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Association of *TP53* and *MDM2* polymorphisms with survival in bladder cancer patients treated with chemoradiotherapy

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Platinum-based chemoradiotherapy (CRT) as bladder conservation therapy has shown promising results for muscle-invasive bladder cancer. However, CRT might diminish survival as a result of the delay in cystectomy for some patients with non-responding bladder tumors. Because the p53 tumor suppression pathway, including its MDM2 counterpart, is important in chemotherapy- and radiotherapy-associated effects, functional polymorphisms in the *TP53* and *MDM2* genes could influence the response to treatment and the prognosis following CRT. We investigated associations between two such polymorphisms, and p53 overexpression, and response or survival in bladder cancer patients treated with CRT. The study group comprised 96 patients who underwent CRT for transitional cell carcinoma of the bladder. Single nucleotide polymorphisms (SNPs) in *TP53* (codon 72, arginine > proline) and *MDM2* (SNP309, T > G) were genotyped using PCR-RFLP, and nuclear expression levels of p53 were examined using immunohistochemistry. None of the genotypes or p53 overexpression was significantly associated with response to CRT. However, patients with *MDM2* T / G + G / G genotypes had improved cancer-specific survival rates after CRT ($P = 0.009$). In multivariate analysis, the *MDM2* T / G + G / G genotypes, and more than two of total variant alleles in *TP53* and *MDM2*, were independently associated with improved cancer-specific survival ($P = 0.031$ and $P = 0.015$, respectively). In addition, *MDM2* genotypes were significantly associated with cystectomy-free survival ($P = 0.030$). These results suggest that the *TP53* and *MDM2* genotypes might be useful prognostic factors following CRT in bladder cancer, helping patient selection for bladder conservation therapy. (*Cancer Sci* 2009; 100: 2376–2382)

The standard treatment for muscle-invasive urinary-bladder cancer is radical cystectomy followed by urinary diversion. However, this procedure is likely to impair the quality of life of the patient.[1] In many areas of cancer treatment, the trend in the 1990s was aimed at organ conservation using combined chemotherapy and radiation with or without conservative local surgery.[2] In invasive bladder cancer, several groups have reported the value of combined-modality therapy, including transurethral resection (TUR), radiation therapy, and platinum-based systemic chemotherapy.[1–3] With these programs, cystectomy is reserved for patients with an incomplete response or local relapse after combined-modality treatment. Five-year survival rates in the range of 50–65% have been published in these series, and approximately three-quarters of the surviving patients maintained their own bladders.[4–6] However, combined-modality therapy is not only potentially toxic but might also diminish survival as a result of the delay in cystectomy for some patients with non-responding bladder tumors.[7] It would therefore be useful to have predictors for response to the therapy and prognosis, to assist with choosing appropriate patients for bladder preservation.[7]

TP53 is a tumor suppressor gene that plays a critical role in safeguarding the integrity of the genome.[8] p53 is a nuclear protein that induces cell cycle arrest, apoptosis, inhibition of angiogenesis/metastasis, and DNA repair. The *TP53* gene is mutated or part of its regulatory circuit is functionally inactivated in almost all cancers, which highlights its importance in preventing tumor development and progression.[8] In addition, the p53 tumor suppression pathway is important in chemotherapy- or radiotherapy-associated effects. MDM2 plays a key role in regulating the p53 pathway by binding directly to the p53 protein, inhibiting its activity and mediating degradation via the ubiquitination system.[9] Overexpression of MDM2 can result in excessive inactivation of p53, diminishing its tumor suppressor function.[9] In bladder cancer, *TP53* mutations have been associated with higher tumor grade and advanced stage, as well as progression of superficial disease to muscle invasion.[10,11] p53 nuclear overexpression appears to be an independent predictor of disease progression and decreased survival after cystectomy. Moreover, bladder cancer patients with mutant *TP53* and/or p53 nuclear overexpression, and MDM2 nuclear overexpression had the worst clinical outcome.[12]

The function of p53 is altered by a common sequence polymorphism within the *TP53* gene that encodes either arginine (Arg) or proline (Pro) at codon 72 in exon 4 (rs1042522).[13,14] The Pro and Arg variants have been reported to differ in functional activity because this polymorphism is located in the proline-rich domain of p53, which is necessary for the growth suppression and apoptosis mediated by p53.[15] The p53 codon 72 Pro form was revealed to be less efficient than its Arg counterpart in induction of apoptosis, inhibition of cancer cell proliferation, and suppression of Ras-induced transformation.[14,16] These functional differences may thus influence cancer risk or treatment outcome. In addition, a common polymorphism in the *MDM2* promoter region, a T to G change at nucleotide 309 in the first intron (single nucleotide polymorphism [SNP] 309, rs2279744), has been shown to increase the affinity for binding stimulatory protein 1, resulting in higher levels of MDM2 RNA and protein and the subsequent attenuation of the p53 pathway.[17] The G allele of SNP309 has been reported to enhance early onset of, and increase risk of, tumorigenesis.[17,18] Increased MDM2 levels may lead to a higher mutation rate and less effective repair of the DNA damage induced by chemotherapeutic drugs and radiation therapy.

Previous reports have revealed significant associations between *TP53* codon 72 and *MDM2* SNP309 polymorphisms and survival in lung, ovarian, peritoneal, or oral carcinoma patients treated with platinum-based chemotherapy or radiotherapy.[19–21] Concerning bladder cancer, Horikawa *et al.*[22]

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showed that the *MDM2* and *TP53* gene polymorphisms affected rates of recurrence after TUR for non-muscle-invasive bladder cancer, and that the *TP53* polymorphism was associated with survival in patients with invasive bladder cancer after radical cystectomy. In addition, Sanchez-Carbayo *et al.*[23] reported the relationship between the *MDM2* genotype and overall survival of bladder cancer patients following TUR or cystectomy. However, until now there have been no reports on the association between *TP53* and *MDM2* gene polymorphisms and clinical response or prognosis in muscle-invasive bladder cancer patients treated with chemotherapy and/or radiotherapy.

In the current study, we hypothesized that decreased function of p53 caused by *TP53* Arg72Pro and *MDM2* SNP309 might lead to a reduced DNA repair capability, giving rise to higher sensitivity to chemotherapeutic drugs and radiation therapy. To address this hypothesis, we investigated whether these functional polymorphisms could influence the clinical response and survival in bladder cancer patients treated with platinum-based chemoradiotherapy (CRT).

Materials and Methods

Patients. This study was approved by the Institutional Ethical Review Committee at the Graduate School of Medicine, Yamaguchi University, and written informed consent was obtained from each patient before blood sampling. The study group comprised 96 patients who underwent CRT for locally muscle-invasive (T2-4N0M0) or high-risk non-muscle-invasive (T1G3) bladder cancer at the Department of Urology, Yamaguchi University Hospital, from January 1995 to September 2006. All patients were native Japanese and the patients' characteristics are presented in Table 1. The median age was 69 (range, 29–89) years and the patients comprised 74 men (77.1%) and 22 women (22.9%). Before treatment, all patients underwent transurethral tumoral and random-mucosal biopsies of the bladder and a computerized tomography (CT) scan of the chest/abdomen/pelvis for staging. Patients were staged according to the Tumor-Nodes-Metastasis (TNM) staging system of the International Union Against Cancer (1997): nine (9.4%) were stage T1G3, 48 (50.0%) were stage T2, 31 (32.3%) were stage T3, and eight (8.3%) were stage T4. All bladder tumors were histopathologically confirmed as transitional cell carcinoma (TCC). Of the 96 tumors, 86 showed TCC only, five showed squamous differentiation, and five showed an adenocarcinoma component. The tumors were graded according to the World Health Organization's classification: the majority (n = 81, 84.4%) were grade 3 and the remaining 15 (15.6%) were grade 2.

Table 1. Patients' characteristics

Age (years)	
Median	69
Range	29–89
Gender (*n*)	
Men	74
Women	22
Performance status (*n*)	
0	43
1	33
2	11
3	0
Unknown	9
Tumor stage (*n*)	
≤T1	9
T2	48
T3	31
T4	8
Tumor grade (*n*)	
2	15
3	81
Histopathology (*n*)	
Pure TCC	86
TCC with SCC	5
TCC with adenocarcinoma	5
Total cisplatin dose (mg)	
Median	240
Range	30–400
Total radiation dose (Gy)	
Median	48.6
Range	30–60.4

TCC, transitional cell carcinoma; SCC, squamous cell carcinoma.

CRT. All patients received combined platinum-based systemic chemotherapy and radiotherapy. The regimen was based on Shipley's method[24] with slight modification. One cycle comprised cisplatin (70 mg/m^2) on day 1, with radiation at 1.8 gray (Gy) per fraction from day 2 to day 5 in the first week and on 5 consecutive days in the second week. Three cycles of CRT were performed where possible; the treatment was halted in patients who received one or two cycles of therapy and showed persistent side effects such as nausea, vomiting, diarrhea, or pancytopenia for 2 weeks, or who refused to continue with CRT.[25,26] Radiotherapy involved 10 MV photons with a four-field technique treating the bladder and pelvic nodes to 32.4 Gy during two cycles, followed by CT-planned whole bladder boost of 16.2 Gy for an additional cycle. Of the 96 patients, 63 (65.6%) completed three cycles of CRT whereas 32 (33.3%) and one (1.0%) received two and one cycles, respectively. The median total doses of cisplatin and radiation were 240 (range, 30–400) mg and 48.6 (range, 30–60.4) Gy, respectively. Four weeks after the completion of CRT, patients were assessed for response by random-mucosal biopsy or TUR and a CT scan. Patients with non-responding tumors were referred for radical or partial cystectomy: salvage cystectomy was undertaken in 16 patients (16.7%). In addition, four patients underwent cystectomy due to the recurrence of a muscle-invasive bladder tumor during the follow-up period, and a total of 20 patients (20.8%) underwent cystectomy during this study. Patients with responding tumors underwent complete resection of the cancer by TUR. A complete response (CR) was defined as no residual tumor detected pathologically, a partial response (PR) as a residual non-muscle-invasive tumor, and no change (NC) as a residual muscle-invasive tumor. CR was observed in 41.7% (40 of 96), PR in 35.4% (34 of 96), and NC in 22.9% (22 of 96). Nine patients with residual carcinoma *in situ* received intravesical instillation of Bacillus Calmette-Guérin. Cystoscopic examination followed by washing cytology was carried out every 3 months for the first 5 years and every 6 months thereafter. Complementary examinations, including chest X-rays and/or CT scans, were carried out every 6 months. The median duration of follow-up was 54 (range, 2–158) months. Nineteen patients suffered bladder cancer-related death during the follow-up. Among these 19 patients, the responses to the initial CRT, 4 weeks after evaluation, were CR in three, PR in eight, and NC in eight patients, and the response rate (CR/PR) in these 19 patients was low (57.9%), compared with those in all the other patients (81.8%).

DNA extraction and genotyping. Venous blood samples were collected from each patient before tumor biopsies were performed. Lymphocyte DNA was extracted using a QIAamp DNA Mini Kit (VWR International, West Chester, PA, USA). SNPs in the *TP53* (codon 72, Arg > Pro) and *MDM2* (SNP309, T > G) genes were genotyped using PCR-RFLP. The methods, including primer sequences and a restriction endonuclease for each polymorphism, have been described previously.[27,28] Briefly, the DNA fragments were amplified from 10 ng of DNA in 10-μL PCR reactions containing 1.5 mM $MgCl_2$, 0.2 mM

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each dNTP, 0.3 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 0.3 μM of each primer. The PCR products were digested with the appropriate restriction endonuclease for each polymorphism. The digested PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide for visualization under ultraviolet light. In order to control the restriction digestion of the PCR products, genotyping assays were randomly repeated and results were checked for concordance. Variant alleles were defined as Pro for *TP53* and G for *MDM2*, in accordance with previous reports.[27,28]

Immunohistochemistry (IHC). Biopsy specimens were selected from the main tumors, and IHC was performed in 87 cases on routinely processed, formalin-fixed paraffin-embedded tissue using the avidin–biotin complex immunoperoxidase technique, as described previously.[11] Briefly, a mouse monoclonal antibody for p53 (DO-7; Dako, Carpinteria, CA, USA) was applied and immunostaining was performed using a Vectastain Universal Quick Kit (Vector Laboratories, Burlingame, CA, USA). A p53-positive prostate cancer that was known to show a positive nuclear reaction for p53 was used as a positive control. A duplicate section where the primary antibody was omitted from the immunohistochemical procedure was used as a negative control. The entire section was screened in order to locate the area with the highest staining intensity. Specimens were considered to be positive for p53 overexpression when more than 20% of nuclei were positively stained.[10,11]

Statistical analysis. Associations between the *TP53* and *MDM2* genotypes and p53 overexpression, and tumor stage, grade, and response to CRT were assessed using the Chi-square test or two-sided Fisher's exact test; the odds ratio or risk ratio (RR) with 95% confidence interval (CI) was calculated. The outcome selected for this study was cancer-specific survival, defined as the time from the start of CRT to the date of death from bladder cancer. Cancer-specific survival was analyzed by plotting Kaplan–Meier curves and the survival probability distributions were compared using the log-rank test. Cystectomy-free survival, defined as the time from the start of CRT to the date of cystectomy or death from bladder cancer, was also analyzed by plotting Kaplan–Meier curves. Categorical variables influencing survival were compared using Cox proportional hazards regression models. Data were processed using JMP software (SAS Institute, Cary, NC, USA), with $P < 0.05$ indicating statistical significance. Variables with $P < 0.05$ in univariate analysis were also assessed for their relationship with cancer-specific survival in multivariate analysis.

Results

The relationship between *TP53* and *MDM2* genotypes or p53 overexpression, and clinical features. Genotype frequencies of *TP53* codon 72 and *MDM2* SNP309 were found to be in Hardy–Weinberg equilibrium. The variant allele frequencies for these polymorphisms were 42.6% and 52.1%, respectively. The mean percentage of positive nuclear staining for p53 by IHC was 33.0%, and the frequency of positive p53 overexpression was 50.6%. There were no significant relationships between the genotypes of *TP53* or *MDM2* and p53 overexpression in bladder cancer patients treated with CRT. No significant associations were detected between the genotypes or p53 overexpression and age or gender. None of the evaluated genotypes was significantly associated with tumor stage or grade (Chi-square test or two-sided Fisher's exact test; Table 2). Positive p53 overexpression tended to be associated with high-stage and high-grade

Table 3. Associations between genotypes of *TP53* and *MDM2* and p53 overexpression, and response to chemoradiotherapy

	Response (*n*)		Risk ratio (95% CI)	*P*- value†
	CR/PR	NC		
TP53 codon 72				
Arg/Arg	22	6	Reference	
Arg/Pro	40	12	1.06 (0.51–2.22)	0.87
Pro/Pro	10	4	1.15 (0.66–2.00)	0.70
Arg/Pro + Pro/Pro	50	16	1.12 (0.52–2.41)	0.77
MDM2 SNP309				
T/T	12	6	Reference	
T/G	45	10	0.56 (0.25–1.26)	0.20
G/G	16	6	0.86 (0.42–1.74)	0.74
T/G + G/G	61	16	0.60 (0.26–1.42)	0.26
p53 overexpression				
Negative	37	6	Reference	
Positive	31	13	1.72 (0.86–3.46)	0.078

†Chi-square test or two-sided Fisher's exact test. CR, complete response; PR, partial response; NC, no change; CI, confidence interval.

Table 2. Associations between genotypes of *TP53* and *MDM2* and p53 overexpression, and tumor stage and grade

	Tumor stage (*n*)		Odds ratio (95% CI)	*P*-values†	Tumor grade (*n*)		Odds ratio (95% CI)	*P*-values†
	T1/T2	T3/T4			G2	G3		
TP53 codon 72								
Arg/Arg	18	10	Reference		3	25	Reference	
Arg/Pro	31	22	1.28 (0.50–3.29)	0.61	11	42	0.46 (0.12–1.80)	0.36
Pro/Pro	9	5	1.00 (0.26–3.82)	1.00	1	13	1.56 (0.15–16.53)	1.00
Arg/Pro + Pro/Pro	40	27	1.22 (0.49–3.03)	0.68	12	55	0.55 (0.14–2.12)	0.54
MDM2 SNP309								
T/T	10	8	Reference		3	15	Reference	
T/G	33	23	0.87 (0.30–2.54)	0.80	9	47	1.04 (0.25–4.36)	1.00
G/G	14	8	0.71 (0.20–2.55)	0.75	3	19	1.27 (0.22–7.20)	1.00
T/G + G/G	47	31	0.82 (0.29–2.32)	0.71	12	66	1.10 (0.28–4.39)	1.00
p53 overexpression								
Negative	30	13	Reference		9	34	Reference	
Positive	23	21	2.11 (0.87–5.08)	0.095	3	41	3.62 (0.91–14.43)	0.069

†Chi-square test or two-sided Fisher's exact test. CI, confidence interval.

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Table 4. Univariate and multivariate regression analyses for predicting cancer-specific survival in bladder cancer patients treated with chemoradiotherapy

Variable (*n*)	Univariate analysis		Multivariate model one†		Multivariate model two‡	
	Risk ratio (95% CI)	*P*-values	Risk ratio (95% CI)	*P*-values	Risk ratio (95% CI)	*P*-values
Age (years)						
<69 (46)	Reference					
≥69 (50)	1.49 (0.93–2.43)	0.10				
Gender						
Men (74)	Reference					
Women (22)	0.96 (0.51–1.61)	0.90				
Tumor stage						
T1/T2 (57)	Reference					
T3/T4 (39)	1.39 (0.88–2.24)	0.16				
Tumor grade						
2 (15)	Reference					
3 (81)	0.79 (0.47–1.48)	0.42				
Histopathology						
Pure TCC (86)	Reference					
Other element (10)	2.21 (1.05–3.99)	0.040	2.05 (0.96–3.77)	0.061	2.56 (1.18–4.91)	0.021
Cisplatin dose (mg)						
<240 (41)	Reference					
≥240 (51)	0.97 (0.61–1.57)	0.91				
Radiation dose (Gy)						
<48.6 (35)	Reference					
≥48.6 (61)	1.49 (0.93–2.54)	0.10				
TP53 codon 72						
Arg/Arg (28)	Reference					
Arg/Pro (52)	0.96 (0.60–1.58)	0.86				
Pro/Pro (14)	0.54 (0.13–1.29)	0.19				
Arg/Pro + Pro/Pro (66)	0.86 (0.55–1.42)	0.55				
MDM2 SNP309						
T/T (18)	Reference					
T/G (55)	0.62 (0.39–1.02)	0.068				
G/G (22)	0.41 (0.16–0.84)	0.014				
T/G + G/G (77)	0.55 (0.35–0.91)	0.021	0.57 (0.36–0.95)	0.031		
Total variant alleles in *TP53* and *MDM2* genes						
≤ 2 (76)	Reference					
>2 (20)	0.42 (0.10–0.93)	0.029			0.38 (0.09–0.86)	0.015
p53 overexpression						
Negative (43)	Reference					
Positive (44)	0.89 (0.55–1.43)	0.63				

†Total number of variant alleles was excluded as a variable in model one. ‡*MDM2* genotype was excluded as a variable in model two.
CI, confidence interval; TCC, transitional cell carcinoma.

tumors in this study, which comprised locally advanced bladder cancer cases ($P = 0.095$ and $P = 0.069$, respectively). The relationships between the genotypes of *TP53* and *MDM2* or p53 overexpression, and the response to CRT are presented in Table 3. No genotypes were significantly associated with response to CRT 4 weeks after evaluation (Chi-square test or two-sided Fisher's exact test). Positive p53 overexpression tended to be associated with a poor response to CRT ($P = 0.078$).

The relationship between *TP53* and *MDM2* genotypes or p53 overexpression, and cancer-specific or cystectomy-free survival. In univariate analysis using the Cox proportional hazards regression model, *MDM2* genotypes and the total number of variant alleles in the two *TP53* and *MDM2* polymorphisms were significantly associated with cancer-specific survival (RR: 0.55, 95% CI: 0.35–0.91, $P = 0.021$ for *MDM2* genotypes; RR: 0.42, 95% CI: 0.10–0.93, $P = 0.029$ for number of variant alleles; Table 4). Thus, patients with *MDM2* T/G + G/G genotypes, or with more than two variant alleles in *TP53* and *MDM2*, had improved cancer-specific survival rates. Cancer-specific survival rates were plotted for the above-mentioned genotypes using Kaplan–Meier survival curves ($P = 0.009$ for *MDM2* genotypes; $P = 0.058$ for the number of variant alleles; log-rank test; Fig. 1). p53 overexpression was not significantly associated with cancer-specific survival (RR: 0.89, 95% CI: 0.55–1.43, $P = 0.63$). Clinical variables were also assessed for their relationship with cancer-specific survival (Table 4). In univariate analysis, tumors with squamous cell carcinoma or adenocarcinoma elements were significantly associated with an unfavorable outcome (RR: 2.21, 95% CI: 1.05–3.99, $P = 0.040$). In multivariate analysis (model one) including other histopathological elements and *MDM2* genotypes, only *MDM2* T/G + G/G genotypes were independently associated with a favorable cancer-specific survival (RR: 0.57, 95% CI: 0.36–0.95, $P = 0.031$). When the number of variant alleles in *TP53* and *MDM2* was used instead of *MDM2* genotypes in multivariate analysis (model two), the histopathology and total variant alleles were independently associated with cancer-specific survival (RR: 2.56, 95% CI: 1.18–4.91, $P = 0.021$ for other elements; RR: 0.38, 95% CI: 0.09–0.86, $P = 0.015$ for more than two variant alleles). Patients with *MDM2* T/G + G/G genotypes had significantly favorable cystectomy-free

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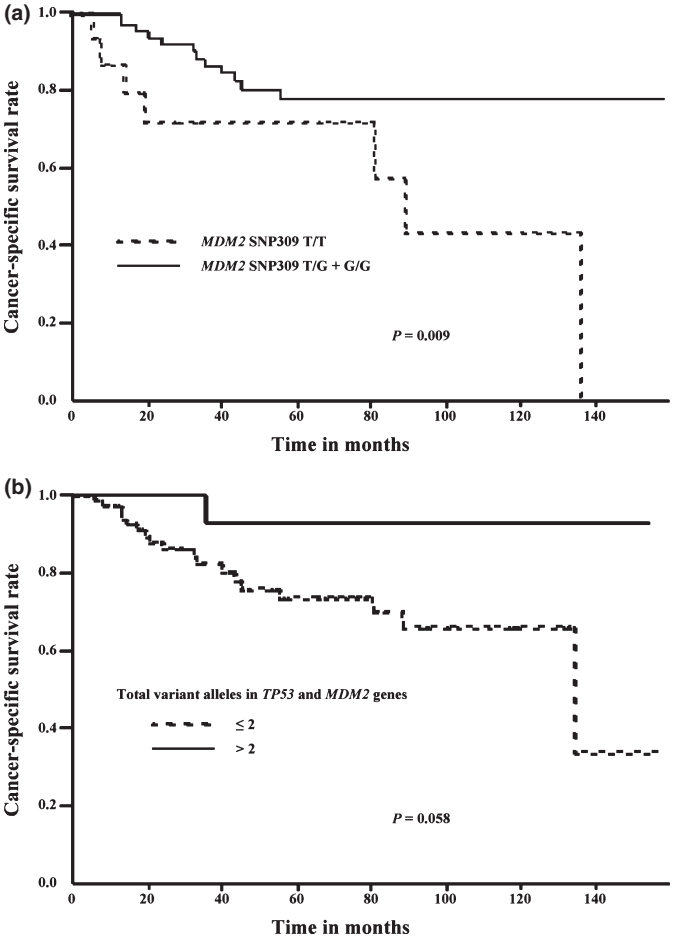

Fig. 1. Kaplan–Meier cancer-specific survival curves for bladder cancer patients treated with chemoradiotherapy, stratified by the *MDM2* SNP309 genotypes (T/T *vs* T/G + G/G; $P = 0.009$, log-rank test; a) and by the total variant alleles in *TP53* and *MDM2* genes (≤2 *vs.* >2; $P = 0.058$, log-rank test; b).

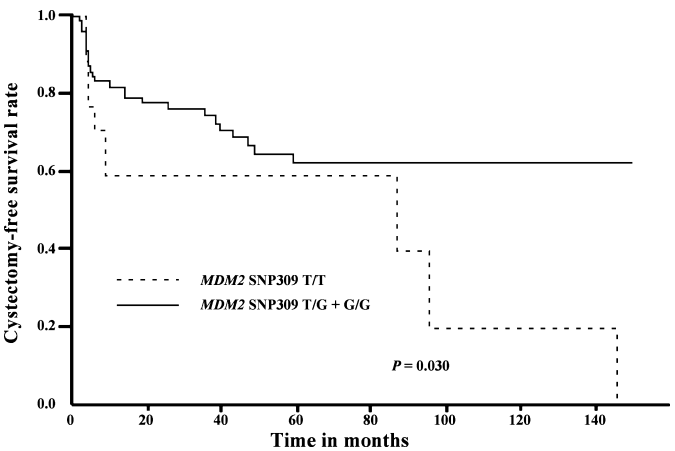

Fig. 2. Kaplan–Meier cystectomy-free survival curves for bladder cancer patients treated with chemoradiotherapy, stratified by the *MDM2* SNP309 genotypes (T/T *vs* T/G + G/G; $P = 0.030$, log-rank test).

survival rates compared with those with *MDM2* T/T ($P = 0.030$; log-rank test; Fig. 2). *TP53* genotypes, the total number of variant alleles in the two *TP53* and *MDM2* polymorphisms or p53 overexpression was not significantly associated with cystectomy-free survival.

Discussion

We found significant associations between the *TP53* codon 72 and *MDM2* SNP309 genotypes and cancer-specific survival in bladder cancer patients treated with CRT. No significant associations were found between these genotypes and tumor stage, grade, or response to CRT. Our data indicated that the *MDM2* T/G + G/G genotypes, or more than two of total variant alleles in *TP53* and *MDM2*, were independently associated with a favorable outcome. In addition, the *MDM2* T/G + G/G genotypes were shown to be significantly associated with a favorable outcome regarding bladder preservation. The *MDM2* SNP309G allele has previously been shown to be associated with higher levels of *MDM2* mRNA.[17] Ko *et al.*[29] observed that *MDM2* mRNA expression was a favorable prognostic factor in non-small-cell lung cancer, though it was not associated with clinicopathological parameters, including tumor grade, tumor stage, tumor type, and TNM values. Tanner *et al.*[30] also stated that *MDM2* mRNA expression was an independent prognostic factor for patients with primary ovarian cancer, who received chemotherapy with carboplatin or cisplatin and cyclophosphamide. Our results are similar to these findings.

Wu *et al.*[31] showed that lymphoblastoid cell lines with *TP53* wild-type alleles had statistically significantly higher DNA repair capacity than cell lines with at least one variant allele. Bond *et al.*[18] proposed that high levels of MDM2, resulting from the G allele of SNP309 and just one wild-type *TP53* allele, produce a severely weakened p53 tumor suppressor pathway resulting in less effective DNA repair processes. Moreover, Ribeiro *et al.*[32] found that a cell line expressing a *TP53* mutation was more sensitive to radiotherapy than lines with wild-type *TP53*, providing support for a model in which loss of p53 function is associated with increased radiosensitivity, possibly due to reduced p53-dependent DNA repair. Cote *et al.* also demonstrated that p53 alterations in bladder cancer resulted in increased sensitivity to chemotherapy that included DNA-damaging agents.[33] Taken together, our findings may be due to a decreased p53-dependent DNA repair capacity. In turn, this might result in a lower rate of repair of tumor cell DNA damaged by platinum agents and radiation, resulting in increased chemoradiosensitivity. We have previously reported the importance of DNA repair capacity in progression and prognosis of renal cell and urothelial carcinomas, including bladder cancer treated with CRT.[11,26,34–36] The present findings support the results of these previous studies.

Although the favorable outcome after CRT in bladder cancer patients with *MDM2* T/G + G/G genotypes is possibly due to an inactivated p53 pathway (by high levels of MDM2 resulting in a decreased p53-dependent DNA repair capacity), p53 nuclear overexpression was not associated with the survival. A possible explanation for this inconsistency is that the association between the *MDM2* genotypes and survival may also be implicated in the p53-independent effects of MDM2. It has been reported that MDM2 has a p53-independent role in the regulation of the DNA double-strand break repair response,[37,38] and DNA damaged by ionizing radiation is repaired mainly by the double-strand break repair pathway.[39] Thus, our findings may possibly be due to a decrease in both the p53-dependent and p53-independent DNA repair capacities conferred by *MDM2* variant genotypes.

In the current study, we did not examine the mutational status of the *TP53* gene because of a lack of available tumor tissue for DNA extraction. Instead, we analyzed the nuclear expression levels of the p53 protein, representing somatic alterations in p53 in bladder cancer cells. However, in bladder cancer, Cordon-Cardo *et al.*[40] showed a strong association between positive p53 immunostaining and the presence of mutations as detected

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by SSCP and DNA sequencing. Esrig *et al.*[41] reported that the immunohistochemical detection of p53 nuclear accumulation was highly associated with mutations in the *TP53* gene, and that immunohistochemical methods might be more sensitive than SSCP for detecting p53 alterations. Moreover, Smith *et al.*[10] stated that *TP53* genetic alterations most commonly manifested nuclear overexpression of the p53 protein with subsequent positive immunostaining. Concerning the associations between somatic alterations in the *TP53* gene in bladder cancer cells and the clinical course of bladder cancer patients treated with CRT, we previously reported that the *TP53* allelic imbalance was not significantly associated with the response to CRT or survival following CRT.[25]

Han *et al.*[19] reported that the *TP53* Pro/Pro and the *MDM2* T/G + G/G genotypes were predictive of shorter survival in advanced non-small-cell lung cancer patients who received chemotherapy. Tu *et al.*[21] also reported that the *MDM2* G/G genotype was associated with poor survival in irradiated patients with oral carcinoma. These results are not consistent with our present findings. This discrepancy might stem from the effects on different cancer types or synergistic effects in CRT whereby the platinum-based chemotherapy interacts with the radiotherapy. It has been proposed that radiation induces free radicals and subsequently the formation of toxic platinum intermediates, which increase cell killing.[42] Moreover, ionizing radiation can increase cellular uptake of platinum; and damage to DNA by ionizing radiation, which would typically be reparable, can become fixed and lethal through cisplatin's free-electron-scavenging capacity.

In conclusion, this is the first report of the association between *TP53* codon 72 and *MDM2* SNP309 polymorphisms and clinical response or prognosis in bladder cancer patients treated with platinum-based CRT. The *MDM2* T/G + G/G genotypes, or more than two of total variant alleles in *TP53* and *MDM2* were shown to be independently associated with a longer cancer-specific survival. In addition, the *MDM2* T/G + G/G genotypes were shown to be significantly associated with a favorable cystectomy-free survival rate. These are possibly due to a decreased p53-dependent and p53-independent DNA repair capacity conferred by these alleles. The results suggest that these genotypes might be useful as prognostic factors after CRT in bladder cancer, helping patient selection for bladder conservation therapy. However, with the limited sample size, our results allow only preliminary conclusions; functional and larger studies, including an assessment of the mutational status of the *TP53* gene, are needed to confirm the prognostic significance of the genotypes in bladder cancer patients treated with CRT.

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