tumor progression. Bladder cancer invasion and metastasis are the main factors of recurrence of bladder cancer. It is also the leading cause of treatment failure and death in patients. Recent researches showed that heparanase (HPa) can specifically degrade sulfate proteoglycan in extracellular matrix, and damage extracellular matrix barrier, so as to promote cellular invasion, cell proliferation and induction of angiogenesis [2–6]. The study also found that Hpa was not directly anchored on the cell surface, but played a role by combining CD222 receptor. CD222 is also called insulin-like growth factor II receptor. It is a multiple structure domain transmembrane glycoprotein. In addition to Hpa, it also can combine insulin-like growth factor II (IGF-II) and lysosomal enzyme, inhibit tumor cell proliferation induced by IGF-II [7–9]. This study used immunohistochemical method to detect the expression of Hpa and CD222 protein in 95 cases of urothelial carcinoma, and to investigate the correlation between expression level of Hpa and its receptor with the metastasis and prognosis of bladder cancer.

Correspondence to: Shuhong Shi. Email: shushuhong2000@163.com

Materials and methods

Materials

General information

We gathered 95 cases of paraffin specimens of bladder cancer removed in operation in Tumor Hospital of Liaoning province from February 2002 to October 2005. All patients were treated without preoperative chemotherapy, radiotherapy and immunotherapy. The pathological diagnosis was transitional cell carcinoma of bladder. Another 20 cases of paraneoplastic bladder tissues (distance was more than 5 cm from neoplastci foci) were considered as the control group. After pathological examination, it was

confirmed as normal bladder tissue. All specimens were fixed in 10% formalin, embedded in paraffin. We made 4 μm continuous slices to spare. There were 77 cases of male and 18 cases of female in 95 cases of bladder cancer patients. The age was 30–76 years old. The average age was 45 years old. Among all patients, 35 cases occurred lymphatic metastasis. According to WHO method, we formulated clinical pathological staging, 57 cases were superficial bladder cancer and 38 cases were invasive bladder cancer. All cases had complete follow-up after operation, the survival rate was calculated from the operation date to death induced by recurrence and metastasis or follow-up date (June 2011). This study was approved by the relevant ethics committees.

Reagents

Kit Rabbit anti-human Hpa polyclonal antibody and mouse anti-human CD222 monoclonal antibody were purchased from Santa Cruz company, USA. SP ELISA Kit (second anti biotin Goat anti pika IgG, normal sheep serum blocking solution, horseradish peroxidase labeled streptavidin solution) were purchased from Maixin Biotechnology Development Co., Ltd., China. 0.01 M citrate antigen repairing buffer (pH6.0), PBS (pH7.2–7.6) buffer solution and DAB enzyme substrate chromogenic reagents were purchased from Maixin Biotechnology Development Co., Ltd., China.

Method

Immunohistochemical detection

In accordance with the operation method in detection kit of immunohistochemical SP method, we used the pressure cooker antigen repairing technique for the detection of Hpa and CD222 expression in bladder cancer. We use PBS buffer instead of the first antibody as negative control.

Judgment standard

The Hpa and CD222 immunohistochemistry results of brown yellow granules emerging in cytoplasm was positive, staining intensity and percentage of positive cells were used to evaluate positive expression. We randomly selected 10 high power field in each section of the bladder cancer tissue under light microscope. We counted 100 cells in per power field. The staining intensity was classified as follows: no coloring was 0, light brown was 1, brown yellow and deeper color was 2. The number of positive cells was graded as: the number of positive cells ≤ 50% was 0, 51%–75% was 1, ≥ 76% was 2. Two scores were added together, the result > 2 was positive.

Statistical analysis

SPSS19.0 was used for statistical analysis. Enumeration data were counted by Chi-square test. We used Ka-plan-Meier method to make survival analysis of follow-up data and determined the difference by Log-rank test. There were statistically different when $P < 0.05$.

Results

Expression of Hpa and CD222 in bladder cancer and paraneoplastic bladder tissues

The expression of Hpa in normal bladder epithelium was negative (Fig. 1a), but its expression in part of lymphocytes and endothelial cells in the bladder interstitium was in different extent of strength. The expression of Hpa can be observed in 68.42% (65/95) in cytoplasm of bladder cancer, it was brown yellow granules (Fig. 1b). There was significant difference in positive expression rate between bladder carcinoma tissues and paraneoplastic bladder tissues ($P < 0.01$). In invasive and noninvasive bladder cancer tissues, the positive rates were 86.84% (33/38) and 56.14% (32/57) respectively, and there was statistically significant difference between them ($P < 0.01$). The positive expression of CD222 in normal bladder epithelium was weak (Fig. 1c), mainly in cytoplasm in bladder cancer tissues, and the positive expression rate was 61.05% (58/95) (Fig. 1d).

The relationship between the expression of Hpa, CD222 and clinical pathological factors

We did Chi-square test for the expression of Hpa, CD222 and its correlation with the clinical pathological features of bladder cancer respectively. The results showed that the expression of Hpa was correlated with tumor size, clinical pathological staging, lymphatic metastasis in bladder cancer patients ($P < 0.05$). In those patients such as the diameter of tumor > 3 cm, invasive tumors and with lymphatic metastasis, the expressions of Hpa were all strong. The expression of CD222 was correlated with clinical pathological staging and lymphatic metastasis in patients with bladder cancer ($P < 0.05$). The expression of CD222 in invasive tumor accompanying lymphatic metastasis in patients was strong (Table 1).

The relationship between the expression of Hpa and CD222

We contrasted with Hpa, CD222 positive and negative data in bladder cancer patients. The results found that the expression of Hpa and CD222 were remarkably consistent. The positive rate of CD222 expression was significantly increased in the patients whose expression of Hpa was positive ($P < 0.05$; Table 2).

The relationship between the expression levels of heparanase, CD222 and 5-year survival rate of bladder cancer: 95 cases of bladder cancer patients all had complete follow-up data. The follow-up period was five years, including 35 cases of death. We used Kaplan-Merier method to analyze the survival period, and did Log-rank sequence test. The results showed that the positive expression of

Table 1 Correlation between the clinico-pathological parameters and the expressions of Hpa and CD222 (*n*)

Parameter	*n*	Heparanase			*P*	CD222			*P*
		+	–	Positive rate (%)		+	–	Positive rate (%)	
Age (years)									
< 45	36	22	14	61.11	0.301	23	13	63.88	0.658
≥ 45	59	42	17	71.17		35	24	59.32	
Tumor size (cm)									
< 3	65	37	18	56.92	0.020	41	24	63.07	0.119
≥ 3	30	27	3	90.00		17	13	56.66	
Pathologic grade									
1	32	15	17	46.87		16	16	53.13	
2	34	25	9	69.44	0.007	20	14	61.76	0.305
3	29	24	5	82.76		22	7	79.31	
Recurrent or not									
Primary	41	22	17	53.66	0.028	21	20	51.21	0.087
Recurrent	59	42	12	77.78		37	17	68.52	
Number of tumors									
Single	25	14	11	56.00	0.158	13	12	52.00	0.280
Multiple	40	50	20	71.43		45	25	64.29	
Clinical stage									
Non-invasive (pTa)	57	32	25	56.14	0.004	30	27	54.38	0.039
Invasive (pT1–pT4)	38	32	6	84.21		28	10	71.05	
Lymphatic metastasis									
No	60	35	25	56.14	0.014	31	29	51.66	0.014
Yes	35	29	6	84.21		27	8	77.14	

+, positive; –, negative

Table 2 Correlation between the expressions of Hpa and CD222 in bladder carcinoma

Parameter	HPa expression				*n*	*P*
	Positive		Negative			
	n	%	*n*	%		
CD222 (+)	46	79.31	12	20.69	58	0.002
CD222 (–)	18	48.65	19	51.35	37	

+, positive; –, negative

the Hpa group showed a significantly poorer 5-year survival rate than the negative expression group, and there was statistically significant difference between them ($P < 0.05$; Fig. 2). The positive expression of the CD222 group showed a significantly lower 5-year survival rate than the negative expression group, but there was no significant difference between them ($P > 0.05$; Fig. 3). The co-expression of the Hpa and CD222 group showed a significantly worse 5-year survival rate than negative expression group, there was statistically significant difference between them ($P < 0.05$; Fig. 4).

Discussion

The extracellular matrix (ECM) and basement membrane (BM) are important barriers from tumor invasion and metastasis. The barriers are mainly consisted of two components: (1) structural proteins, including collagen, laminin, fibronectin and vitreous binding and etc. (2) glycosaminoglycan. Its main component is heparan sulfate proteoglycan (HSPG). Hpa is an endogenous beta glucuronide incision enzyme. It can specifically degrade heparan sulfate proteoglycan in extracellular matrix, destroy extracellular matrix barrier, and make some growth factors binding to heparan sulfate (VEGF, bFGF) released. Thus can promote cell invasion, also promote cell proliferation and inducting angiogenesis, which may play a role in tumor invasion and metastasis [10–12].

Recent studies indicated that Hpa highly expressed in many tumors such as breast cancer, gastric cancer, pancreatic cancer, bladder cancer, ovarian cancer, lung cancer, head, neck and oral carcinoma. It positively correlated with tumor invasion and metastasis, and negatively correlated with the prognosis of the patients. A study on several oral cancer cell lines and human oral mucosal epithelial carcinoma from Leiser *et al* [13] found that Hpa activity and mRNA level increased and correlated with metastasis ability. Zheng *et al* [14] indicated that Hpa expression in gastric cancer tissue was positively corre-

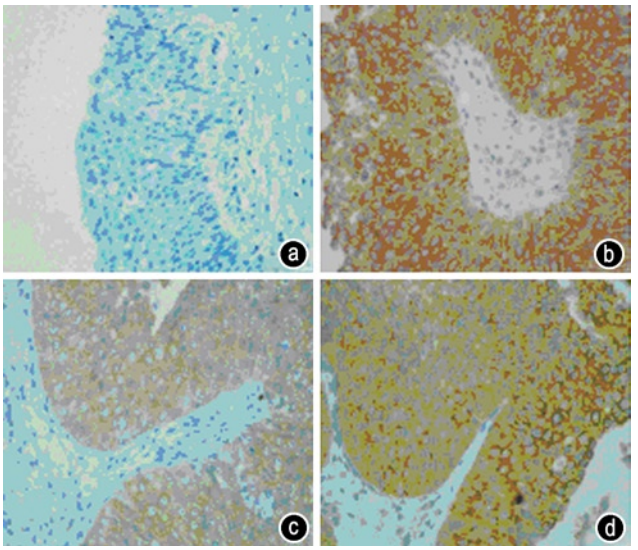

Fig. 1 Expression of HPa and CD222 in bladder cancer and paraneoplastic tissues. (a) Negative expression of HPa in paraneoplastic tissue; (b) Positive expression of Hpa in bladder carcinoma tissue; (c) Weakly positive expression of CD222 in paraneoplastic tissue; (d) Positive expression of CD222 in bladder carcinoma tissue

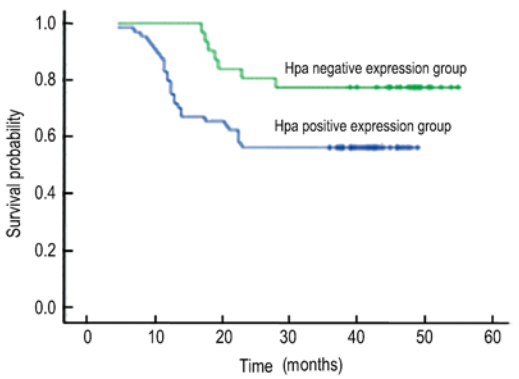

Fig. 2 Overall survival curves. Prognosis was significantly better in the group without Hpa expression than in the group with Hpa expression (Log-rank test; $P = 0.027$, $P < 0.05$)

lated with the degree of tumor invasion and lymphatic metastasis. Gohji *et al* [15] found that the expression of Hpa protein in bladder cancer tissues invasive of the muscular layer was obviously higher than in that only confined to the mucosa. The expression in primary bladder cancer tissues accompanying with lymphatic metastasis was significantly higher than that without metastasis. The expression in high clinical staging of cancer tissue was higher than that in low clinical staging. The results of this study showed that Hpa was correlated with tumor size, histological grade, lymphatic metastasis and 5-year survival rate of bladder cancer ($P < 0.05$). This was basically consistent with above research results.

The present study showed that Hpa was not directly

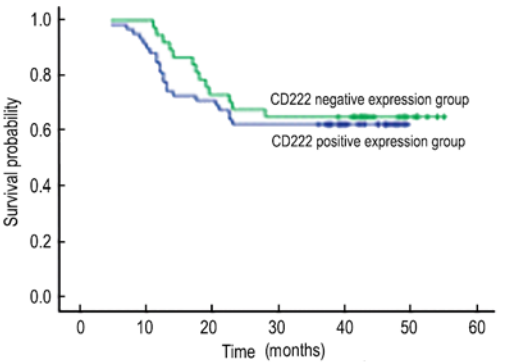

Fig. 3 Overall survival curves. Overall survival was not associated with CD222 expression (log-rank test, $P = 0.0.593$; $P > 0.05$)

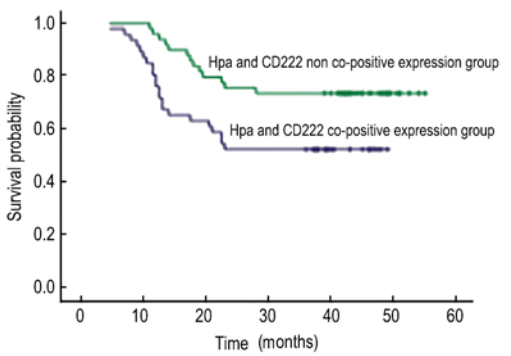

Fig. 4 Overall survival curves. Overall survival rates were lower in patients with co-expression of Hpa and CD222 than in patients without Hpa and CD222 expression (Log-rank test; $P = 0.016$, $P < 0.05$)

acted on the cell surface, but it exerted its degradation by combining the CD22 receptor. Wood *et al* [16] found that after Hpa was combined with L cells expressing CD222 receptor in mice, the ability to destroy the extracellular matrix and basement membrane was 3–4 times of not expressing CD222 receptor. Hpa combining with CD222 can significantly promote the degradation of extracellular matrix. The results of this study also showed that the expression of Hpa and CD222 was remarkably consistent ($P < 0.05$), the 5-year survival rate in the positive co-expression group of Hpa and CD222 was significantly lower than that in the negative group ($P < 0.05$). These indicated that Hpa and CD222 co-expression in bladder cancer was closely related with the prognosis of patients.

The results of this study also showed the expression of CD222 was obviously correlated with clinical pathological staging in bladder cancer and lymphatic metastasis, but not correlated with the size of the tumor. Moreover, the positive expression of the CD222 group did not show a significant difference in 5-year survival rate compared with the negative expression group, and the positive expression group and 5-year survival rate were not significantly different from that in the negative expression

group. This also showed that the CD222 had a regulatory role in the occurrence and development of the of bladder cancer. But it alone as the detection index of bladder cancer was of little significance. Only combined detection of Hpa and CD222 can really help to judge the metastasis and prognosis of bladder cancer.

In conclusion, CD222 may mediate the activation of Hpa in bladder cancer, combined detection of Hpa and CD222 is expected to become a new index of judging the recurrence and prognosis of patients with bladder cancer.

References

1. Nargund VH, Tanabalan CK, Kabir MN, *et al*. Management of non-muscle-invasive (superficial) bladder cancer. Semin Oncol, 2012, 39: 559–572.
2. Chen Z, Zheng XD. The expressions and significance of Hpa and bFGF in oral squamous-cell carcinoma. Chinese-German J Clin Oncol, 2009, 8: 46–49.
3. Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest, 2001, 108: 341–347.
4. Vlodavsky I, Eldor A, Haimovitz-Friedman, *et al*. Expression of heparanase by platelets and circulating cells of the immune system: possible involvement in diapedesis and extravasation. Invasion Metastasis, 1992, 12: 112–127.
5. Pikas DS., Li JP, Vlodavsky I, *et al*. Substrate specificity of heparanases from human hepatoma and platelets. J Biol Chem, 1998, 273: 18770–18777.
6. Freeman C, Parish CR. Human platelet heparanase: purification, characterization and catalytic activity. Biochem J, 1998, 330: 1341–1350.
7. Ilan N, Elkin M, Vlodavsky I, *et al.* Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. Int J Biochem Cell Biol, 2006, 38: 2018–2039.
8. Leksa V, Pfisterer K, Ondrovicová G, *et al.* Dissecting mannose 6-phosphate-insulin-like growth factor 2 receptor complexes that control activation and uptake of plasminogen in cells. J Biol Chem, 2012, 287: 22450–22462.
9. Harkness L, Christiansen H, Nehlin J, *et al*. Identification of a membrane proteomic signature for human embryonic stem cells independent of culture conditions. Stem Cell Res, 2008, 1: 219–227.
10. Thompson CA, Purushothaman A, Ramani VC, *et al*. Heparanase regulates secretion, composition and function of tumor cell-derived exosomes. J Biol Chem, 2013, 288: 10093–10099.
11. Sanderson RD, Yang Y, Kelly T, *et al*. Enzymatic remodeling of heparan sulfate proteoglycans within the tumor microenvironment: growth regulation and the prospect of new cancer. Cell Biochem, 2005, 96: 897–905.
12. Nilasaroya A, Martens PJ, Whitelock JM, *et al.* Enzymatic degradation of heparin-modified hydrogels and its effect on bioactivity. Biomaterials, 2012, 33: 5534–5540.
13. Leiser Y, Abu-El-Naaj I, Sabo E, *et al*. Prognostic value of heparanase expression and cellular localization in oral cancer. Head Neck, 2011, 33: 871–877.
14. Zheng L, Pu J, Jiang G, *et al.* Abnormal expression of early growth response 1 in gastriccancer: association with tumor invasion, metastasis and heparanase transcription. Pathol Int, 2010, 60: 268–277.
15. Gohji K, Okamoto M, Kitazawa S, *et al.* Heparanase protein and gene expression in bladder cancer. J Urol, 2001, 166: 1286–1290.
16. Wood RJ, Hulett MD. Cell surface-expressed cation-independent mannose 6-phosphate receptor (CD222) binds enzymatically active heparanase independently of mannose-6 phosphate to promote extracellular matrix degradation. J Biol Chem, 2008, 283: 4165–4176.