The Value of p21 and p53 Overexpression in Predicting the Outcome After Systemic Chemotherapy of Urothelial Cancer

Feliksas Jankevičius*§, Peter Goebell#, Mayumi Kushima&, Wolfgang A. Schulz*, Rolf Ackermann* and Bernd J Schmitz-Dräger*$

**Department of Urology, Düsseldorf University School of Medicine, Germany*

§ **Current address:**
Department of Urology
Lithuanian Oncology Center
Santariskiu 1,
LT-2600 Vilnius, Lithuania
& **Current address:**
Department of Urology,
Saseikai General Hospital
Hiroosa-cho 1,
Shimotoba Fushimi-ku,
Kyoto 612-8473, Japan
 # Current address:
Department of Urology,
University of Essen
Hufelandstr. 55,
45122 Essen, Germany
$ **Current address:**
Urology, EuromedClinic
Europa-Allee 1,
D-90763 Fürth, Germany

Objectives: Indicators that may predict the outcome of chemotherapy in urothelial cancer are highly desirable. Since the wild type p53 tumor suppressor gene induces apoptotic cascade and downregulates cell cycle transition in response to DNA damage, the inactivation of p53 or its downstream effector, WAF1/p21 gene, might alter the sensitivity of tumor cells to chemotherapy. In this study we investigated if alterations in nuclear expression of the p53 and p21 genes may mark different chemosensitivity of urothelial tumors.

Methods: Archival formalin-fixed paraffin-embedded tumor sections from 60 patients with urothelial cancer who underwent cisplatin-based chemotherapy for locally advanced or/and metastatic urothelial cancer were stained for p53 and p21. The results of immunohistochemistry were compared with response to chemotherapy and overall survival.

Results: Of the 60 tumors stained, 35 (58%) showed P53 accumulation and 25 (42%) expressed detectable p21. No association between p53 accumulation and expression of p21 was observed. A comparison of the incidence of complete and partial remissions following inductive chemotherapy (n = 39) demonstrated that patients with intact p53 responded initially significantly better (70% *vs*. 31%, respectively, $P < 0.05$). However, no difference in terms of overall survival was observed with regards to p53 immunostaining (median: 12 months *vs*. 17 months for p53-positive and p53-negative tumors, respectively). P21 expression was neither related to the initial response nor to overall survival following inductive chemotherapy. In contrast, the outcome of adjuvant chemotherapy (n = 21) correlated with the IHC results and in particular with p21 expression. Median overall survival for patients with p21-positive or p21-negative tumors was 60 months *vs*. 21 months, respectively ($P < .005$ in Kaplan-Meier analysis).

Conclusion: Although no correlation was observed between tumor p53/p21 status and chemosensitivity in patients who underwent inductive chemotherapy, patients with p21-positive tumors had a significantly longer overall survival following adjuvant chemotherapy with respect to the patients with p21-negative tumors. p21 immunohistochemical expression has been shown as a significant prognostic indicator of response to adjuvant chemotherapy, and therefore deserves further analysis by larger prospective studies.

Key words: p21, p53, chemotherapy, transitional all carcinoma, survival

INTRODUCTION

Cellular resistance to cytotoxic drugs is a limiting factor in human cancer therapy. The combination of methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC) chemotherapy has been shown to be efficacious in primary bladder, nodal or metastatic transitional cell carcinoma (TCC) (1), although the rates of complete remissions reported vary from 15% to 36% (2). Moreover, high toxicity and severe side effects of the M-VAC regimen have been reported, with drug-related death in up to 4% of the cases (3). Therefore, indicators identifying nonresponding bladder cancer patients prior to chemotherapy which

could spare them exerting, costly and useless treatment are urgently needed.

The role of molecular markers in predicting the response of cancer to treatment is poorly defined. Induction of apoptosis in tumor cells has been reported to be an important mechanism of chemotherapy-induced cell death. An intact *p53* tumor suppressor gene is required for efficient activation of apoptosis in response to DNA damage *in vitro* (4). However, the role of p53 inactivation regarding cellular chemosensitivity remains controversial. Accumulation of the P53 protein demonstrated no correlation with response to cisplatin-based chemotherapy in non-small cell lung and colorectal cancers (5, 6) but with a beneficial effect of adjuvant chemotherapy in breast cancer (7).

While generally correlated with poor outcome in patients with bladder cancer (8, 9, 10), nuclear accumulation of *p53* has been shown to be of negative prognostic value concerning survival after neoadjuvant chemotherapy (11). However, no association of p53 accumulation with response to systemic chemotherapy in patients with non-metastatic TCC was observed (12).

The more recent discovery that cell-cycle checkpoints can regulate chemosensitivity of tumor cells (13) has prompted interest in the *WAF1/p21* gene. This gene is transcriptionally activated by p53 and mediates the p53-dependent G_1 arrest following DNA damage (14). One known function of this G_1 checkpoint is to allow DNA repair before replication (15). Since P53 alterations would be expected to result in loss of p21 induction and thereby in a failure to arrest cell-cycle (16), we hypothesized that the p21 status might predict the tumor response to cytotoxic therapy.

This study was performed to analyze the correlation between immunohistochemical expression of p53 and p21 in primary urothelial tumors and (a) clinical response to systemic chemotherapy in metastatic TCC and (b) overall survival in patients undergoing chemotherapy for TCC.

MATERIALS AND METHODS

Patients

Sixty patients who underwent systemic chemotherapy for locally advanced and/or metastatic urothelial cancer at the Urology Department of the Duesseldorf University School of Medicine were identified from archival records and included in this retrospective study. The study group included 46 males and 14 females with a mean age of 66 years (range, 48 to 86 years). With respect to the primary lesion, 50 patients had bladder cancer, 7 TCC of the upper urinary tract, and another 3 had TCC in the bladder and ureter.

The patients were followed for 1 to 56 months. All tumor specimens were restaged and classified according the TNM system (17). Grading was performed according to the criteria of the World Health Organization (18).

All patients underwent at least two cycles (range, 2–12) of cisplatin-based chemotherapy (M-VAC, MC (methotrexate, cisplatin) or CISCA (cisplatin, cyclophosphamide, doxorubicin) chemotherapeutic regimen). Thirty-nine patients underwent cytotoxic therapy as induction therapy for progressive disease (tumor progression in form of local tumor recurrence or distant metastases), and 21 patients in an adjuvant setting after exenterative surgery for locally advanced tumors (pT3b, pT4) with or without positive pelvic lymph nodes.

Immunostaining

Archival formalin-fixed paraffin-embedded primary site tumor specimens with the highest tumor grade and maximal invasion were carefully selected for all 60 representative patients for immunohistochemical (IHC) evaluation. All tumor samples were obtained prior to chemotherapy. Monoclonal antibodies (mAb) AB-6, recognizing both wild-type and mutant p53 (Dianova, Hamburg, Germany) and WAF1/Ab-1 binding to p21 (Ab-1, clone EA10, Oncogene Science, Cambridge, MA) were used for immunostaining. The sections were dewaxed in xylene, rehydrated in a series of graded alcohols and treated with 1% hydrogen peroxidase in methanol to block endogenous peroxidase. Next, the slides were microwaved three times for 10 min at 650W in 0.01M citrate-buffered solution (pH 6.0). After washing in PBS, the sections were incubated for 1 h with 20% normal horse serum. The specific primary mAb AB6 at a dilution of 1:100 or WAF1/Ab-1 at a dilution of 1:50 were added and the sections were incubated at room temperature overnight. For immunoperoxidase staining the biotin/avidin peroxidase detection system was used (Vectastain® ABC kit, Burlingame, CA). Visualization of antibody binding was performed using Diaminobenzidine (Sigma Chemical, St. Louis). Slides were counterstained with Mayer's hemalum (Merck, Darmstadt, Germany), dehydrated, cleared, and mounted. In addition to routine negative and positive controls negative internal controls omitting the primary antibodies were performed for each slide.

Nuclear overexpression of p53 and p21 was noted only in tumor cells and was evaluated by determining the percentage of nuclear staining within several representative areas of each tumor. Since the fraction

of ≥20% positively stained nuclei has been shown to have the best correlation with p53 gene mutations (19), this cut-off value was used to discriminate between p53-positive and p53-negative tumors. Because of considerable intratumoral heterogeneity in staining for P21, a lesion was considered positive, if nuclear staining of more than 5% of tumor cells was observed in at least one region of the tumor (20). The two independent investigators evaluating the results of IHC were blinded to the treatment outcome.

Statistical Evaluation

The study endpoints were clinical and/or pathological responses to chemotherapy, and overall survival. The clinical responses were classified according to the WHO classification (21). Patient survival was recorded from the date of initiation of chemotherapy to the time of death or censoring. The log-rank test was used to determine the correlation between imunohistochemical expression of p53 and p21 response to chemotherapy or patient survival. The Kaplan-Meier method was used to calculate the survival function.

RESULTS

Of the 60 tumors studied, 36 (60%) showed p53 accumulation and 25 (42%) expressed detectable p21. Except for a high incidence of p53 positivity (50%) for the six T1 tumors p53 immunoreactivity increased with tumor stage. No correlation between tumor stage and p21 staining was observed (Table 1). One third of the patients had p53-positive tumors negative for p21 (p53 + / p21-) and 25% of the patients had p53- positive tumors still expressing p21. Among the p53-negative tumors the corresponding fractions were 25% and 17%, respectively.

Overall, a 48% tumor response rate (8 (20%) CR and 11 (28%) PR) was observed after inductive chemotherapy (Table 2). These responses, however, did not translate in overall survival in these patients, which in no case exceeded 14 months. In contrast, patients treated with adjuvant chemotherapy showed a significantly better overall survival compared to those not receiving chemotherapy (median survival 48 months; P = 0.0003).

Nuclear accumulation of p53 and p21 was assessed for correlation with response after inductive chemotherapy (Table 2). Among 22 patients with p53-positive tumors 7 (32%) achieved tumor remission (CR or PR) compared to 12 out of 17 patients (70%) with p53-negative tumors. This difference was statistically significant ($p(\chi^2) < 0.05$). In contrast, no difference in response to chemotherapy was observed between p21-positive and p21-negative tumors (50% *vs.* 48%, respectively). Further subdividing the tumors according to their p53/p21 phenotype demonstrated that among p53-positive cancers, tumors expressing p21 (p21+) demonstrated a better response rate than tumors with no p21 expression (40% *vs*. 25%), although the difference did not achieve

Table 1. **Immunohistochemical detection of p53 and p21 with regard to tumor stage**

Tumor Phenotype	Tumor Stage				
	pT1 (n = 6)	pT2 (n = 14)	pT3 (n = 33)	pT4 (n = 7)	Total (n = 60)
p53-positive*	3 (50%)	5 (36%)	21 (64%)	7 (100%)	36 (60%)
p21-positive**	3 (50%)	6 (43%)	12 (36%)	4 (57%)	25 (42%)

*>20% of tumor cells with positive nuclear staining;**>5% of tumor cells with positive nuclear staining.

Table 2. **Response of the bladder tumors to inductive chemotherapy in relation to p53/p21 expression (n = 39)**

p53/p21 status	Tumor Response*			
	CR (%)	PR (%)	NS (%)	PD (%)
p53+ tumors (n = 22)	2 (9)	5 (23)	9 (41)	6 (27)
p53- tumors (n = 17)	6 (35)	6 (35)	1 (6)	4 (24)
p21+ tumors (n =16)	4 (25)	4 (25)	2 (13)	6 (37)
p21- tumors (n = 23)	4 (17)	7 (30)	8 (35)	4 (17)
p53+/p21+ tumors (n = 10)	1 (10)	3 (30)	2 (20)	4 (40)
p53-/p21+ tumors (n = 6)	3 (50)	1 (17)	0	2 (33)
p53+/p21- tumors (n = 11)	3 (27)	5 (46)	1 (9)	2 (18)
p53+/p21- tumors (n = 12)	1 (8)	2 (17)	7 (58)	2 (17)
Total (%)	8 (20)	11 (28)	10 (26)	10 (26)

* Response: CR, complete response; PR, partial response; NS, no change; PD, progressive disease.

statistical significance because of the small number of patients in each subgroup.

Despite a correlation between the immunohistochemical results and initial response, no difference in overall survival following inductive chemotherapy was found between the patients with p53-positive and p53-negative primary tumors. Median overall survival for patients with altered p53 expression was 12 months compared with 17 months for patients whose primary tumors did not show immunoreactivity. Similarly, the outcomes of inductive chemotherapy did not relate to p21 immunostaining (11 months vs. 14 months for p21-positive and p21-negative tumors, respectively).

Different results were obtained when the results of immunohistochemistry were correlated with the outcomes of adjuvant chemotherapy. The accumulation of p53 in the primary tumor was related to a shorter overall survival. The median duration of survival in 8 patients with p53- positive tumors after adjuvant therapy was 23 months compared with 60 months for 13 patients with p53-negative tumors. This difference, however, was not statistically significant in the Kaplan-Meier analysis (Fig. 1A). A significant difference in overall survival was observed when correlating the outcome of adjuvant chemotherapy with p21 positivity. Longer median overall survival was related to p21 positive staining (60 months *vs*. 21 months for p21-positive and p21-negative tumors, respectively). This difference was significant ($P < .005$) in the Kaplan Meier analysis (Fig. 1B).

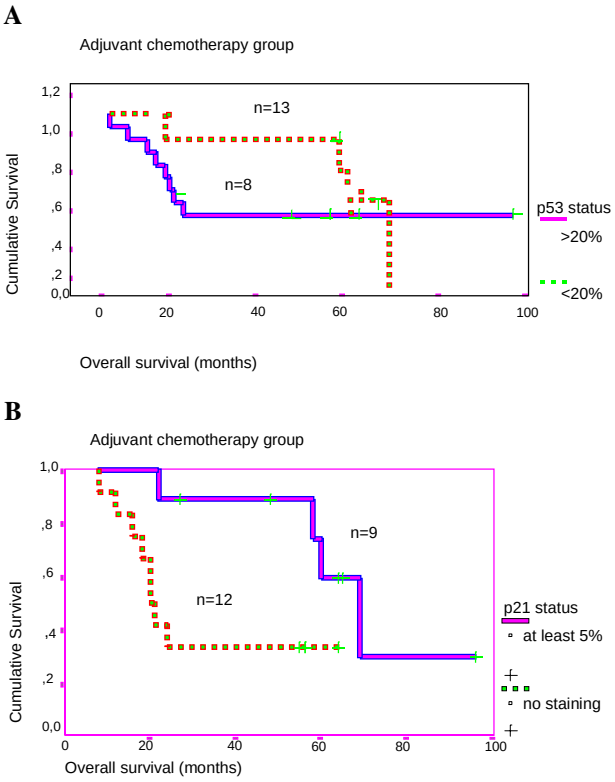

Fig. Kaplan-Meier survival curves for p53-positive and p53-negative urothelial cancers (A) and p21-positive and p21-negative urothelial cancers (B)

DISCUSSION

Recent evidence suggests that many forms of chemotherapy may exert their cytotoxic effects by inducing apoptosis (22). The tumor suppressor gene p53 appears to be crucial in control of DNA damage. Loss of p53 function in cells that initiate apoptosis has been reported as a possible cause of resistance to cytotoxic treatment (23). A mechanism suggesting that anticancer agents induce p53-dependent apoptosis might help explain why tumors derived from p53-mutant cells can be resistant to anticancer drugs; however, it provides no insight as to why such drugs often can be used to successfully treat these tumors (24).

p21 is a downstream mediator of p53 function and is a crucial protein in cell-cycle control. The most important function of p21 is probably the inhibition of a G1 cyclin-dependent kinase which phosphorylate pRb and related family members, leading to a $G–G_1$ arrest of the cell cycle. By increasing p21 levels, p53 can inhibit cell proliferation in response to DNA damage, arrest cells in G1 phase and also directly inhibit DNA replication (25). *In vitro* studies demonstrated that p21 deficient cells displayed a markedly different response to DNA-damaging agents compared to cells with intact p21 (26).

A strong association between p53 protein abnormalities and down-regulation of p21 has been reported in human tumors (27). However, it has been observed that p21 can also be induced by a p53-independent pathway (28,29). No significant correlation betweeen p53 accumulation and expression of p21 has been observed in transitional cell carcinoma (20, 30). This suggests that induction of p21 by factors other than p53 may be important in urothelial cell growth regulation. Therefore, maintainance of p21 expression in tumors with abnormal p53 might explain their different chemosensitivity, and examination of both p53 and p21 protein expression may be important to predict the sensitivity of human cancer to cytostatic drugs.

In this study the expression of p53 and p21 in a series of advanced urothelial cancers was examined by means of immunohistochemistry and compared with the patients' response to cisplatin-based chemotherapy and overall survival. The 58% (35/60) incidence of p53-positive immunostaining observed in our study exceeds the values previously reported in urothelial cancer (8–10). Of note, however, in

the current study immunostaining was performed in the primary site tumor specimens from patients who after definitive surgery further developed cancer progression in form of distant metastases (induction therapy group) or had extravesical tumor extension or nodal disease at the time of surgery (adjuvant therapy group). Therefore our study concerning aggressive urothelial carcinomas and the high incidence of p53 accumulation probably reflects the biological nature of these tumors. Among the tumors, 42% (25/60) were positive for p21. No significant association between p53 abnormalities and overexpression of p21 was observed ($p < 0.3$). P21 expression was detected in 43% (15/35) of p53-positive tumors and in 40% of tumors lacking p53 accummulation. The association of p21 overexpression and p53 accumulation did not acquire significance even if only strong staining for p21 (defined as > > 20 % of nuclei positive) was considered (data not shown). Similar results were obtained in the study of Stein et al, who observed p21 nuclear reactivity in 42% of 101 p53-positive bladder tumors (31). Interestingly, down-regulation of p21 was also recorded in 15/20 (60%) of tumors lacking p53 staining. The way in which p21 might be suppressed in these tumors is unclear. In some cases of urothelial carcinoma point mutations can be responsible for this phenomenon (32).

A comparison of the incidence of CR and PR in patients with or without p53 accumulation (32% *vs*. 70%, respectively) demonstrated that tumors with intact p53 responded initially significantly better to induction chemotherapy, whereas p21 expression showed no apparent relation with the response to inductive anticancer therapy. This observation is in line with the concept that p53-dependent apoptosis modulates the cytoxicity of anticancer agents and that the absence of wild-type p53 function results in cellular resistance to chemotherapy (24). However, the correlation between p53 overexpression and tumor response to inductive chemotherapy did not extend to the overall survival of these patients. The median overall survival of those patients who underwent inductive chemotherapy for metastatic disease and did not express P53 protein only slightly exceeded the survival of patients with p53-positive tumors. The discrepancy in the predicitve value of p53 expression observed for chemotherapeutic response and overall survival suggests that in patients with extensive metastatic disease a combination of clinical and biological parameters determines the outcome. Although the relatively small number of patients and the heterogeneity of the primary sites of urothelial cancer in the induction therapy group does not allow firm conclusions; the observation that patients with urothelial tumors without p53 accumulation survived only marginally better than patients with tumors positive for p53 suggests that patients with progressive disease and altered p53 immunostaining should not be excluded from cytotoxic chemotherapy treatment.

A comparison of the outcome of patients after adjuvant chemotherapy with the immunohistochemical results indicated a significant predictive value of molecular markers. Patients who underwent adjuvant chemotherapy and expressed p21 protein survived significantly longer than those with p21-negative tumors (Fig. 1B). Furthermore, a trend for an improved survival not reaching significance, presumably due to the small numbers, was found for patients without p53 accumulation in the primary tumor (Fig. 1A). This suggests that a combination of both markers might be even more predictive. Although in this study the number of patients in each group was too small to address this point conclusively, it appears that patients with p53-/p21+ tumors might represent the subgroup with the best survival (data not shown). The worst overall survival was observed for p53+/p21- cases (data not shown) fitting the idea that this tumor phenotype with mutated p53 and impaired G1 checkpoint is not able to activate the apoptotic cascade in response to DNA damage and must be considered to be at high risk for treatment failure. Expression of p21 may partially compensate the deleterious effect of p53 alterations. The induction of p21 in p53+/p21+ tumors may occur through p53-independent mechanisms (33) or these tumors could carry overexpression of non-mutated p53 (33).

Interestingly, the correlation between survival probability and immunohistochemical tumor phenotype observed in the adjuvant chemotherapy group is in concordance with data obtained in a recent Japanese study from Koga et al, who also observed an improved chemotherapeutic response in the group of p53 -/p21+ bladder cancer patients treated with cisplatin and pirarubicin combination (34).

The difference in the therapeutic significance of molecular markers between the inductive and adjuvant chemotherapy groups observed in our study may be related to tumor volume, since the extension of disease has been reported previously to be a major negative prognostic determinant for survival in patients with advanced bladder cancer treated with M-VAC chemotherapy (35). In patients with a lower tumor volume the biological markers may be more important to predict the benefit of chemotherapy.

In summary, although the conclusions drawn from this retrospective study were based on a relatively small number of patients, the data suggest that the value of p53/p21 immunohistochemical detection to predict im-

proved survival after adjuvant chemotherapy of patients with urothelial cancer is worthwhile to assess in further larger prospective studies.

Received 9 January 2002
Accepted 7 March 2002

References

1. Geller NL, Sternberg CN, Pennenberg D, Scher H, Yagoda A. Prognostic factors for survival of patients with advanced urothelial tumors treated with methotrexate, vinblastine, doxorubicine and cisplatin chemotherapy. Cancer 1991; 67: 1525.
2. Loehrer PJ, Einhorn LH, Elson PJ, Crawford ED, Kuebler P, Tannock I, Raghavan D, Stuart-Harris R, Sarosdy MF, Lowe BA. A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. J Clin Oncol 1992; 10(7): 1066–73.
3. Igawa M, Kadena K, Ueda M, Usui T. Association between patients characteristics and treatment history, and toxicity associated with methotrexate, vinblastine, doxorubicin, and cisplatin (M-VAC) for advanced urothelial cancer. British J Urol 1994; 73: 263–7.
4. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1994; 74: 957–67.
5. Junker K, Wiethege T, Muller KM, Thomas M. *p53* tumour-suppressor gene in non-small cell lung cancer with neoadjuvant therapy. J Cancer Res Clin Oncol 2000; 126: 238–45.
6. Cascinu S, Catalano V, Aschele C, Barni S, Debernardis D, Gallo L, Bandelloni R, Staciolli MP, Baldell AM, Brenna A, Valenti A, Murretto P, Catalano G. Immunohistochemical determination of p53 protein does not predict clinical responce in advanced colorectal cancer witj low thymidylate synthase expression receiving a bolus 5-fluorouracil-leucoverin combination. Ann Oncol 2000; 11: 1053–6.
7. Stal O, Stenmark-Askmalm M, Wingren S, Rutqvist LE, Skoog L, Ferraud L, Sullivan S, Carstensen J, Nordenskjold B. p53 expression and the results of adjuvant therapy of breast cancer. Acta Oncol 1995; 34: 767–70.
8. Lipponen PK. Over-expression of p53 nuclear oncoprotein in transitional-cell bladder cancer and its prognostic value. Int J Cancer 1993; 53: 365–70.
9. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, Nichols PW, Skinner DG, Jones PA, Cote RJ. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994; 331: 1259–64.
10. Kuczyk MA, Bokemeyer C, Serth J, Hervatin C, Oelke M, Höfner K, Tan HK, Jonas U. p53 overexpression as a prognostic factor for advanced stage bladder cancer. Eur J Cancer 1995; 31A: 2243–47.
11. Sarkis AS, Bajorin DF, Reuter VE, Herr HW, Netto G, Zhang Z-F, Schultz PK, Cordon-Cardo C, Scher HI. Prognostic value of p53 nuclear overexpression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J Clin Oncol 1995; 13: 1384–90.
12. Qureshi KN, Griffiths TR, Robinson MC, March C, Roberts JT, Hall RR, Lunec J, Neal DE. TP53 accumulation predicts improved survival in patients resistant to systemic cisplatin-based chemotherapy for muscle-invasive bladder cancer. Clin Cancer Res 1999; 5(11): 3500–7.
13. Hartwell LH, Kastan MB. Cell cycle control and cancer. Science 1994; 266: 1821–8.
14. Waldman T, Kinzler K, Vogelstein B. p21 is necessary for the p53-mediated G1 arrest in human cancer cells. Cancer Res 1995; 55: 5187–90.
15. Dulic V, Kaufman WK, Wilson SJ, Tisty TD, Lees E, Harper JW, Elledge SJ, Reed SI. p53-dependent inhibition of cycline-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. Cell 1994; 76: 1014–24.
16. Sarkar S, Julicher KP, Burger MS, Della Valle V, Larsen CJ, Yeager TR, Grossman TB, Nickels RW, Protzel C, Jarrard DF, Reznikoff CA. Different combinations of genetic/epigenetic alterations inactivate the p53 and pRb pathways in invasive human bladder cancers. Cancer Res 2000; 15; 60(14): 3862–71.
17. Sin LH, Wittekind Ch (Eds.). TNM Classification of Malignant Tumours. Fifth Edition. Wiley-Liss, 1997.
18. Mostofi FK, Sobin LH, Torlini H. Histological typing of urinary bladder tumors. In *International Histological Classification of Tumors*. No 10. Geneva: World Health Organization, 1973.
19. Cordon-Cardo C, Dalbagni G, Saez GT, Oliver MR, Zhang ZF, Rosai J, Reuter VE, Pellicer A. p53 mutations in human bladder cancer: Genotypic versus phenotypic patterns. Int J Cancer 1994, 56: 347–53.
20. Clasen S, Schulz WA, Gerharz CD, Grimm MO, Christoph F, Schmitz-Dräger BJ. Frequent and heterogeneous expression of cyclin-dependent kinase inhibitor WAF1/p21 protein and mRNA in urothelial carcinoma. Br J Cancer 1998; 77: 515–21.
21. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207–14.
22. Kerr JFR, Winterford CM, Harmon BV. Apoptosis: its significance in cancer and cancer therapy. Cancer 1994; 73: 2013–26.
23. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. New Engl J Med 1993; 329: 1318–27.
24. Waldman T, Zhang Y, Dillehay L, Kinzler K, Vogelstein B, Williams J. Cell-cycle arrest versus cell death in cancer therapy. Nature Med 1997; 9: 1034–6.
25. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a ppotential mediator of p53 tumor suppression. Cell 1993; 75: 817–25.
26. Waldman T, Lengauer C, Kinzler KW, Vogelstein B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature 1996; 381: 713–6.
27. Bukholm I, Nesland Jm, Käresen R, Jacobsen U, Borresen AL. Relationship between abnormal p53 protein and failure to express p21. J Pathol 1997; 181: 140–5.
28. Zhang W, Grasso L, McClain CD, Gambel AM, Cha Y, Travali S, Deisseroth AB, Mercer E. p53-indepen-

dent induction of WAF1/CIP1 in human leucemia cells is correlated with growth arrest accompanying monocyte/macrophage differentiation. Cancer Res 1995; 55: 668–74.
29. Jakus J, Yeudall AW. Growth-inhibitory concentrations of EGF induce p21 (WAF1/CIP1) and alter cell-cyle control in squamous-carcinoma cells. Oncogene 1996; 12: 2369–76.
30. Jahnson S, Karlsson MG. Tumor mapping of regional immunostaining for p21, p53, and mdm2 in locally advanced bladder carcinoma. Cancer 2000; 89(3): 619–29.
31. Stein JP, Ginsberg DA, Grossfeld GD, Esrig D, Freeman J, Dickinson MG, Groshen S, Nichols PA, Jones PA, Skinner DG, Cote RJ. The effect of P21 expression on tumor progression in p53 altered bladder cancer. J Urol 1996; 155(Suppl): 628A.
32. Malkowicz SB, Tomaszewski JE, Linnenbach AJ, Cangiano TA, Maruta Y, McGarvey TW. Novel $p21^{WAF1/CIP1}$ mutations in superficial and invasive transitional cell carcinomas. Oncogene 1996; 13: 1831–183.
33. Peng HQ, Hogg D, Malkin D. Mutations of p53 gene do not occur in testis cancer. Cancer Res 1993; 53: 3574–9 .
34. Koga F, Kitachara S, Arai K, Honda M, Sumi S, Yoshida K. Negative p53/positive p21 immunostaining is a predictor of a favorable response to chemotherapy in patients with locally advanced bladder cancer. Jpn Cancer Res 2000; 91(4): 416–23.
35. Schultz P, Herr HW, Zhang ZF, Bajorin DF, Seidman A, Sarkis A, Fair WR, Scherr D, Bosl GJ, Scher HI. Neoadjuvant chemotherapy for invasive bladder cancer: prognostic factors for survival of patients treated with M-VAC with 5-years follow-up. J Clin Oncol 1994; 12: 1394–401.

Feliksas Jankevičius, Peteris Goebellis,
Mayumi Kushima, Wolfgangas A. Schulzas,
Rolfas Ackermannas, Bernd'as J Schmitz-Drägeris

S a n t r a u k a

Apoptozės indukcija ir ląstelės ciklo kontrolės reguliavimas yra svarbūs mechanizmai, lemiantys chemoterapijos sukeltą ląstelės žūtį. Efektyviam apoptozės suaktyvinimui reikalingas nepakitęs naviko augimą slopinantis p53 genas. p53 netiesiogiai aktyvuoja WAF1/p21 geną, kuris, esant DNR pakenkimui, atsako už ląstelės ciklo sustabdymą G1 fazėje. Tokiu būdu p53 ir p21 ekspresija gali koreliuoti su urotelio vėžio jautrumu citotoksinei terapijai.

Metodai. Šioje retrospektyvinėje studijoje p53 ir p21 ekspresija buvo įvertinta imunohistochemijos (IHC) būdu archyvinių navikų mėginiuose, gautuose iš 60 ligonių, sergančių vietiškai pažengusiu ar metastaziniu urotelio vėžiu, kuriems buvo taikyta polichemoterapija su cisplatina. IHC rezultatai buvo palyginti su šių ligonių atsaku į gydymą ir išgyvenimu.

Rezultatai. Trisdešimt penki navikai (58%) parodė p53 akumuliaciją ir 25 (42%) navikuose buvo aptikta p21 ekspresija. Priklausomybės ryšio tarp p53 akumuliacijos ir p21 ekspresijos aptikti nepavyko. Pilnų ir dalinių remisijų dažnio po indukcinės polichemoterapijos (n = 39) analizė parodė, kad ligonių su nepakitusiu p53 atsakas į gydymą buvo geresnis negu ligonių su p53 hiperekskrecija (atitinkamai 70% ir 31%, p < 0,05). Tačiau p53 statusas neturėjo didesnės įtakos šių ligonių išgyvenimui (ligoniai su p53 neigiamais ir p53 teigiamais navikais vidutiniškai išgyveno atitinkamai 17 ir 12 mėnesių). Indukcinės chemoterapijos grupėje p21 ekspresija neturėjo ryšio nei su ligonių atsaku į gydymą, nei su jų išgyvenimu. Priešingas vaizdas stebėtas adjuvantinės chemoterapijos grupėje (n = 21). Gydymo efektas turėjo aiškų ryšį su imunohistochemijos rezultatais ir ypač su p21 ekspresija. Ligonių su p21 teigiamais ir p21 neigiamais navikais išgyvenimo vidurkis sudarė atitinkamai 60 ir 21 mėnesį (p < 0,005 Kaplano-Meierio analizėje).

Išvados. Šioje studijoje ligoniai su p21 teigiamais navikais po adjuvantinės chemoterapijos išgyveno gerokai ilgiau negu ligoniai su p21 neigiamais navikais. Studijos rezultatų analizė parodė p21 imunohistocheminę ekspresiją kaip nepriklausomą statistiškai reikšmingą teigiamo adjuvantinės chemoterapijos poveikio prognostinį indikatorių, todėl ji turėtų būti tiriama didesnėse apžvalginėse studijose.

Raktažodžiai: p21, p53, chemoterapija, urotelio vėžys