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Prognostic Value of p16 in Locally Advanced Prostate Cancer: A Study Based on Radiation Therapy Oncology Group Protocol 9202

Arnab Chakravarti, **Michelle DeSilvio**, **Min Zhang**, **David Grignon**, **Seth Rosenthal**, **Sucha O. Asbell**, **Gerald Hanks**, **Howard M. Sandler**, **Li-Yan Khor**, **Alan Pollack**, and **William Shipley**
From the Massachusetts General Hospital/Harvard Medical School, Boston, MA; American College of Radiology; Albert Einstein Medical Center; Fox Chase Cancer Center, Philadelphia, PA; Harper Hospital, Detroit; University of Michigan Medical Center, Ann Arbor, MI; and Radiological Associates of Sacramento, Sacramento, CA.

Abstract

Purpose—Deregulation of the retinoblastoma (RB) pathway is commonly found in virtually all known human tumors. p16, the upstream regulator of RB, is among the most commonly affected member of this pathway. In the present study, we examined the prognostic value of p16 expression in men with locally advanced prostate cancer who were enrolled on Radiation Therapy Oncology Group protocol 9202.

Patients and Methods—RTOG 9202 was a phase III randomized study comparing long-term (LT) versus short-term (ST) androgen-deprivation therapy (AD). Of the 1,514 eligible cases, 612 patients had adequate tumor material for p16 analysis. Expression levels of p16 were determined by immunohistochemistry (IHC). IHC staining was scored quantitatively using an image analysis system.

Results—On multivariate analysis, intact p16 expression was significantly associated with decreased rate of distant metastases ($P = .0332$) when both STAD and LTAD treatment arms were considered together. For patients with intact (high levels of immunostaining) p16 (mean p16 index > 81.3%), LTAD plus radiotherapy (RT) significantly improved prostate cancer survival (PCS) compared with STAD plus RT ($P = .0008$) and reduced the frequency of distant metastasis ($P = .0069$) compared with STAD plus RT. In contrast, for patients with tumors demonstrating p16 loss (low levels of immunostaining, mean p16 index ≤ 81.3%), LTAD plus RT significantly improved biochemical no evidence of disease survival over STAD ($P < .0001$) primarily by decreasing the frequency of local progression ($P = .02$), as opposed to distant metastasis, which was the case in the high-p16 cohort.

Conclusion—Low levels of p16 on image analysis appear to be associated with a significantly higher risk of distant metastases among all study patients. p16 expression levels also appear to identify patients with locally advanced prostate cancer with distinct patterns of failure after LTAD.

Address reprint requests to Arnab Chakravarti, MD, Massachusetts General Hospital, Department of Radiation Oncology, 100 Blossom St, Cox 3, Boston, MA 02114; achakravarti@partners.org.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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AUTHOR CONTRIBUTIONS
Conception and design: David Grignon, Seth Rosenthal, Sucha O. Asbell, Gerald Hanks, Howard M. Sandler, William Shipley
Collection and assembly of data: Arnab Chakravarti, Min Zhang, Li-Yan Khor, Alan Pollack
Data analysis and interpretation: Michelle DeSilvio,
Manuscript writing: Arnab Chakravarti

INTRODUCTION

Deregulation of the retinoblastoma protein (pRB) tumor suppressor pathway is commonly found in virtually all human tumor types.[1,2] It is thought that the primary function of this pathway is to prevent uncontrolled cellular proliferation by regulating the G1/S cell cycle checkpoint. Additional functions of this pathway such as regulation of apoptosis and transcriptional control are becoming better understood.[3] pRB pathway deregulation can occur at the level of pRB itself, or further upstream, including the cyclin-dependent kinases (CDKs) or CDK inhibitors such as p16. CDKs phosphorylate pRB, which, in turn, leads to dissociation from E2F family members. Free E2F can increase transcription of key genes, leading to S phase progression and increased cellular proliferation. We previously investigated the prognostic value of pRB pathway molecules in patients with locally advanced prostate cancers treated on Radiation Therapy Oncology Group (RTOG) 8610.[4] RTOG 8610 was a phase III randomized study that randomly assigned patients with locally advanced prostate cancers (T2–T4) without evidence of distant metastasis to receive goserelin (3.6 mg) every 4 weeks and flutamide (250 mg) three times per day for 2 months before radiation therapy compared with radiation therapy alone.[5] We found that low levels of p16 immunostaining (Fig 1) were significantly associated with reduced disease-specific survival ($P = .0078$), and increased risk of local failure ($P = .0035$) and distant metastasis ($P = .026$). Given these important findings, we proceeded to retrospectively validate p16 as a prognostic marker in locally advanced prostate cancer using tumor specimens from RTOG 9202.

PATIENTS AND METHODS

Study Population

For this analysis, a subset of patients entered in RTOG 9202 who had sufficient pathologic material available was studied. Tables 1–3 illustrate the differences between patients with p16 data versus those without p16 data with regard to pretreatment characteristics, outcome, and follow-up. The only significant difference that emerged was that patients treated by long-term (LT) versus short-term (ST) androgen-deprivation therapy (AD) had a significantly higher rate of p16 determination than did patients in the STAD group. However, there were no significant differences with regards to outcome or follow-up time between the two groups.

All patients were treated according to the guidelines of RTOG 9202. All patients received external-beam radiotherapy (EBRT) to the whole pelvis followed by a boost to the prostate. With regard to hormone therapy, before EBRT, all patients received monthly flutamide 250 mg orally tid with monthly goserelin acetate 3.6 mg subcutaneously until EBRT was completed. The patients were then randomly assigned to receive no further treatment (STAD plus RT) or to receive goserelin acetate 3.6 mg subcutaneously monthly for an additional 2 years after the completion of EBRT (LTAD plus RT).[6]

Immunohistochemical Technique

Tissues received in the RTOG tissue bank consisted of needle biopsies of prostate cancer preserved in buffered formalin. The tissues were promptly fixed after the biopsy procedure. For immunohistochemistry (IHC), the unstained slides were routinely deparaffinized in xylene. Antigen retrieval was accomplished by heating the sections in 10mmol/L citrate buffer pH = 6.0 for 50 minutes using a pressure cooker (BioCare Medical, Walnut Creek, CA). After antigen retrieval, samples were placed on an autostainer (DakoCytomation, Glostrup, Denmark) and incubated with antibody directed against p16 (DakoCytomation, 1:100 dilution for 10 minutes). Biotinylated secondary antibody was applied for 10 minutes, followed by incubation with streptavidin peroxidase (DAKO LSAB2, k0675) for 10 minutes. The slides were then rinsed and stained with diaminobenzidine chromogen solution (ResGen Invitrogen Corporation,

Carlsbad, CA) and counterstained with routine hematoxylin. Staining was accomplished using a DAKO autostainer. Negative staining controls consisted of slides stained with omission of antibody. Positive controls included normal prostatic epithelium. Scoring of IHC was performed using high-power quantitative image analysis. This was accomplished with the Chromavision ACIS image analysis system (Clarient Inc, San Juan Capistrano, CA) to determine the intensity of staining and the percentage of cells staining positively. The scoring pathologist was blinded to both clinical outcome and treatment assignment in conducting this assessment.

Definition of End Points

The failure event for overall survival (OS) was defined as death resulting from any cause. The failure event for prostate cancer–specific survival (PCSS) was death certified as resulting from prostate cancer, death resulting from complications of treatment, and death resulting from unknown causes with active malignancy (clinical disease relapse) or from another cancer with documented bone metastasis attributed to prostate cancer before the appearance of the second independent cancer. Local failure was assessed by palpation and defined as an increase in tumor volume by 25% or local persistence of tumor beyond 18 months. Distant metastasis was defined as radiographic or clinical evidence of hematogenous spread. The definition of biochemical failure (BF) was modeled after the American Society of Therapeutic Radiology and Oncology consensus definition. BF was defined as three consecutive rises or the institution of hormone treatment for a rising prostate-specific antigen (PSA) or a post-treatment PSA nadir level more than 4.0 ng/mL. Failure for the biochemical no evidence of disease (bNED) end point is defined as a BF, local failure, distant metastases failure, or death resulting from any cause. All time events, with the exception of BF, were measured from the date of random assignment to the date of their occurrence or last follow-up. BF was measured from the date of random assignment to the midpoint date between the postirradiation date of nadir PSA and the date of the first of the three consecutive rises.

Statistical Analysis

All pretreatment characteristics were dichotomized. Age was dichotomized by the median age in the entire cohort, PSA and stage were dichotomized by their stratification groupings, and Gleason score was dichotomized as 2 to 6 versus 7 to 10. Statistical comparisons to assess whether missing p16 data were dependent on pretreatment characteristics, assigned treatment, or outcome and were carried out using the χ^2 test and the Cox proportional hazards model.[1] p16 mean index percentage was dichotomized as 81.3% or less versus more than 81.3%, and p16 intensity score was dichotomized as 180.3 or less versus more than 180.3. Cox proportional hazards models were utilized to identify the impact of p16 expression as both a continuous and categoric variable on OS, PCSS, distant metastasis, local progression, and biochemical progression. OS and PCSS were estimated using the Kaplan-Meier method.[2,7] Local progression, distant metastasis, and biochemical progression failure rates were calculated using the cumulative incidence method[8] because these end points are cause specific and patients could die without experiencing the event of interest.

RESULTS

Assessment of Missing Data

Of the 1,514 eligible and analyzable cases, 612 cases (40.4%) had determined p16 data. Age, combined institutional Gleason score, PSA, clinical T stage, outcome, and follow-up were not statistically different between those with and without p16 data (Tables 1–3). There was a statistically significant difference in the proportion of patients randomly assigned to the LTAD plus RT arm that had p16 data compared with those without (53.4% *v* 47.2%; $P = .018$). This suggests that the subset of cases with p16 is not necessarily a random representation of the

entire RTOG 9202 study population and may not be generalizable outside the subset of cases with p16 data.

Patient Characteristics

Table 4 stratifies patient characteristics by mean index percentage (cut point = 81.3%) and median intensity score (cut point = 180.3), respectively. Patients younger than 70 years are significantly more apt to have reduced expression of p16 compared with patients 70 years of age and older, on the basis of both mean index percentage and median intensity score cut points (P = .03 and .01, respectively). Other pretreatment characteristics such as Gleason score, PSA, and T stage did not appear to be significantly associated with either mean index percentage or median intensity score cut points, respectively.

Univariate Analysis of Survival End Points

There were no significant associations with either OS, PCSS, distant metastasis, local progression, or biochemical progression, using either the p16 mean index of 81.3% or p16 intensity score of 180.3 as a cut point.

Univariate analysis of p16 staining stratified by mean index percentage, with a cut point of 81.3%, reveals a nonsignificant difference in distant metastasis–free survival in patients with low p16 levels compared with those with higher levels (P = .099).

Multivariable Analysis of Survival End Points

Multivariable analysis of OS, focusing on the independent variable p16 (both the p16 intensity score [Table 5] continuously and with a cut point of 180.3 and the p16 mean index percentage [Table 6] continuously and with a cut point of 81.3%) adjusting for age, institutional combined Gleason score, PSA, assigned treatment was performed. In both models for continuous p16 and dichotomized p16 intensity score, only age greater than 70 years was associated with significantly worse OS time, with a hazard ratio (HR) of 1.51 (95% CI, 1.09 to 2.10; P = .013) and 1.56 (95% CI, 1.12 to 2.16; P = .008), respectively (results not shown). Also, the results from continuous and dichotomized p16 mean index percentage showed that age older than 70 years was associated with significantly worse OS time, with an HR of 1.55 (95% CI, 1.22 to 2.15; P = .008) and 1.56 (95% CI, 1.13 to 2.16; P = .008), respectively (results not shown).

Multivariable analysis of PCSS focusing on the independent variable p16 and adjusting for age, institutional combined Gleason score, PSA, clinical stage, and assigned treatment revealed that clinical stage (T2 *v* T3) and assigned treatment (LTAD plus RT *v* STAD plus RT) were the only variables associated with improved PCSS. Multivariable analysis of distant metastasis focusing on the independent variable p16 adjusting for age, institutional combined Gleason score, PSA, clinical stage, and assigned treatment revealed that assigned treatment (LTAD *v* STAD, P = .009), combined Gleason score (2 to 6 *v* 7 to 10; P = .01), and p16 mean index percentage of more than 81.3% (P = .03) were significantly associated with higher rates of distant metastasis (Tables 7 and 8). p16 intensity scores with a 180.3 cut point were not found to be associated with the subsequent development of distant metastasis, however. Multivariable analysis of local progression, focusing on the independent variable p16, adjusting for age, institutional combined Gleason score, PSA, clinical stage, and assigned treatment on local progression revealed that assigned treatment (LTAD plus RT *v* STAD plus RT) was the only factor significantly associated with reduced local progression. Multivariable analysis of bNED, focusing on the independent variable p16 and adjusting for age, institutional combined Gleason score, PSA, clinical stage, and assigned treatment revealed that a PSA of 30 or less and assignment to LTAD plus RT significantly decreased biochemical progression (P < .0001 for both).

We next performed multivariable analyses of OS, PCSS, distant metastases, local failure, and biochemical failure, focusing on the independent variables of assigned treatment and p16 intensity score (≤ 180.3 *v* > 180.3), adjusting for age, institutional combined Gleason score, PSA, and T stage. Table 7 demonstrates that LTAD significantly increased PCSS and decreased biochemical progression in patients regardless of p16 intensity score. However, LTAD significantly reduced local progression only in patients with p16 intensity scores of 180.3 or less (*P* = .0266). Likewise, LTAD significantly reduced distant metastasis only in patients with p16 intensity scores more than 180.3 (*P* = .0158). Table 8 demonstrates similar results in a multivariable analysis of assigned treatment by p16 mean index percentage (≤ 81.3% *v* > 81.3%). These data suggest that LTAD improves bNED survival in patients with p16 loss primarily by reducing the frequency of local progression, whereas in patients with higher levels of p16, LTAD improves bNED survival primarily by reducing the frequency of distant metastasis.

In our multivariate Cox proportional hazards model, our data suggest that, in p16 index, there was a significant effect of LTAD in the high-p16 group of patients. Table 9 illustrates that there is a strong trend toward decreased biochemical progression among patients treated by LTAD with high p16 compared with those with low p16 (*P* = .07). Further, among patients treated by LTAD, both PCSS and distant metastasis–free survival were significantly improved in patients whose tumors had high p16 compared with those with low p16 (*P* = .05 and .02, respectively), as assessed by the p16 mean index percentage cut point of 81.3%.

We next investigated whether there may be a direct interaction between p16 levels and treatment. In this analysis, no significant associations were found. Therefore, no definitive conclusions can be made with regard to whether, indeed, specific groups of patients may derive significant benefit from LTAD plus RT versus STAD plus RT based exclusively on p16 levels. This also underscores an important limitation of the current study, which is that it represents a retrospective analysis of a prospective phase III trial. To validate the true prognostic and predictive value of p16 levels, studies in which patients are stratified prospectively based on p16 expression levels need to be conducted. RTOG is planning such a future study to investigate the prognostic and predictive value of p16 in the setting of prostate cancer. Short of this type of class I evidence, retrospective data, even when performed on a prospective phase III study data set, carry important limitations.

DISCUSSION

This study demonstrates that p16 expression as determined by quantitative IHC may play an important role in identifying patients with locally advanced prostate cancer treated by RT plus AD who are at high risk for the subsequent development of distant metastasis. Our multivariate Cox proportional hazards data also suggest that patients with high versus low p16 appear to have significantly improved distant metastasis–free survival and PCSS times within the LTAD arm. Further, the mechanisms by which LTAD plus RT improves bNED survival over STAD plus RT appear to be significantly associated with p16 expression levels. For tumors that have greater loss of p16 (eg, mean indices < 81.3%), it appears that LTAD plus RT improves bNED survival over STAD plus RT primarily by decreasing the frequency of local failure. Unfortunately, although patients with low p16 levels are at especially high risk for the subsequent development of distant metastasis, it does not appear that LTAD reduces this risk appreciably for this high-risk cohort of patients compared with STAD.

Although the present study represents a retrospective correlative analysis performed on a prospective phase III study, the large patient numbers and the quantification of IHC data can serve only to enhance the accuracy of the data. Further, because this article serves as independent confirmation of the prognostic value of p16 from a previous correlative study

performed on RTOG 8610,[4] the prognostic value of p16 in this setting should be seriously considered. It is important to acknowledge that the present study represents a subgroup analysis that may not represent the entire cohort of patients enrolled on RTOG 9202. Further, it is important to acknowledge that the test for interaction between p16 levels and treatment revealed no significant interactions in the present study. Therefore, the data presented in this study are insufficient to select which prostate cancer patients should receive LTAD versus STAD on the basis of p16 expression patterns. Indeed, RTOG is planning future prospective studies in the setting of prostate cancer in which the prognostic and predictive values of molecular markers such as p16 will be used as stratification variables and rigorously evaluated.

Although it is possible that p16 expression serves merely as a marker for distant metastasis, a direct mechanistic connection between p16 loss and distant metastasis in prostate cancer cannot be entirely ruled out. It is curious that reports from other histologic tumor subtypes also suggest that p16 loss is associated with an increased risk for the subsequent development of distant metastasis and adverse prognosis.[9–12] Although the mechanistic conclusions that can be drawn from a correlative study like the present are inherently limited, the importance for identifying therapeutic strategies for reducing the subsequent development of distant metastasis cannot be overstated for high-risk patients, especially those with low p16 levels. The observation that LTAD plus RT versus STAD plus RT improves bNED survival in patients with high p16 (mean index percentage > 81.3%), primarily by reducing the frequency of distant metastasis, suggests that the underlying biologic mechanisms for the development of distant metastasis may be inherently different in these patients compared with those with low p16 levels. Although the data from RTOG 9202 reveals that LTAD is superior to STAD for the general population of patients with locally advanced prostate cancer, the present study highlights several opportunities for further increasing the efficacy of LTAD. Because patients with low levels of p16 have a higher risk for the subsequent development of distant metastasis, which does not appear to be significantly mitigated by LTAD, patients in this population may be prime candidates for biotherapeutic approaches that have been shown to be efficacious in reducing metastatic potential in preclinical models of prostate cancer. These include antiangiogenic strategies in combination with AD and RT for those patients with locally advanced prostate cancers with low levels of p16. There are also increasing data that a more direct link between p16 expression and angiogenesis may exist, providing a mechanism for the observed association of p16 loss with the development of distant metastasis in many tumor types. [13–17] Harada et al[18] reported that restoration of wild-type p16 in p16-depleted gliomas was associated with downregulation of VEGF levels and resultant inhibition of tumor neovascularization. A subsequent study reported that demethylation of the p16 promoter (which results in increased expression of p16 protein levels) results in downregulation of VEGF in human lung cancer models.[19] Therefore, a more effective treatment approach for locally advanced prostate cancers demonstrating p16 loss may involve combining LTAD plus RT with antiangiogenic therapies such as avastin or other classes of antiangiogenic agents. For patients with high levels of p16, strategies to improve local control hold the promise of adding to the benefit of LTAD plus RT. To this end, biotherapeutic strategies to enhance apoptotic cell death through targeting important growth factor receptors that signal through critical prosurvival signal transduction pathways such as the phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT) pathway may prove to be a promising strategy in combination with LTAD plus RT for patients with intact p16 expression. Inhibition of central antiapoptotic molecules such as bcl-2 and Survivin may prove to be promising as well in improving local control in these patients.

In summary, the results of this study suggest that low p16 levels are associated with an increased risk for the subsequent development of distant metastasis in all patients with locally advanced prostate cancers. These data also suggest that patients with locally advanced prostate cancer with high versus low p16 levels treated by LTAD plus RT have significantly improved PCSS and distant metastasis–free survival times along with a strong trend towards improved bNED

survival on multivariate analysis. To more accurately assess the interactions between assigned treatment and p16 for all of the efficacy end points, RTOG is planning future studies prospectively stratifying patients on the basis of molecular marker data, including p16 expression, which will be correlated with the outcome variables described in this report.

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References

1. Harbour JW, Dean DC. The pRB/E2F pathway: Expanding roles and emerging paradigms. Genes Dev 2000;14:2393–2409. [PubMed: 11018009]
2. Nevins JR. The Rb/E2F pathway and cancer. Hum Mol Genet 2001;10:699–703. [PubMed: 11257102]
3. Pan H, Yin C, Dyson NJ, et al. Key roles for E2F1 in signaling p53-dependent apoptosis and in cell division within developing tumors. Mol Cell 1998;2:283–292. [PubMed: 9774967]
4. Chakravarti A, Heydon K, Wu C-L, et al. Loss of p16 expression is of prognostic significance in locally advanced prostate cancer: An analysis from the Radiation Therapy Oncology Group protocol 8610. J Clin Oncol 2003;21:3328–3334. [PubMed: 12947069]
5. Pilepich MV, Krall JM, Al-Sarraf M, et al. Androgen deprivation with radiation therapy compared with radiation therapy alone for locally advanced prostatic carcinoma: A randomized comparative trial of the Radiation Therapy Oncology Group. Urology 1995;45:616–623. [PubMed: 7716842]
6. Hanks GE, Pajak TF, Porter AC, et al. Phase III trial of long-term adjuvant androgen deprivation after neoadjuvant hormonal cytoreduction and radiotherapy in locally advanced carcinoma of the prostate: The Radiation Therapy Oncology Group protocol 9202. J Clin Oncol 2003;21:3972–3978. [PubMed: 14581419]
7. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481.
8. Kalbfleish, JD.; Prentice, RL. The Statistical Analysis of Failure Times Data. New York, NY: John Wiley; 1980.
9. Takeuchi H, Ozawa S, Ando N, et al. Altered p16/MTS1/CDKN2 and cyclin D1/PRAD-1 gene expression is associated with prognosis of squamous cell carcinoma of the esophagus. Clin Cancer Res 1997;3:2229–2236. [PubMed: 9815619]
10. Namazie A, Alavi S, Olopade O, et al. Cyclin D1 amplification and p16 (MTS1/CDK4I) deletion correlate with poor prognosis in head and neck tumors. Laryngoscope 2002;112:472–481. [PubMed: 12148857]
11. Gessner C, Liebers U, Kuhn H, et al. BAX and p16INK4a are independent positive prognostic markers for advanced tumour stage of nonsmall cell lung cancer. Eur Respir J 2002;19:134–140. [PubMed: 11843312]
12. Jarrard DF, Bova GS, Ewing CM, et al. Deletional, mutational, and methylation analyses of CDKN2 (p16/MTS1) in primary and metastatic prostate cancer. Genes Chromosomes Cancer 1997;19:90–96. [PubMed: 9171999]
13. Li J, Wang E, Rinaldo F, et al. Upregulation of VEGF-C by androgen depletion: The involvement of IGF-1R-FOXO pathway. Oncogene 2005;24:5510–5520. [PubMed: 15897888]
14. Niculescu T, Weerth S, Soane L, et al. Effects of membrane attack complex of complement on apoptosis in experimental autoimmune encephalomyelitis. Ann N Y Acad Sci 2003;1010:530–533. [PubMed: 15033785]
15. Boddy JL, Fox SB, Han C, et al. The androgen receptor is significantly associated with vascular endothelial growth factor and hypoxia sensing via hypoxia-inducible factors HIF-1a, HIF-2a, and the prolyl hydroxylases in human prostate cancer. Clin Cancer Res 2005;11:7658–7663. [PubMed: 16278385]

16. Lara PN Jr, Twardowski P, Quinn DI. Angiogenesis-targeted therapies in prostate cancer. Clin Prostate Cancer 2004;3:165–173. [PubMed: 15636683]

17. Gustavsson H, Welen K, Damber JE. Transition of an androgen-dependent human prostate cancer cell line into an androgen-independent subline is associated with increased angiogenesis. Prostate 2005;62:364–373. [PubMed: 15389782]

18. Harada H, Nakagawa K, Iwata S, et al. Restoration of wild-type p16 down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human gliomas. Cancer Res 1999;59:3783–3789. [PubMed: 10446996]

19. Miki K, Shimizu E, Yano S, et al. Demethylation by 5-aza-deoxycytidine (5-azadC) of p16INK4A gene results in downregulation of vascular endothelial growth factor expression in human lung cancer cell lines. Oncol Res 2001;12:335–342. [PubMed: 11589304]

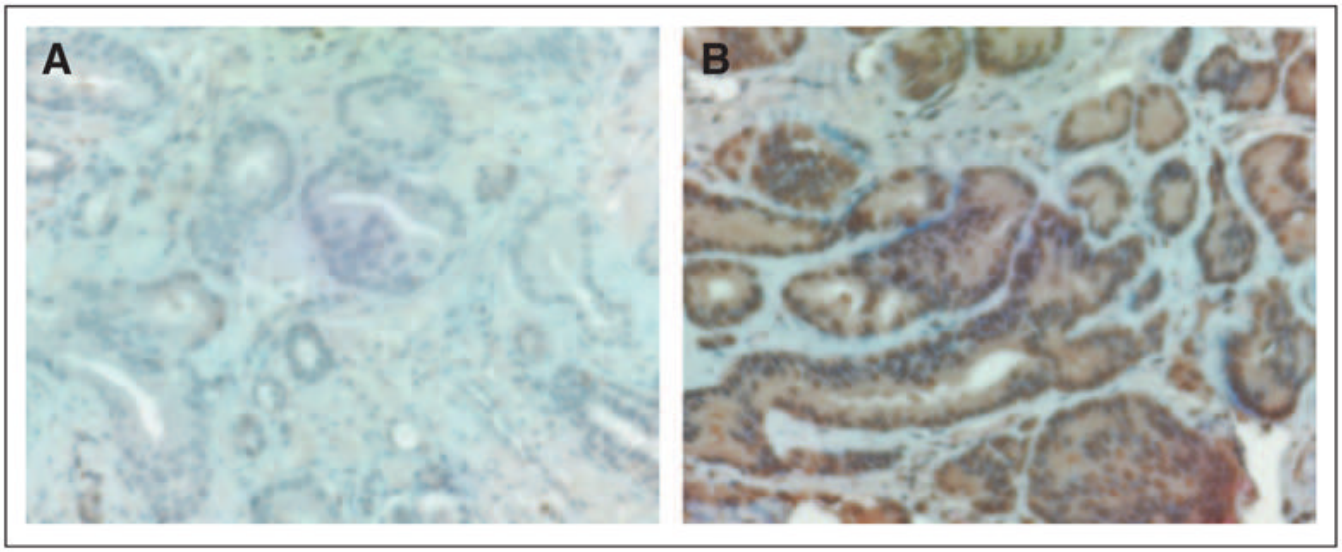

Fig 1.
Representative stained slides for (A) p16-negative and (B) p16-positive immunostaining.

Table 1

Pretreatment Characteristics of Eligible Patients Entered Onto RTOG Protocol 9202

	Missing p16 Data (n = 902)		Determined p16 Data (n = 612)		
Characteristic	**No.**	**%**	**No.**	**%**	**χ^2 Test *P***
Age, years					.764
< 70	405	44.9	270	44.1	
≥ 70	497	55.1	342	55.9	
Median	70		70		
Range	43–88		43–88		
Combined institutional Gleason score*					.162
2–6	360	42.4	217	38.6	
7–10	490	57.6	345	61.4	
PSA, ng/mL†					.603
≤ 30	607	67.3	404	66.0	
> 30	295	32.7	208	34.0	
Median	20		20.4		
Range	0.2–280		0.1–295		
Clinical stage					.481
T2c	402	44.6	284	46.4	
T3–4	500	55.4	328	53.6	
Assigned treatment					.018
LTAD + RT	426	47.2	327	53.4	
STAD + RT	476	52.8	285	46.6	

Abbreviations: RTOG, Radiation Therapy Oncology Group; PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

*Fifty-two and 50 cases with missing and determined p16 data, respectively, have unknown combined institutional Gleason scores.

†Three and two cases with missing and determined p16 data, respectively, have unknown PSA.

Table 2

Univariate Cox Proportional Hazards Models

End Point	Assigned Treatment	p16	No. of Patients	No. of Failures	HR*	95% CI	*P*
Overall survival	LTAD + RT	Missing	426	130	0.88	0.67 to 1.15	.36
		Determined	327	91			
	STAD + RT	Missing	476	141	1.08	0.83 to 1.41	.58
		Determined	285	86			
Prostate cancer–specific survival	LTAD + RT	Missing	426	33	0.84	0.49 to 1.45	.39
		Determined	327	22			
	STAD + RT	Missing	476	50	1.30	0.85 to 2.00	.22
		Determined	285	37			
Distant metastasis	LTAD + RT	Missing	426	53	0.75	0.61 to 1.43	.10
		Determined	327	36			
	STAD + RT	Missing	476	84	1.05	0.74 to 1.50	.77
		Determined	285	50			
Local progression	LTAD + RT	Missing	426	28	0.96	0.55 to 1.67	.87
		Determined	327	22			
	STAD + RT	Missing	476	55	1.26	0.84 to 1.90	.26
		Determined	285	40			
Biochemical progression†	LTAD + RT	Missing	422	102	0.85	0.63 to 1.15	.30
		Determined	322	73			
	STAD + RT	Missing	474	226	1.12	0.91 to 1.39	.28
		Determined	284	141			

Abbreviations: HR, hazard ratio; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

*The HR ratio for determined p16 data as compared with missing p16 data.

†There are six missing and six determined p16 cases with unknown biochemical progression status.

Table 3

Median Follow-Up Time

	Missing p16 Data			Determined p16 Data		
Characteristic	**LTAD + RT**	**STAD + RT**	**Total**	**LTAD + RT**	**STAD + RT**	**Total**
Total No. of patients	426	475	901	327	285	612
Follow-up, months						
Median	70.6	71.9	71.2	71.4	70.3	70.6
Range	2.1–107	2.8–106	2.1–107	0.46–105	1.2–105	0.46–105
No. of patients alive	296	334	630	236	199	435
Follow-up, months						
Median	76.3	76.3	76.3	75.7	75.3	75.7
Range	11.6–107	2.96–103	2.96–107	21.4–105	18.8–105	18.8–105

Abbreviations: LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

* There is one missing p16 case with unknown survival time.

Table 4

Pretreatment Characteristics of Eligible Patients With Determined p16 Data (n = 612)

	p16 Mean Index %					p16 Intensity Score				
	≤ 81.3 (n = 306)		> 81. 3(n = 306)			≤ 180.3 (n = 307)		> 180.3 (n = 305)		
Characteristic	**No.**	**%**	**No.**	**%**	**χ^2 Test *P***	**No.**	**%**	**No.**	**%**	**χ^2 Test *P***
Age, years					.034					.007
< 70	148	48.4	122	39.9		152	49.5	118	38.7	
≥ 70	158	51.6	184	6.1		155	5.5	187	61.3	
Median	70		71			70		71		
Range	43–88		51–88			43–88		51–84		
Combined institutional Gleason score*					.277					.703
2–6	111	36.4	125	4.9		116	37.8	120	39.4	
7–10	195	63.6	181	59.1		191	62.2	185	60.6	
PSA, ng/mL†					.733					.014
≤ 30	204	66.7	200	65.4		217	70.7	187	61.3	
> 30	102	33.3	106	34.6		90	29.3	118	38.7	
Median	20.2		20.8			19.5		22.8		
Range	0.9–295.0		0.1–152.0			0.9–295.0		0.1–219.7		
Clinical stage					.746					.931
T2c	140	45.8	144	47.1		143	46.6	141	46.2	
T3–4	166	54.2	162	52.9		164	53.4	164	53.8	
Assigned treatment					.808					.996
LTAD + RT	165	53.9	162	52.9		164	53.4	163	53.4	
STAD + RT	141	46.1	144	47.1		143	46.6	142	46.6	

Abbreviations: PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

*Twenty-three and 27 cases with mean index ≤ 81.3% and > 81.3% data, respectively, have unknown combined institutional Gleason scores.

†Nineteen and 31 cases with intensity score ≤ 180.3 and > 180.3 data, respectively, have unknown combined institutional Gleason scores.

Table 5

Multivariate Cox Proportional Hazards Models* p16 Intensity Score (n = 562)

	Continuous Intensity Score			Dichotomized Intensity Score		
End Point	**HR†**	**95% CI**	***P***	**HR‡**	**95% CI**	***P***
Overall survival	1.18	0.95 to 1.48	.14	1.07	0.78 to 1.47	.66
Prostate cancer–specific survival	1.45	0.90 to 2.34	.12	1.49	0.84 to 2.65	.17
Distant metastases	0.99	0.77 to 1.29	.99	1.04	0.65 to 1.65	.88
Local progression	0.82	0.64 to 1.05	.11	1.04	0.62 to 1.76	.87
Biochemical progression	0.98	0.83 to 1.16	.82	1.07	0.81 to 1.43	.63

Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

*Adjusted for age (≤ 70 *v* > 70 years), combined institutional Gleason score (≤ 6 versus > 6), PSA (≤ 30 *v* > 30), clinical T stage (T2 *v* T3) and assigned treatment (LTAD + RT *v* STAD + RT). Adjustments for age were made only for the overall survival model.

†The HR for a unit change of 50 in the p16 continuous intensity score.

‡The HR for the dichotomized intensity score of ≤ 180.3 *v* > 180.3.

Table 6

Multivariate Cox Proportional Hazards Models* p16 Mean Index % (n = 562)

	Index % (Continuous)			Index % (Dichotomous)		
End Point	**HR†**	**95% CI**	***P***	**HR‡**	**95% CI**	***P***
Overall survival	1.07	0.95 to 1.22	.28	1.10	0.80 to 1.49	.57
Prostate cancer–specific survival	1.01	0.82 to 1.25	.90	1.04	0.61 to 1.79	.88
Distant metastases	0.88	0.75 to 1.02	.10	0.61	0.38 to 0.98	.04
Local progression	0.89	0.75 to 1.07	.21	0.75	0.44 to 1.26	.27
Biochemical progression	0.98	0.88 to 1.09	.69	0.91	0.69 to 1.21	.52

Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

*Adjusted for age (≤ 70 *v* > 70 years), combined institutional Gleason score (≤ 6 versus > 6), PSA (≤ 30 *v* > 30), clinical T stage (T2 *v* T3) and assigned treatment (LTAD + RT *v* STAD + RT). Adjustments for age were made only for the overall survival model.

†The HR for a unit change of 50 in the p16 continuous intensity score

‡The HR for the dichotomized index % of ≤ 81.3% *v* > 81.3%.

Table 7

Multivariate Cox Proportional Hazards Models p16 Intensity Score (n = 562)

		p16 Intensity Score ≤ 180.3			p16 Intensity Score > 180.3		
End Point	**Assigned Treatment**	**HR***	**95% CI**	***P***	**HR***	**95% CI**	***P***
Overall survival	LTAD + RT *v* STAD + RT	1.21	0.76 to 1.93	.4181	1.21	0.79 to 1.84	.3820
Prostate cancer–specific survival	LTAD + RT *v* STAD + RT	3.03	1.12 to 8.19	.0285	2.55	1.20 to 5.4	.0145
Distant metastases	LTAD + RT *v* STAD + RT	1.47	0.77 to 2.82	.2484	2.29	1.17 to 4.49	.0158
Local progression	LTAD + RT *v* STAD + RT	2.41	1.11 to 5.23	.0266	1.97	0.93 to 4.16	.0748
Biochemical progression	LTAD + RT *v* STAD + RT	3.45	2.24 to 5.32	> .0001	3.59	2.34 to 5.50	> .0001

Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

* The HR for assigned treatment of STAD + RT compared with LTAD + RT, after adjusting for age (≤ 70 *v* > 70 years), combined institutional Gleason score (≤ 6 *v* > 6), PSA (≤ 30 *v* > 30), clinical T stage (T2 *v* T3), and assigned treatment (LTAD + RT *v* STAD + RT). Adjustments for age were made only for the overall survival model.

Table 8

Multivariate Cox Proportional Hazards Models p16 Mean Index % (n = 562)

		p16 Index ≤ 81.3%			p16 Index > 81.3%		
End Point	**Assigned Treatment**	**HR***	**95% CI**	***P***	**HR***	**95% CI**	***P***
Overall survival	LTAD + RT *v* STAD + RT	1.30	0.83 to 2.05	.2504	1.17	0.76 to 1.80	.4865
Prostate cancer–specific survival	LTAD + RT *v* STAD + RT	1.56	0.72 to 3.39	.2601	6.51	2.18 to 19.41	.0008
Distant metastases	LTAD + RT *v* STAD + RT	1.41	0.79 to 2.50	.2463	3.28	1.39 to 7.75	.0069
Local progression	LTAD + RT *v* STAD + RT	2.41	1.18 to 4.93	.0161	2.00	0.88 to 4.53	.0973
Biochemical progression	LTAD + RT *v* STAD + RT	3.02	2.01 to 4.55	> .0001	4.26	2.68 to 6.77	< .0001

Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen; LT, long term; AD, androgen-deprivation therapy; RT, radiotherapy; ST, short term.

* The HR for assigned treatment of STAD + RT compared with LTAD + RT, after adjusting for age (≤ 70 *v* > 70 years), combined institutional Gleason score (≤ 6 *v* > 6), PSA (≤ 30 *v* > 30), clinical T stage (T2 *v* T3) and assigned treatment (LTAD + RT *v* STAD + RT). Adjustments for age were made only for the overall survival model.

Table 9

Multivariate Cox Proportional Hazards Models Assigned Treatment (n = 562)

		LTAD+RT			STAD+RT		
End Point	**p16 Index (%)**	**HR***	**95% CI**	***P***	**HR***	**95% CI**	***P***
Overall survival	≤ 81.3 *v* > 81.3	1.13	0.72 to 1.75	.6004	1.04	0.67 to 1.62	.8611
Prostate cancer–specific survival	≤ 81.3 *v* > 81.3	0.32	0.10 to 1.02	.0533	1.46	0.74 to 2.89	.2752
Distant metastases	≤ 81.3 *v* > 81.3	0.35	0.15 to .82	.0154	0.85	0.47 to 1.54	.5980
Local progression	≤ 81.3 *v* > 81.3	0.88	0.37 to 2.10	.7723	0.72	0.38 to 1.38	.3227
Biochemical progression	≤ 81.3 *v* > 81.3	0.62	0.37 to 1.04	.0712	1.02	0.72 to 1.44	.8999

Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen; AD, androgen-deprivation therapy; LT, long term; ST, short term; RT, radiotherapy.

* The HR for assigned treatment of STAD + RT compared with LTAD + RT, after adjusting for age (≤ 70 *v* > 70 years), combined institutional Gleason score (≤ 6 *v* > 6), PSA (≤ 30 *v* > 30), clinical T stage (T2 *v* T3) and assigned treatment (LTAD + RT *v* STAD + RT). Adjustments for age were made only for the overall survival model.