SPANDIDOS PUBLICATIONS

Evaluation of Ki67, p53 and angiogenesis in patients enrolled in a randomized study of neoadjuvant chemotherapy with or without cystectomy: A Southwest Oncology Group study

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Received March 8, 2006; Accepted May 25, 2006

Abstract. In this prospective biomarker study, we evaluated the prognostic significance of Ki67, p53 and angiogenesis in patients with locally advanced bladder cancer. The patients were volunteers from a Southwest Oncology Group trial of locally advanced bladder cancer who were randomized to treatment with neoadjuvant chemotherapy plus cystectomy or cystectomy alone. Tissue specimens were obtained prior to neoadjuvant chemotherapy from 42 patients randomized to receive the combination-treatment arm and 52 randomized to cystectomy alone. The statistical power of the study was quite limited by the small sample size. The biomarkers were assayed by immunohistochemistry. Angiogenesis was determined using anti-CD34 immunostaining. Patients whose tumors had increased Ki67 expression had better progression-free survival that was marginally significant, p=0.063. The median survival in those with higher Ki67 expression was 73 months, and in those with lower expression was 38 months. However, this did not achieve statistical significance, p=0.25. There was a suggestion of worse survival among patients whose tumors exhibited altered p53 staining [hazard ratio (HR) = 1.48; p=0.15], but there was no difference in progression-free survival (HR=1.02; p=0.93). The enumeration of tumor microvessels did not provide prognostic information.

Introduction

Patients with locally advanced bladder cancer have a poor prognosis despite treatment with radical cystectomy. Even with aggressive surgical extirpation, 56% of patients with pathologic stage T3 disease (invasion of perivesical tissue) have tumor recurrence, which occurs most commonly as distant metastases (1). In 1987, the Southwest Oncology Group (SWOG) initiated a study to determine if neoadjuvant chemotherapy would improve the outcome for patients with locally advanced bladder cancer. Patients were randomized to 3 cycles of M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) and cystectomy or cystectomy alone. In 1994, the NCI Bladder Cancer Marker Network and SWOG initiated a companion trial to study three promising indicators of prognosis. The clinical trial has been published and demonstrated that patients receiving neoadjuvant chemotherapy and radical cystectomy lived 2.6 years longer than patients treated with cystectomy alone (2). The results of the companion biomarker study evaluating Ki67, p53 and angiogenesis in these patients are now being reported.

Materials and methods

Patients eligible for this study were enrolled in the Southwest Oncology Group study 8710 (neoadjuvant M-VAC and radical cystectomy vs. radical cystectomy alone). Patients had T2-T4a bladder cancer and had tissue specimens available for histologic assessment prior to receiving neoadjuvant chemotherapy. This ancillary study (S9458) registered 104 patients, and 94 patients had valid slides submitted for analysis.

Immunostaining for Ki67 was performed using monoclonal antibody Ki67 (Dako, Carpenteria, CA) at a dilution of 1:20 and an avidin-biotin immunoperoxidase protocol. The percentage of positively staining tumor nuclei was determined by counting 1000 cells in those areas with the highest concentration of stained cells (3).

Immunostaining for p53 was performed using monoclonal antibody clone Pab1801 (Oncogene/Calbiochem Laboratories, Cambridge, MA) and an avidin-biotin immunoperoxidase

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Key words: bladder cancer, p53, Ki67, angiogenesis

Table I. Patient characteristics and biomarker distribution (n=94).

Age median (min, max)	64 (39, 80)
Male (%)	79
Race (%)	
White	88
African American	12
Clinical stage (%)	
T2	40
T3-T4	60
Treatment arm (%)	
MVAC + cystectomy	45
Cystectomy alone	55
Ki67 median (min, max)	38.4 (5.0, 77.9)
No. missing	8
Angiogenesis median (min, max)	92.0 (12.0, 264.0)
No. missing	4
p53 positive (%)	40
No. missing	6

protocol. The cut-off for a positive test was ≥20% of the tumor cells showing positive nuclear staining (4).

Angiogenesis was determined by counting microvessels. Immunostaining was performed using the anti-CD34 antibody HPCA-1 (Becton-Dickinson Immunocytometry Systems, San Jose, CA) and an avidin-biotin immunoperoxidase protocol. Light microscopy was used to identify regions within or immediately adjacent to the tumor that contained the greatest microvessel density. Any stained endothelial cell that was isolated from adjacent microvessels or other connective tissue elements was considered a microvessel. Microvessel counts were performed on a x200 microscopic field (5).

The methods of Kaplan and Meier were used to estimate the survival and progression-free survival curves, and the log-rank test p-value is reported. Hazard ratios and corresponding confidence intervals are obtained from univariate proportional hazard regression models.

Results

Ninety-four patients met all eligibility criteria. The median age of the eligible patients was 64 years (range 39-80 years); there were 20 women and 74 men. The distribution of the three biomarkers and patient characteristics are shown in Table I. Ki67 immunostaining was split into quartiles based on the data from the 86 patients for whom valid data were available. It was later dichotomized at the median when the two lower quartiles behaved similarly as did the two upper quartiles. p53 immunostaining was categorized as positive or negative. Angiogenesis was initially split into quartiles based on the data from the 90 patients for whom valid values were available and then subsequently dichotomized at the median value.

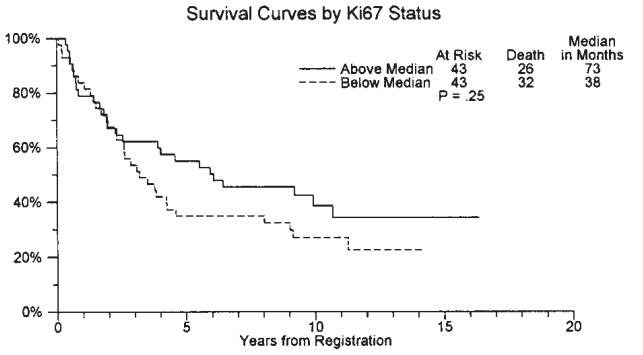

Figure 1. Survival of patients registered to SWOG 9458 by Ki67 dichotomized at the median.

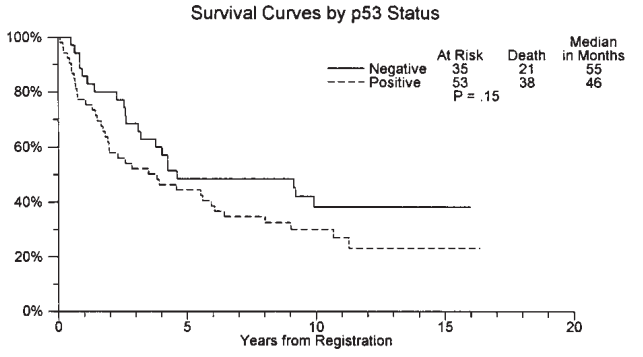

Figure 2. Survival of patients registered to SWOG 9458 by p53 status.

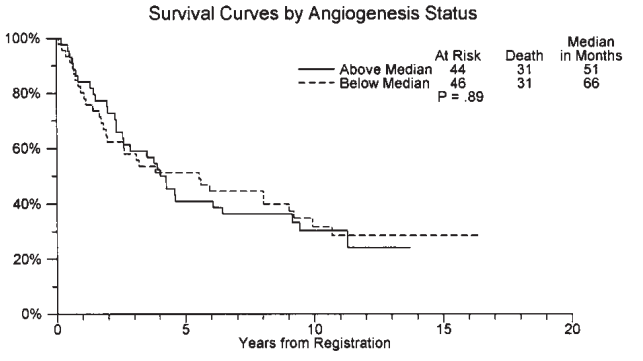

Figure 3. Survival of patients registered to SWOG 9458 by angiogenesis dichotomized at the median.

There was no association between Ki67, p53 or angiogenesis and sex, growth pattern, grade and stage (data not shown).

Ki67 immunostaining dichotomized at the median was not statistically significantly associated with survival (Fig. 1). The median survival for patients whose tumors were above the median was 73 months vs. 38 months in those whose tumors were below the median, p=0.25. Ki67 immunostaining was marginally significantly associated with progression-free survival, p=0.063. Patients whose tumors exhibited low Ki67 staining had a median progression-free survival of 12 months vs. 66 months for those whose tumors exhibited high Ki67 staining. Immunostaining for p53 was not correlated with survival, p=0.15 (Fig. 2) or progression-free survival, p=0.93

Results of univariate proportional hazards regression models.

	Progression-free survival		Survival	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Ki67 above median vs. below	0.62 (0.37, 1.03)	0.063	0.74 (0.44, 1.24)	0.25
p53 positive	1.02 (0.61, 1.71)	0.93	1.48 (0.87, 2.53)	0.15
Angiogenesis above median vs. below	1.01 (0.62, 1.64)	0.99	1.04 (0.63, 1.70)	0.89

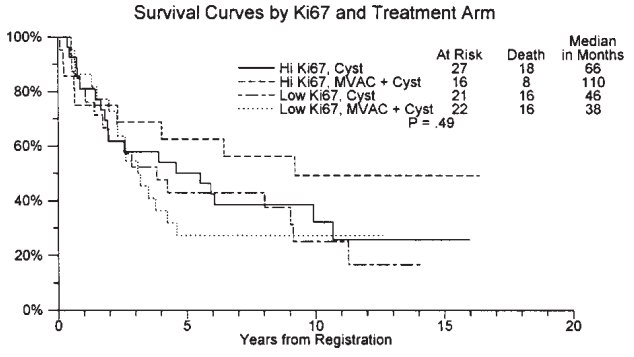

Figure 4. Survival of patients registered to SWOG 9458 by Ki67 dichotomized at the median and randomization arm.

Angiogenesis also did not correlate with survival, p=0.89 (Fig. 3), or progression-free survival, p=0.99. Hazard ratios and corresponding confidence intervals for Ki67, p53 and angiogenesis predicting survival and progression-free survival can be found in Table II.

Descriptive exploratory analyses were also performed based on the arm to which the patients were randomized. There were 52 randomized to cystectomy alone and 42 to neoadjuvant chemotherapy and cystectomy. Patients whose tumors exhibited high Ki67 staining appeared to have better survival regardless of the treatment arm to which they were randomized (Fig. 4). However, the small numbers in each group produced wide confidence intervals for the hazard ratios.

Discussion

Prognostic information is helpful to both patient and physician in guiding a variety of decisions that may have a profound effect on both quantity and quality of life. Stage strongly correlates with survival in patients with bladder cancer who are treated by cystectomy (6). However, within a given stage there is significant variation in patient outcome. Efforts to improve prediction for individuals have led to the evaluation of numerous molecules as potential biomarkers for progression. Nevertheless, the translation of these observations to the clinic has yet to be accomplished.

The TP53 tumor suppressor gene and its protein, p53, is an archetype of the problems associated with prognostic biomarker development in bladder cancer (7). Retrospective analyses examining specimens from selected patients are unlikely to provide reliable validation of any biomarker. The obvious solution is to test a promising biomarker in a prospective clinical trial. This report describes the outcome of such an effort. Three promising biomarkers were assessed that are involved in pathways known to be important in bladder cancer - proliferation, genomic instability and angiogenesis. The Ki67 protein is essential for cell proliferation and provides diagnostic information in some circumstances (8). The role of Ki67 as a biomarker for prognosis is less certain. The TP53 tumor suppressor gene has been called the guardian of the genome and has been extensively studied in bladder cancer with promising but not validated use as a prognostic biomarker (9). Angiogenesis, characterized by the development of new microvessels, is associated with the malignant phenotype and is being examined as a therapeutic target (10).

We found that patients whose tumors exhibited greater Ki67 expression had better progression-free survival that approached statistical significance. Overall survival was also better but was not statistically significant. This effect was seen in both randomization arms. Ki67 has been inconsistently associated with prognosis, but, when there was an association, increased expression was usually associated with poor outcome (11). Our finding that Ki67 overexpression was associated with prolonged progression-free survival was unexpected but has previously been reported in colon cancer (12). This finding will need to be evaluated in other patient populations. Although the subgroups are small, the data suggest that patients with Ki67 overexpressing tumors may benefit from neoadjuvant chemotherapy. This hypothesis can be further examined in an ongoing Southwest Oncology Group study.

Patients whose tumors exhibited altered p53 expression had shorter survival and progression-free survival, but this did not achieve statistical significance. The role of p53 as a prognostic indicator remains to be demonstrated. We did not find that angiogenesis was associated with survival or progression-free survival. Angiogenesis has been examined in many tumors and is prognostic in some but not in others. A recent review supports our finding that angiogenesis does not provide prognostic information in bladder cancer (13).

This study unexpectedly found that Ki67 overexpression was associated with longer progression-free survival. Immunohistochemistry of p53 may be an indicator of poor prognosis but requires further confirmation. Angiogenesis does not appear to be a useful prognostic indicator for bladder cancer. In

interpreting these results, it is important to remember that the power of the reported analyses is low because specimens were not obtained from the majority of the patients enrolled in the clinical trial.

Acknowledgements

This investigation was supported in part by the following PHS Cooperative Agreement grant numbers awarded by the National Cancer Institute, DHHS: CA38926, CA32102, CA22433, CA20319, CA76447, CA46441, CA42777, CA58416, CA46282, CA46113, CA14028, CA58658, CA04920, CA58415, CA58882, CA45377, CA63845, CA45560, CA35262, CA35431.

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