

# MDR1/P-Glycoprotein Overexpression in Bladder Transitional Cell Carcinoma and its Correlation with Expression of Survivin and Fas

Changli Wu  
Wenlan Zhang  
Jiwu Chang  
Zuohui Zhao  
Guang Sun  
Ruifa Han

Tianjin Urological Department, Tianjin Institute of Urologic Surgery, the Second Hospital of Tianjin Medical University, Tianjin 300211, China.

Correspondence to: Changli Wu  
E-mail: wucl2003@163.com

This work was supported by the grant from the Education Administration Development Foundation of Tianjin City, China (No.20030304).

Received March 1, 2006; accepted April 24, 2006.

CJCO <http://www.cjco.cn> E-mail: cocr@eyou.com  
Tel (Fax): 86-22-2352-2919

**OBJECTIVE** To explore the expression of the MDR1/P-glycoprotein, Fas and survivin and to examine their correlation with the biologic behavior of bladder transitional cell carcinoma (BTCC).

**METHODS** Immunohistochemistry was used to examine the expression of P-gp, survivin and Fas in BTCC (n=64) and normal bladder mucosa (n=12).

**RESULTS** The expression level of P-gp and survivin in BTCC was higher compared to normal bladder mucosa ( $P<0.01$ ) and their expression was strongly correlated with clinical grading ( $P<0.01$ ). In BTCC and normal bladder mucosa Fas expression was 50% and 100%, respectively ( $P<0.01$ ). Recurrent BTCC showed higher expression than primary BTCC ( $P<0.01$ ) and the expression of P-gp in BTCC had a reverse correlation with Fas expression but no correlation with survivin expression.

**CONCLUSUON** The MDR of BTCC was strongly correlated with the expression of P-gp and Fas, but was not correlated with survivin expression. Thus, enhancing cancer sensitivity to chemotherapy by reversing multidrug resistance with reversal agents or up-regulating Fas expression by apoptotic enhancing agents, might be a potential therapy to prevent tumor recurrence and invasiveness.

**KEYWORDS:** bladder transitional cell carcinoma, P-glycoprotein multidrug resistance, survivin, Fas.

Bladder transitional cell carcinomas (BTCC) are the most common urologic tumors in China and the sixth most common malignancy in developed countries.<sup>[1]</sup> One characteristic feature of BTCC is the high recurrence incidence which is about 75~80% after operation. Combined surgical management, including radical cystectomy and transurethral resection and post-operation intravesical chemotherapy, are the main therapeutic approaches. Drug resistance is a persistent problem for cancer chemotherapy and also is a serious obstacle for successful multimodality therapy. Induction of cancer cell apoptosis is an important mechanism for effective chemotherapy.<sup>[2]</sup> Up to now, there has been a lack of agreement about the association between cancer cell apoptosis and multidrug resistance so their precise underlying relationship remains an open question.<sup>[3,4]</sup> In the present study, we examined the expression of the MDR1/P-glycoprotein, Fas and survivin by immunohistochemistry in normal and cancerous bladder tissue. An association between the expression of MDR1/P-gp, survivin, Fas and the clinicopathologic parameters of the bladder cancers was also examined.

## MATERIALS AND METHODS

### Clinical specimens

Seventy-seven paraffin-embedded tissue specimens were obtained, after informed consent, from 76 patients treated at The No.2 Hospital of Tianjin Medical University between April 2004 and December 2004. All the specimens were pathologically verified to be cancerous or normal bladder tissue. Among them, 64 cases were shown to be bladder transitional cell carcinoma. The cases were comprised of 40 males and 24 females and 48 primary and 16 recurrent tumors. Their ages ranged from 34 to 86 years with an average of 51. According to the World Health Organization/International Society of Urologic Pathology (ISUP) (1998 classification), 12 were Grade 1, 28 Grade 2 and 24 Grade 3 BTCC. Based on the 1997 American Joint Committee on Cancer-International Union Against Cancer (AJCC-UICC) TNM system, 42 specimens were classified as Stage Ta-T1 and 22 as Stage T2-T4. In addition, 12 specimens were normal bladder tissues from transurethral resection of the prostate from patients with benign prostatic hyperplasia. Their ages ranged from 59 to 78 with an average of 68.

### Method

#### Immunohistochemical staining

Consecutive 4  $\mu$ m thick sections were cut from the

paraffin blocks, after which En Vision immunostaining was used. The following antibodies were applied to the sections: MDR1/P-glycoprotein (1:50, mouse monoclonal antibody, ZM-0189, ZSBO, Beijing, China), Fas (1:100, mouse monoclonal antibody, ZM-0295, ZSBO, Beijing, China), survivin (1:80, rabbit polyclonal antibody, BA1420, BOSTER, Wuhan, China). The color reaction was developed with the addition of DAB. All procedures were implemented according to the supplier's instructions. For negative controls, sections were processed as the above but with PBS instead of the primary antibodies.

#### Evaluation of MDR1/P-glycoprotein, Fas and survivin staining

The immunoreactivity to survivin was localized in the cytoplasm, while Fas and P-gp were localized in the cytoplasm and cell membrane. The staining was graded according to the proportion of positive cells and the degree of staining intensity achieved. The former was defined as follows: <10% as 0, 10~25% as 1, 25~50% as 2,  $\geq$ 50% as 3 and the latter was: - (background) as 0, +(weak yellow) as 1, ++ (yellow) as 2, +++(brown) as 3. The sum  $\leq$ 2 was defined as negative expression,  $\geq$ 3 as positive expression.

#### Statistical analysis

All data were analyzed with SPSS 11.5 statistical software (including chi-square or adjusted chi-square) and  $P < 0.05$  was considered to have statistical significance.

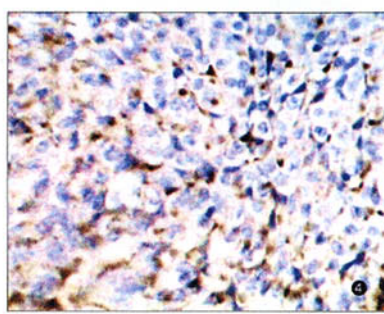
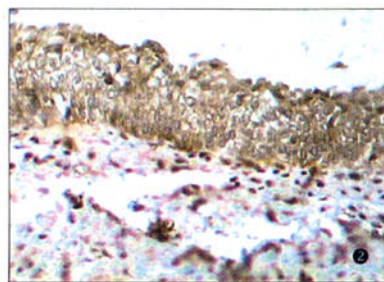
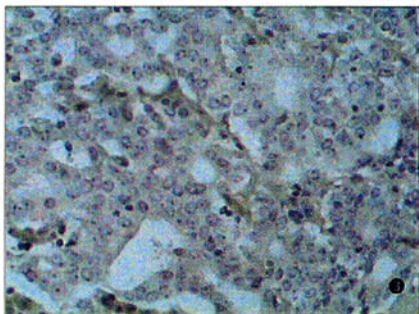
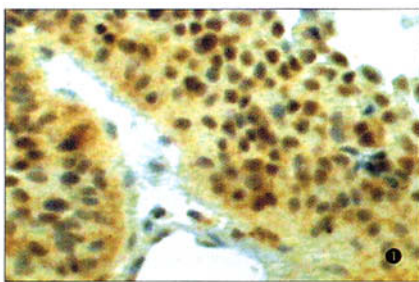


Fig.1. Survivin positive expression in grade 2 BTCC ( $\times$  400).

Fig.3. Fas positive expression in grade 2 BTCC ( $\times$  200).

Fig.2. Fas positive expression in normal bladder ( $\times$  200).

Fig.4. P-GP positive expression in grade 2 BTCC ( $\times$  200).

**Table 1. Expression of P-gp, survivin and Fas in BTCC and correlation with clinical pathological characteristics**

Clinical pathological parameters		Case (n)	P-GP (%)	Survivin (%)	Fas (%)
Sex	Male	49	26 (53.1%)	46(93.9%)	24(48.9%)
	Female	15	8 (53.3%)	14(93.3%)	8(53.3%)
$\chi^2$			0.985	0.939	0.087
Coefficient			0.020	0.106	0.037
Age	<55	21	10 (47.6%)	19(90.5%)	10(47.6%)
	>55	43	24 (55.8%)	41(95.3%)	22(51.1%)
$\chi^2$			0.381	0.572	0.071
Coefficient			0.077	0.094	0.033
Grading	G1	12	3(25.0%)	9(75.0%)	5(41.7%)
	G2	28	11(39.3%)	27(96.4%)	13(46.4%)
	G3	24	20(83.3%)**	24(100%)**	14(58.3%)
$\chi^2$			14.760	9.143	1.143
Coefficient			0.433	0.354	0.132
Stage	T0-1	42	22(52.4%)	38(90.5%)	20(47.6%)
	T2-4	22	12(54.5%)	22(100%)	12(54.5%)
$\chi^2$			0.027	2.235	0.277
Coefficient			0.021	0.184	0.066
Tumor No	One	24	11(45.8%)	22(91.7%)	10(41.6%)
	Multiple	40	23(57.5%)	38(95%)	22(55.0%)
$\chi^2$			0.820	0.284	1.067
Coefficient			0.112	0.067	0.128
Recurrence	Primary	48	19(39.5%)	44(91.6%)	21(43.7%)
	Recurrent	16	15(93.7%)**	16(100%)	11(68.7%)
$\chi^2$			14.139	1.422	3.000
Coefficient			0.425	0.147	0.212
Total			34(53.1%)	60(93.8%)	32(50.0%)

\*\* $P < 0.05$ 

## RESULTS

### Expression of MDR1/P-glycoprotein, Fas and survivin in BTCC

The results of the immunohistochemical staining were as follows: the MDR1/P-glycoprotein, Fas and survivin staining was positive in 53.1, 50.1 and 93.8% of the cases of bladder transitional cell carcinomas compared to 3, 12 and 0% respectively of the tissues from normal bladders. Survivin was more highly expressed in bladder transitional cell carcinoma than in normal bladder tissue ( $\chi^2=53.437$ ,  $P=0.001$ ), but expression of Fas was higher in normal bladder compared to cancerous bladder ( $\chi^2=3.2$ ,  $P=0.074$ ). However expression of MDR1/P-glycoprotein did not differ significantly between normal and cancerous tissues (Figs.1~4) ( $\chi^2=3.2$ ,  $P=0.074$ ), but MDR1/P-glycoprotein expression did significantly correlate with pathological grading and tu-

mor recurrence ( $P < 0.01$ ). Survivin correlated only with pathological grading ( $P < 0.01$ ). Fas expression had no correlation with the clinical pathological characteristics ( $P > 0.05$ , Table 1).

### Expression of the MDR1/P-glycoprotein in BTCC and its correlation with Fas and survivin

The expression of P-gp in BTCC showed a reverse correlation with Fas expression, but no correlation with survivin expression (Table 2). The survivin expression level were increased gradually with an ascending pathological grade.

## DISCUSSION

The appearance of tumor cells resistant to multiple anticancer agents is a serious obstacle in cancer treatment. The overexpression of MDR1/P-glycoprotein is

**Table 2. MDR1/P-glycoprotein expression correlation with survivin and Fas**

	Survivin		Fas	
	+	-	+	-
P-GP(+)	32	2	10	24
P-GP(-)	28	2	22	8**
$\chi^2$	5.34		12.298	
Coefficient	0.016		-0.401	

\*\* $P < 0.01$ 

one of most critical molecular proteins for limiting drug resistance. Overexpression of P-gp has been found in human bladder cancer cells selected by drug resistance against P-gp-targeting drugs.<sup>[5]</sup> In patients with bladder cancers, expression of P-gp is often increased after chemoradiotherapeutic treatment.<sup>[6]</sup> Furthermore, recent studies have found that over-expression of the MDR1 gene occurred more frequently in recurrent bladder cancers.<sup>[7,8]</sup> In our study, we found that the expression of the MDR1/P-glycoprotein was markedly elevated in recurrent bladder cancers after intravesical chemotherapy compared to untreated primary tumors, indicating that this increased expression of the MDR1/P-glycoprotein might be partly involved in drug resistance and intravesical recurrence in these patients. Moreover, over-expression of the MDR1/P-glycoprotein was significantly correlated with different pathological grading in these bladder cancers but not with their clinical stage. These findings are consistent with previous studies.<sup>[9]</sup> Results from this study provide further support for enhancing chemotherapy sensitivity through the use of agents such as Verapamil, CyclosporinA, etc. that reverse resistance.

The formation of multidrug resistance in cancer cells is a multiple gene regulation process, that might involve overexpression of an anti-apoptotic gene and/or abnormal expression of the apoptosis-regulating gene. Recent studies have shown that changes in apoptosis-regulating genes correlated with a multidrug-resistance mechanism, such as Bcl-2 and bax.<sup>[10]</sup> Survivin is a recently discovered protein belonging to a gene family of inhibitors of apoptosis (IAP). It is selectively expressed in cancer cells but undetectable in nonproliferating normal tissues, suggesting an important role in tumorigenesis. In our study, increased expression of survivin was positively correlated with an ascending pathological grade. Furthermore, there was no significant correlation between MDR1/P-glycoprotein and survivin expression, findings which are consistent with a previous study.<sup>[11]</sup>

万方数据

Fas (CD95/APO-1) is a cell-surface "death receptor" that mediates apoptosis upon engagement by its ligand. Tumor cells frequently exhibit decreased expression of Fas. In our study, MDR1/P-glycoprotein expression was reversely correlated with Fas, indicating that chemotherapy resistance was possibly associated with Fas-mediated apoptotic inhibition. One study found that Fas gene transduction could reverse the MDR of the human drug-resistant gastric cancer cell, SGC7901/VCR.<sup>[12]</sup>

In conclusion, this study further demonstrates that MDR1/P-glycoprotein-mediated multidrug resistance might be an important reason of chemotherapy failure and tumor recurrence in bladder transitional cell carcinomas. The formation of multidrug resistance was possibly associated with Fas-mediated apoptotic inhibition, whereas no definite association was shown with the anti-apoptotic protein survivin. Thus, enhancing cancer sensitivity to chemotherapy, reversal of multidrug resistance by agents that reverse multidrug resistance or up-regulating Fas expression by apoptotic agents, might be a potential goal to prevent tumor recurrence and invasiveness.

## REFERENCES

- Landis SH, Murray T, Bolden S, et al. Cancer statistics. CA Cancer J Clin. 1999;49:8-31.
- McKnight JJ, Gray SB, O'Kane HF, et al. Apoptosis and chemotherapy for bladder cancer. J Urol. 2005;173:683-690.
- Friedrich K, Wieder T, Von Haefen C, et al. Overexpression of caspase-3 restores sensitivity for drug-induced apoptosis in breast cancer cell lines with acquired drug resistance. Oncogene. 2001;20:2749-2760.
- Radetzki S, Kohne CH, von Haefen C, et al. The apoptosis promoting Bcl-2 homologues Bak and Nbk/Bik overcome drug resistance in Mdr-1-negative and Mdr-1-overexpressing breast cancer cell lines. Oncogene. 2002; 21:227-238.
- Hasegawa S, Abe T, Naito S, et al. Expression of multidrug resistance-associated protein (MRP), MDR1 and DNA topoisomerase II in human multidrug-resistant bladder cancer cell lines. Br J Cancer. 1995;71:907-913.
- Nakagawa M, Emoto A, Nasu N, et al. Clinical significance of multi-drug resistance associated protein and P-glycoprotein in patients with bladder cancer. J Urol. 1997; 157:1260-1265.
- Cao L, Duchrow M, Windhovel U, et al. Expression of MDR1 mRNA and encoding P-glycoprotein in archival formalin-fixed paraffin-embedded gall bladder cancer tissues. Eur J Cancer. 1998;34:1612-1617.
- Tada Y, Wada M, Migita T, et al. Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin. Int J Cancer. 2002;98:630-635.

- 9 Yuan JL, Wang H, Wang PF, et al. Expression of P-glycoprotein in transitional cell carcinoma of urinary bladder and its significance in tumor recurrence. *Journal of Third Military Medical University* (Chinese). 2000;22:1176 – 1178.
- 10 Yakirevich E, Sabo E, Naroditsky I, et al. Multidrug resistance-related phenotype and apoptosis-related protein expression in ovarian serous carcinomas. *Gynecol Oncol*. 2006;100:152–159.
- 11 Sagol O, Yavuzsen T, Oztop I, et al. The effect of apoptotic activity, survivin, Ki-67, and P-glycoprotein expression on prognosis in pancreatic carcinoma. *Pancreas*. 2005;30:343–348.
- 12 Yin F, Shi YQ, Zhao B, et al. Suppression of P-gp induced multiple resistance in a drug resistant gastric cancer cell line by overexpression of Fas. *World J Gastroenterol*. 2000;6:664–670.