APE1 and XRCC1 Protein Expression Levels Predict Cancer-Specific Survival Following Radical Radiotherapy in Bladder Cancer

Sei C. Sak,[1] Patricia Harnden,[2] Colin F. Johnston,[1] Alan B. Paul,[3] and Anne E. Kiltie[1]

Abstract

Introduction: Radiotherapy offers the potential of bladder preservation in muscle-invasive bladder cancer, but only a proportion of tumors respond, and there are no accurate predictive methods. The ability of tumor cells to repair DNA damage induced by ionizing radiation influences radiosensitivity. We therefore investigated the prognostic value of the DNA repair proteins APE1 and XRCC1 in patients with muscle-invasive bladder cancer treated by radical radiotherapy.

Materials and Methods: The tumors of 90 patients with muscle-invasive transitional cell carcinoma and known clinical outcomes were immunostained with APE1 and XRCC1 antibodies. Levels of protein expression were assessed as a percentage of tumor cells with positive nuclear staining (1,000 cells per tumor).

Results: The median percentage of nuclear staining for APE1 was 98.7% (range, 42.2-100%) and for XRCC1 was 96.5% (range, 0.6-99.6%). High expression levels of APE1 or XRCC1 ($\geq$95% positivity) were associated with improved patient cancer-specific survival (log-rank, $P = 0.02$ and 0.006, respectively). In a multivariate Cox regression model, APE1 and XRCC1 expression and hydronephrosis were the only independent predictors of patient survival.

Conclusions: Expression levels of both APE1 and XRCC1 proteins were strongly associated with patient outcome following radiotherapy, separating patients with good outcome from the 50% with poor outcome (82% and 44%, 3-year cause-specific survival, respectively). If prospectively validated, this simple test could be incorporated into clinical practice to select patients likely to respond to radiotherapy and consider alternative forms of therapy for those unlikely to respond.

Bladder cancer, predominantly transitional cell carcinoma (TCC), is the fourth commonest cancer in the United Kingdom and the United States and causes 4,700 deaths a year in the United Kingdom (1). Radical radiotherapy and cystectomy are the two main curative treatment options for nonmetastatic, muscle-invasive TCC (stages T2-T4a). More recently, chemotherapy has been added to treatment regimens with the aim of improving outcomes (2). Bladder-conserving therapy using combinations of chemotherapy and radiotherapy have shown promising results (3, 4), but not all tumors respond to radiotherapy. Thus, the ability to predict tumor radiosensitivity would be very helpful in patient selection for bladder conservation therapy. At present, the tools for predicting radiosensitivity are limited to clinical tumor-node-metastasis staging, grade, and presence of hydronephrosis (5, 6), but these are of limited value in predicting outcomes of individual patients.

Ionizing radiation kills cells by inducing DNA damage such as base damage, single-strand breaks (SSB), double-strand breaks (DSB), and DNA-interstrand cross-links (7). DNA DSBs are the lethal lesions. Using immunohistochemical staining, Harima et al. (8) and Wilson et al. (9) showed that high levels of the DSB repair proteins Ku70 and Ku80 in preradiation cervical cancer tumors were associated with radioresistance. Komura et al. (10) also showed a similar association between Ku70 protein expression and rectal cancer.

Ionizing radiation causes much more DNA base damage and SSB than DSB. These lesions are repaired by the base excision repair (BER) pathway, and BER proteins include X-ray repair cross complement group 1 protein (XRCC1) and human AP endonuclease (APE1). Few studies have investigated BER proteins in radiotherapy patients. Robertson et al. (11) and Herring et al. (12) showed that high APE1 expression levels were associated with radioresistance in germ cell tumors and cervical cancer, respectively, but this has not been examined in bladder cancer. Furthermore, XRCC1, which has no intrinsic enzymatic function but acts as a scaffold for other BER proteins including APE1, Pol Beta, PARP, PNK, and ligase 3, has to our knowledge only been investigated by immunohistochemistry in human tumors once before. In this study of pancreatic adenocarcinomas (13), reduced XRCC1 expression was seen in 75% of cancer samples when compared with epithelial cells in the nonneoplastic pancreas. However, XRCC1 expression has not been investigated in cohorts of patients treated by radiotherapy to determine its predictive value for radiosensitivity.

Authors' Affiliations: [1]Cancer Research UK Clinical Centre, [2]Pathology and [3]Urology, St. James's University Hospital, Leeds, United Kingdom

Received 1/7/05; revised 5/20/05; accepted 6/3/05.

Grant support: Yorkshire Cancer Research (S.C. Sak, A.E. Kiltie, and this study), Leeds Teaching Hospitals NHS Trust (P. Harnden and A.B. Paul), Cancer Research UK (C.F. Johnston, A.E. Kiltie, and this study), and Royal College of Radiologists (A.E. Kiltie).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Anne E. Kiltie, Cancer Research UK Clinical Centre, St. James's University Hospital, Leeds, LS9 7TF United Kingdom. Phone: 44-113-206-4908; Fax: 44-113-242-9886; E-mail: anne.kiltie@cancer.org.uk.

doi:10.1158/1078-0432.CCR-05-0045

We therefore examined the relationship between XRCC1 and APE1 protein expression and cancer-specific survival in patients with muscle-invasive TCC treated by radical radiotherapy. We found that high APE1 and XRCC1 expression separated patients with good outcome from the 50% with poor outcome, with 82% and 44% 3-year cause-specific survivals, respectively ($P < 0.001$). This statistically and clinically significant result, if validated prospectively and by other groups, could be incorporated into clinical practice to inform patient choice in the treatment of muscle-invasive bladder cancer.

Materials and Methods

Patients and radiotherapy. This study was approved by the local ethical committee. One hundred twenty-nine patients treated by urologists in the Leeds metropolitan area for bladder cancer were given radical radiotherapy at the regional clinical oncology center, Leeds, United Kingdom, from 1995 to 2000. Of these, 116 patients had muscle-invasive bladder TCC and 13 patients had high-risk superficial disease (G3pT1). The current study group comprised the 90 patients with muscle-invasive disease for whom histologic specimens were available from transurethral resection of the bladder tumor before radiotherapy. The median age was 75 years (range, 44-99 years), and there were 68 men and 22 women. All patients received computerized tomography–planned radical radiotherapy to a dose ranging from 50 to 55 Gy in 20 fractions over 4 weeks, to the whole bladder with a 1.5-cm margin on the tumor-bearing wall and 1 cm to the remainder of the bladder.

All 90 patients were staged by computerized tomography scan: 46 were stage T2, 39 stage T3, and five stage T4. Pathology was reviewed by a single pathologist (P.H.) who had no knowledge of clinical outcomes. Tumors were graded on their worst component and the majority (n = 70) were poorly differentiated (grade 3) and the remaining 20 were moderately differentiated (grade 2). Of the 82 tumors with a transitional cell element, 24 showed TCC only, 56 showed squamous differentiation, occasionally admixed with mucinous (n = 2) or small cell (n = 2) areas, and the final two showed an additional small cell component only. Six tumors were squamous, one of which had additional small cell areas. The final two were undifferentiated or sarcomatoid. Seventeen of the patients had previously documented superficial bladder tumors before developing muscle-invasive disease.

The median duration of follow-up in surviving patients was 42 months (range, 11-92 months). The overall and cancer-specific survivals at 3 years for the entire cohort were 53.5% and 63.1%, respectively. Clinical response was assessed by check cystoscopy in 87.8% (79 of 90) of patients; the remaining 11 were not assessable by cystoscopy because of patient debility or rapid disease progression. Nine of these patients died of disease (median survival, 5.5 months) and two died of other causes. Complete response was observed in 62.2% (56 of 90), partial response in 15.6% (14 of 90), and progressive disease in 10% (9 of 90). Complete response was defined as no residual tumor detected, partial response as residual noninvasive tumor, and progressive disease as residual muscle-invasive tumor. Salvage cystectomy was undertaken in 10 patients (11%). This was done for intractable bleeding in one patient, and the cystectomy showed noninvasive papillary TCC only. One patient developed nodal metastases 9 months after first presentation and cystectomy, which showed muscle-invasive carcinoma, was done for local symptom control. In the remainder, residual or recurrent invasive carcinoma was found either in the first-check cystoscopy after radiotherapy (n = 4) or on subsequent follow-up (n = 4). The cystectomy showed no evidence of residual carcinoma (n = 1), carcinoma *in situ* only (n = 1), or invasive carcinoma (pT1, n = 3; pT3a, n = 3).

Immunohistochemical staining for APE1 and XRCC1. Immunohistochemical staining was done using a standard streptavidin-biotin complex method. The formalin-fixed, paraffin wax–embedded tumor sections (4 μm thick) were dewaxed for 15 minutes in xylene and hydrated by passage through a graded ethanol series to tap water. Sections were then treated with 0.3% of hydrogen peroxidase in water for 10 minutes. After treatment, they were boiled in citrate buffer solution (10 mmol/L, pH 6.0) using high-power microwave for 12 minutes for antigen retrieval. After cooling on ice for 5 minutes, sections were blocked with normal rabbit serum (DAKO, Glostrup, Denmark) diluted 1:10 in TBS for 5 minutes before incubating with primary mouse monoclonal antibodies anti-APE1 (Abcam, Cambridge, United Kingdom) at a dilution of 1:5,000, or anti-XRCC1 (Biocarta, San Diego, CA) at 1:100 for 1 hour at room temperature. Sections were then incubated with biotinylated rabbit anti-mouse antibody for 30 minutes followed by incubation with streptavidin-biotin horseradish peroxidase complex (DAKO) for a further 30 minutes. All sections were counterstained with hematoxylin for 15 seconds. Reactivity was visualized with 3,3′-diaminobenzidine as substrate, yielding a brown reactive product. Sections of breast carcinoma and tonsil were used as positive controls for APE1 and XRCC1, respectively. Negative controls were obtained by omitting the primary antibody in each case.

Quantification of APE1 and XRCC1 protein expression. Stained specimens were assessed using an Olympus BX50 microscope/camera system. Digital images of cells from the muscle-invasive tumor components were captured randomly (magnification, ×600). The number of cells with nuclei positive for APE1 and XRCC1 was determined by scoring 10 microscopic fields of 100 tumor cells each, without prior knowledge of clinical data or radiosensitivity. A total of 1,000 tumor nuclei were counted and the expression levels for APE1 and XRCC1 were determined as the percentage of positive cells. All measurements were done by the same operator (S.C.S). Five percent of sections were counted twice to assess the reproducibility of the measurements and variability in the quantification of protein expression was <2%.

Histopathology. H&E-stained sections were examined in detail for a range of morphologic features including presence of squamous or other tumor differentiation, tumor necrosis (absent and/or present), carcinoma *in situ* (absent and/or present), host response, defined as inflammatory cells in contact with tumor cells (absent and/or present), and vascular invasion (absent and/or present).

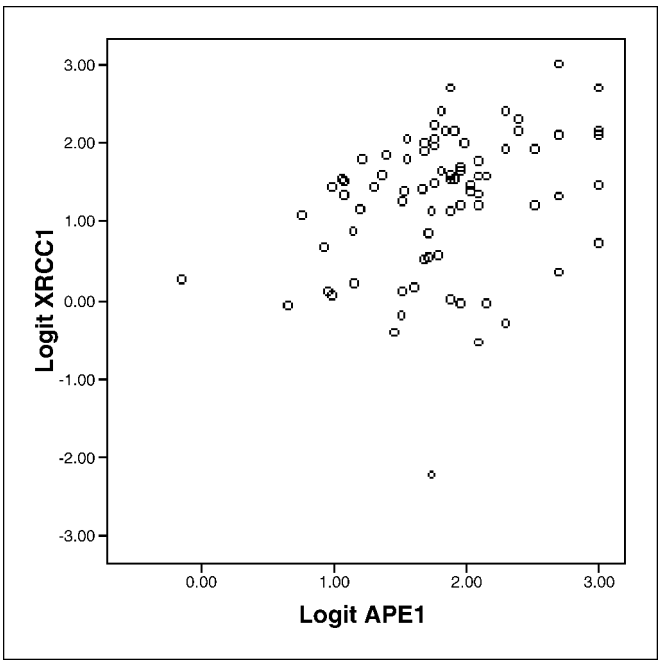

Fig. 1. Scatterplot for logit-transformed XRCC1 versus logit-transformed APE1 protein expression.

Statistical analysis. The statistical analysis was done using the Statistical Analysis System version 8 software. Data are presented as medians with 25% to 75% confidence intervals. Grouped data were compared using the Mann-Whitney *U* test for nonparametric distributions.

The outcome selected for this study was cancer-specific survival, defined as the time from the start of radiotherapy to the date of death from muscle-invasive TCC. The thresholds of APE1 and XRCC1 influencing cancer-specific survival were analyzed by plotting Kaplan-Meier curves. The survival probability distributions were compared using the log-rank test. Categorical variables influencing survival were compared using Cox proportional hazards regression.

Results

Association of APE1 and XRCC1 with clinical prognostic features. The APE1 protein showed extensive and strong nuclear expression in muscle-invasive TCC, with a median percentage of positive nuclear staining of 98.7% (range, 42.2-100%). The expression of XRCC1 was more variable, with a median percentage nuclear staining in invasive carcinoma of 96.5% (range, 0.6-99.9%). Logit transformation was done to normalize the distribution of APE1 and XRCC1 expression. We then assessed the correlation between APE1 and XRCC1 protein expression by plotting the logit-transformed APE1 and XRCC1 expression (Fig. 1). We found there was a highly significant correlation between APE1 and XRCC1 expression (Spearman ρ, $P = 0.002$).

The relationships between APE1 and XRCC1 protein expression and clinical prognostic features such as tumor stage, grade, and response to radiotherapy are presented in Table 1. The XRCC1 protein expression was significantly lower in tumors with high clinical T stage (i.e., T3/T4 compared with T2 tumors; $P = 0.008$). However, APE1 protein expression was not associated with tumor T stage. There was no relationship between the APE1 and XRCC1 protein expression and tumor grade and clinical response to radical radiotherapy at first-check cystoscopy.

Association of APE1 and XRCC1 with patient cancer-specific survivals. Using the Cox regression model, expression levels of each protein were significantly associated with cancer-specific survival ($P = 0.04$ for APE1 and 0.008 for XRCC1) such that tumors with higher APE1 and XRCC1 protein expression levels had improved cancer-specific survivals. A threshold of 95% was applied to both proteins to stratify expression into high (>95%) or low (≤95%). This threshold was chosen as in a subgroup of 39 cases initially studied this gave the best discrimination levels for both APE1 and XRCC1 markers. Cancer-specific survival

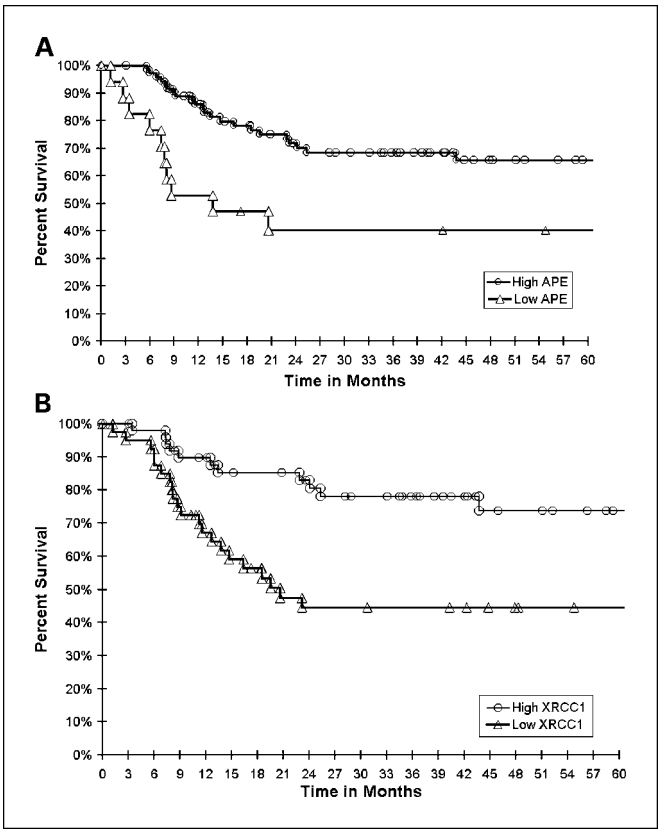

Fig. 2. Kaplan-Meier cancer-specific survival plot for muscle-invasive TCC patients treated by radical radiotherapy. *A*, high versus low APE1 expression; *B*, high versus low XRCC1 expression.

Table 1. Relationship between APE1 and XRCC1 protein expression and tumor stage, grade, and response at first-check cystoscopy

Factors	Median protein expression for APE1 as % (confidence interval, 25-75%)	Mann-Whitney *U* (*P*)	Median protein expression for XRCC1 as % (confidence interval, 25-75%)	Mann-Whitney *U* (*P*)
Stage				
T2	98.7 (97.6-99.5)	0.75	97.4 (94.3-99.1)	0.008
T3/T4	98.8 (96.7-99.8)		93.2 (94.9-97.8)	
Grade				
2	98.5 (96.7-99.8)	0.97	96.3 (69.0-97.5)	0.72
3	98.7 (97.3-99.6)		96.7 (80.5-98.9)	
Response at first-check cystoscopy				
CR/PR	98.7 (98.0-99.7)	0.13	96.7 (82.7-98.9)	0.20
PD/RP	98.0 (92.3-99.2)		91.7 (56.0-98.0)	

NOTE: Rapid progression describes those patients where cystoscopy was not done ($n = 11$, see text for details).
Abbreviations: CR/PR, complete response/partial response; PD/RP, progressive disease/rapid progression.

was plotted for both APE1 and XRCC1 using Kaplan-Meier survival curves (Fig. 2A and B). High expression levels of APE1 or XRCC1 were significantly associated with improved cancer-specific survival compared with low expression (log-rank, P = 0.004 and 0.002, respectively). Because both proteins were associated with improved survival, we further stratified patients into four distinct groups depending on staining for APE1 and XRCC1 using the same thresholds: high APE1 and high XRCC1 (n = 45), high APE1 and low XRCC1 (n = 28), low APE1 and high XRCC1 (n = 5), and low APE1 and low XRCC1 (n = 12). High expression for both proteins was associated with improved survival compared with remaining groups (Fig. 3A). Therefore, we combined the remaining groups together and repeated the Kaplan-Meier analysis (Fig. 3B). The combined approach resulted in improved prediction of patient outcome with high expression levels of both proteins being associated with better cancer-specific survival (log-rank, $P < 0.001$).

The clinical response to radical radiotherapy at first-check cystoscopy was also assessed in the combined model for APE1 and XRCC1 by comparing tumors with high APE1 and high XRCC1 with the remaining groups. Tumors with high APE1 and high XRCC1 expression levels had a relatively favorable response to radiotherapy compared with the three remaining groups (χ^2, P = 0.04; Table 2).

Multivariate analysis of clinical variables, tumor morphology, and APE1 and XRCC1 expression levels. Clinical variables and pathologic factors were also assessed for their relationship with cancer-specific survival in univariate and multivariate regression analysis (Table 3). In univariate analysis, the absence of hydronephrosis, absence of tumor necrosis, the presence of a host response, and high APE1 and XRCC1 expression were significantly associated with favorable patient outcome. In a multivariate analysis (model one) including all significant factors (hydronephrosis, necrosis, host response, APE1 expression levels, and XRCC1 expression levels), only hydronephrosis, high APE1 expression, and high XRCC1 expression were marginally associated with improved patient survival (P = 0.06, 0.06, and 0.054, respectively). When combined APE1/XRCC1 levels were used instead of single APE1 and XRCC1 expression levels in the second multivariate analysis, combined APE1 and XRCC1 levels proved to be highly statistically significant (P = 0.005) along with hydronephrosis (P = 0.04) as independent predictors for patient cancer-specific survival.

Table 2. Relationship between combined APE1 and XRCC1 protein expression and response at first-check cystoscopy

Factors	High APE1 and XRCC1	Other	*P*
Response at first-check cystoscopy			
CR/PR (n = 70)	39	31	0.04
PD/RP (n = 20)	6	14	

Abbreviations: CR/PR, complete response/partial response; PD/RP, progressive disease/rapid progression.

Discussion

As base damage and SSBs are the commonest forms of ionizing radiation–induced DNA damage, we selected two interacting BER enzymes, APE1 and XRCC1, to investigate the potential role of DNA repair enzymes in determining response to radiotherapy as, to our knowledge, there have been no previous studies in cohorts of patients with muscle-invasive TCC. We have shown that high levels of APE1 and XRCC1 protein expression were associated with improved cancer-specific survival in these patients. These findings seem to contradict our working hypothesis that high levels of expression of DNA repair proteins would be associated with radioresistance and hence poorer survival, as shown previously (9–12). They also seem to contradict the findings of Hu et al. (14) whose work suggested that single nucleotide polymorphisms in APE1 and XRCC1 may contribute to ionizing radiation hypersensitivity.

One possible explanation is that the reduced expression of XRCC1 and APE1 merely reflects the poorly differentiated nature of tumor cells in more aggressive tumors (15). Cells from aggressive tumors with extensive genomic instability (16) could contain chromosomal aberrations resulting in failure of transcription of genes, including DNA repair genes, thus resulting in lower protein expression of the gene products. Against this argument is the observation[4] that APE levels (particularly cytoplasmic levels) are higher in higher-grade

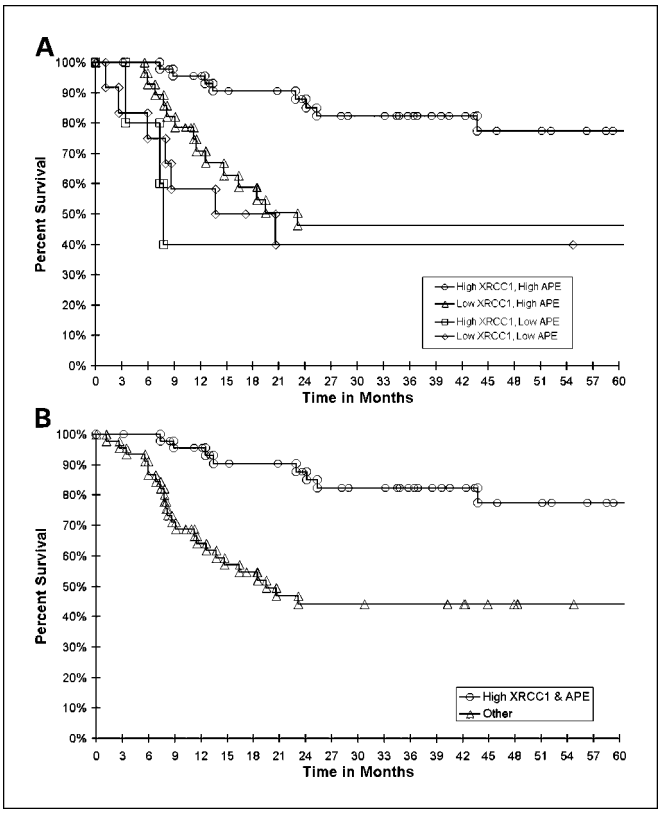

Fig. 3. Kaplan-Meier cancer-specific survival plot for muscle-invasive TCC patients treated by radical radiotherapy. *A*, patients stratified by combined APE1 and XRCC1 expression. *B*, survival advantage of high APE1 and XRCC1 expression versus other groups combined (P = 0.0003).

[4] M.R. Kelley, personal communication.

Table 3. Univariate and multivariate regression analysis of clinical and molecular variables predicting cancer-specific survival in 90 patients with muscle-invasive TCC treated with radical radiotherapy

Categories	Variable (*n*)	Univariate model		Multivariate model one		Multivariate model two	
		P	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)
Clinical	Age (y)						
	<75 (45)		1.02 (0.52-2.00)				
	≥75 (45)	0.94					
	Tumor stage						
	T2 (46)		1.51 (0.76-2.99)				
	T3/T4 (44)	0.24					
	Tumor grade						
	2 (20)		1.21 (0.53-2.77)				
	3 (70)	0.66					
	Hydronephrosis						
	Absent (70)		4.10 (1.94-8.65)	0.06	2.31 (0.97-5.50)	0.04	2.36 (1.05-5.30)
	Present (14)	0.0001					
	NA (6)						
	Dose (Gy)						
	50 (16)						
	52 (25)	0.86	0.92 (0.36-2.37)				
	55 (49)	0.29	0.62 (0.25-1.51)				
	Volume (mL)						
	<800 (45)		1.47 (0.74-2.92)				
	≥800 (44)	0.27					
	NA (1)						
	Salvage cystectomy						
	Yes (10)		1.62 (0.49-5.30)				
	No (80)	0.43					
Tumor morphologies	Tumor differentiation						
	Pure TCC (24)						
	Sq elements (62)	0.83	1.09 (0.48-2.44)				
	Other diff (4)	0.12	2.87 (0.76-10.87)				
	Tumor necrosis						
	Absent (43)		2.28 (1.13-4.62)	0.09	1.96 (0.90-4.31)	0.11	1.85 (0.87-3.93)
	Present (43)	0.02					
	ND (4)						
	CIS						
	Absent (31)		0.96 (0.44-2.12)				
	Present (26)	0.93					
	ND (33)						
	Host response						
	Absent (19)		0.45 (0.22-0.93)	0.39	1.32 (0.70-2.48)	0.08	0.52 (0.25-1.09)
	Present (70)	0.03					
	ND (1)						
	Vascular invasion						
	Absent (40)		1.28 (0.64-2.57)				
	Present (47)	0.48					
	ND (3)						
Molecular markers	APE1						
	Low (17)		0.36 (0.17-0.74)	0.06	0.47 (0.21-1.03)		
	High (73)	0.004					

(*Continued on the following page*)

Table 3. Univariate and multivariate regression analysis of clinical and molecular variables predicting cancer-specific survival in 90 patients with muscle-invasive TCC treated with radical radiotherapy (Cont'd)

Categories	Variable (*n*)	Univariate model		Multivariate model one		Multivariate model two	
		P	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)
	XRCC1						
	Low (40)		0.37 (0.18-0.77)	0.054	0.45 (0.20-1.02)		
	High (50)	0.002					
	Combined APE1 and XRCC1 model						
	All other (45)		0.27 (0.13-0.58)			0.005	0.32 (0.15-0.72)
	High/High (45)	0.0003					

Abbreviations: NA, not assessable; ND, not determined (due to lack of appropriate tumor tissue in paraffin sections); Sq elements, squamous elements; other diff, other differentiation (see text for details); CIS, carcinoma *in situ*.

tumors. Although we found no correlation between APE1 and XRCC1 and tumor grade, this may be because most (78%) of the tumors in this study were grade 3, as graded on their worst component. This would be resolved by investigating the relationship between protein expression and outcomes in patients treated by other modalities such as cystectomy, to determine whether APE1 and XRCC1 expression is a general prognostic factor reflecting the aggressiveness of the tumor or a predictive factor specific to radiotherapy. This work is under way.

In addition, APE1 is an important reduction-oxidation activator of the DNA binding of various transcription factors, such that increased APE1 could lead to an increase in the reduction-oxidation reduction of downstream stress response factors like activator protein, nuclear factor-κB, and p53 and hence a greater effect of the radiotherapy (17).

An alternative explanation involves the DNA repair mechanisms which are activated following DNA damage caused by radiotherapy. Ionizing radiation induces various forms of DNA damage. The most common are base damage (2,000-3,000 per cell per gray) and SSBs (1,000 per cell per gray) followed by DSBs (40 per cell per gray) and interstrand cross-links. Different forms of DNA damage require different repair mechanisms (e.g., BER for base damage and SSB and homologous recombination and nonhomologous end joining for DSB; ref. 18). Although radiation produces more base lesions and SSB, DSB are most lethal to the cell. The process of repair of two damaged bases or a damaged base and SSB that are close together on opposite DNA strands can result in formation of a new DSB due to cleavage of both backbones (19). Thus, repair of base damage by BER can actually increase the number of DSB produced following treatment with radiotherapy. This finding is supported by studies showing that when irradiated cells are allowed time to repair, the number of DSB actually increases (20–22). Hence, repairing base lesions and SSB can potentially convert nonlethal lesions into lethal DSB. Thus, deficiency in BER may actually enhance cell survival following treatment with ionizing radiation as fewer DSB are produced. Furthermore, Blaisdell and Wallace (23) have shown that bacteria deficient in DNA glycosylase (a BER enzyme) are more resistant to radiation than repair-competent bacteria.

APE1 initiates the repair of abasic sites generated during the repair of damaged bases by catalyzing the hydrolic cleavage of the phosphodiester bone immediately 5′ to the AP site thereby creating a SSB. XRCC1 physically interacts with APE1 and stimulates its enzymatic activity (24). Therefore, low levels of the BER proteins APE1 and XRCC1 may reflect poorer BER and hence reduced conversion of nonlethal damage into lethal DSB. This may explain why tumors with low APE1 and XRCC1 protein expression are resistant to radiotherapy thus resulting in poorer patient survival. Horima et al. (8), Wilson et al. (9), and Kumora et al. (10) investigated the DSB repair protein Ku and found that higher protein levels were associated with decreased radiosensitivity, as more DSB were repaired, which would be consistent with our findings in BER proteins.

Tumor stage, grade, and the presence of hydronephrosis are currently the standard clinical predictors of outcome in muscle-invasive bladder cancer (6, 25) but are not used in the selection of patients for different treatment modalities. Our results are very similar to the recent Edinburgh series using the same radiotherapy technique (26), and the only clinical variable that predicted for outcome was the presence of hydronephrosis. However, although hydronephrosis was a strong predictor of poor outcome, hydronephrosis is uncommon (14% of evaluable patients in our series) and it therefore of limited value. There is therefore a need for morphologic or molecular markers applicable to all patients to predict individual outcomes.

Quilty et al. (27) showed in a large series of patients treated with radiotherapy in the 1970s that patients with poorly differentiated tumors (grade 3) responded better to radiotherapy than those with well-differentiated tumors (grade 1). This is in contrast to results of cystectomy series (28) and in theory could be exploitable in selecting patients for radiotherapy or chemoradiotherapy versus surgery. However, as seen in our series, the vast majority of patients with muscle-invasive bladder cancers have grade 3 tumors; thus, the prognostic value of grade 1 may merely reflect its preponderance in superficial tumors. Our in-depth study of morphology on H&E-stained sections showed that tumor necrosis and host response were significant prognostic variables on univariate but not on multivariate analysis. There is therefore a need for additional independent markers of prognosis following radiotherapy.

Immunohistochemistry is available in most diagnostic pathology laboratories and prognostic markers such as those

described here could be included at marginal costs, although some additional time is required for cell counting (~30 minutes per case). Our results require prospective validation but seem to provide levels of discrimination similar to previous immunohistochemical studies, although the directions of results in such studies have been contradictory. Qureshi et al. (29) found that cancer-specific survival at 3 years after radiotherapy was ~76% for patients with positive staining for both p21 and p53 (16 of 68 patients, 24%) versus 32% if one or both markers were negative. This contrasts with the poorer outcome associated with positive staining for these two markers in 82 muscle-invasive bladder carcinomas treated with neoadjuvant chemotherapy and either radiotherapy or cystectomy (30). That is, at 36 months, the disease-free survivals were ~69% for either marker negative versus 16% for both markers positive (17 of 77 patients, 22%). Our combined APE1 and XRCC1 expression splits the cohort exactly in half, to predict 82% versus 44% 3-year cause-specific survivals. Our test selected a larger proportion of patients who had better outcome (50% of patients) following radical radiotherapy compared with the studies of Qureshi et al. (29) and Garcia del Muro et al. (ref. 30; 24% and 22% of patients, respectively). With the ability to predict outcome in half of the cohort, if validated, our test could potentially be very useful in patient counseling and selection for radiotherapy.

Conclusions

High protein expression levels of both APE1 and XRCC1 were associated with improved patient survival in muscle-invasive bladder cancer following radical radiotherapy, and a combined score selected the 50% of patients with an 82% 3-year cause-specific survival following radiotherapy. If validated in further studies, immunohistochemical staining for these proteins could be added to the routine patient work-up in muscle-invasive bladder cancer and would inform patient choice regarding bladder-sparing treatment versus cystectomy.

Acknowledgments

We thank Ailsa Rose and Filomena Esteves for their expert technical assistance and Dr. M.R. Kelley for helpful discussions.

References

1. Office of National Statistics. Cancer Statistics registrations 2000 series MB1, no. 31. London: Office of National Statistics; 2000.
2. Chester JD, Hall GD, Forster M, Protheroe AS. Systemic chemotherapy for patients with bladder cancer: current controversies and future directions. Cancer Treat Rev 2004;30:343–58.
3. Michaelson MD, Shipley WU, Heney NM, Zietman AL, Kaufman DS. Selective bladder preservation for muscle-invasive transitional cell carcinoma of the urinary bladder. Br J Cancer 2004;90:578–81.
4. Rodel C, Grabenbauser G, Kuhn R, et al. Combined-modality treatment and selective organ preservation in invasive bladder cancer: long-term results. J Clin Oncol 2002;20:3061–71.
5. Greven KM, Solin LJ, Hanks GE. Prognostic factors in patients with bladder carcinoma treated with definitive irradiation. Cancer 1990;65:908–12.
6. Shipley WU, Kaufman DS, Zehr E, et al. Selective bladder preservation by combined modality protocol treatment: long-term outcomes of 190 patients with invasive bladder cancer. Urology 2002;60:62–7; discussion 67–8.
7. Ward J, Evans J, Limoli C, Calabro-Jones P. Radiation and hydrogen peroxide induced free radical damage to DNA. Cancer 1987;55:105–12.
8. Harima Y, Sawada S, Miyazaki Y, et al. Expression of Ku80 in cervical cancer correlates with response to radiotherapy and survival. Am J Clin Oncol 2003;26:e80–5.
9. Wilson CR, Davidson SE, Margison GP, Jackson SP, Hendry JH, West CM. Expression of Ku70 correlates with survival in carcinoma of the cervix. Br J Cancer 2000;83:1702–6.
10. Komuro Y, Watanabe T, Hosoi Y, et al. Prediction of tumor radiosensitivity in rectal carcinoma based on p53 and Ku70 expression. J Exp Clin Cancer Res 2003;22:223–8.
11. Robertson KA, Bullock HA, Xu Y, et al. Altered expression of Ape1/ref-1 in germ cell tumors and overexpression in NT2 cells confers resistance to bleomycin and radiation. Cancer Res 2001;61:2220–5.
12. Herring CJ, West CM, Wilks DP, et al. Levels of the DNA repair enzyme human apurinic/apyrimidinic endonuclease (APE1, APEX, Ref-1) are associated with the intrinsic radiosensitivity of cervical cancers. Br J Cancer 1998;78:1128–33.
13. Crogorac-Jurcevic T, Efthumiou E, Neilsen T, et al. Expression profiling of microdissected pancreatic adenocarcinomas. Oncogene 2002;21:4587–94.
14. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001;22:917–22.
15. Kruger S, Thorns C, Bohle A, Feller AC. Prognostic significance of a grading system considering tumor heterogeneity in muscle-invasive urothelial carcinoma of the urinary bladder. Int Urol Nephrol 2003;35:169–73.
16. Rockwell S, Yuan J, Peretz S, Glazer PM. Genomic instability in cancer. Novartis Found Symp 2001;240:133–42; discussion 142–51.
17. Evans AR, Limp-Foster M, Kelly MR. Going APE over ref-1. Mutat Res 2000;461:83–108.
18. Lindahl T. Molecular biology: ensuring error-free DNA repair. Nature 2004;427:598.
19. Ward J. DNA damage produced by ionising radiation in mammalian cells: identities, mechanisms of formation, and repairability. Prog Nucleic Acid Res Mol Biol 1988;35:95–125.
20. Bonura T, Smith K, Kaplan H. Enzymatic induction of DNA double-strand breaks in γ-irradiated mammalian cells. Proc Natl Acad Sci U S A 1975;72:4265–9.
21. Dugle D, Gilliespie C, Chapman J. DNA strand breaks, repair, and survival in X-irradiated mammalian cells. Proc Natl Acad Sci U S A 1976;73:809–12.
22. Ahnstrom G, Bryant P. DNA double-strand breaks generated by the repair of X-ray damage in Chinese hamster cells. Int J Radiat Biol 1982;41:671–6.
23. Blaisdell J, Wallace S. Abortive base-excision repair of radiation-induced clustered DNA lesions in *Escherichia coli*. Proc Natl Acad Sci U S A 2001;98:7426–30.
24. Vidal AE, Boiteaux S, Hickson ID, et al. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. EMBO 2001;20:6530–9.
25. Stein JP, Lieskovsky G, Cote R, et al. Radical cystectomy in the treatment of invasive bladder cancer: long term results in 1,054 patients. J Clin Oncol 2001;19:666–75.
26. Borgaonkar S, Jain A, Bollina P, et al. Radical radiotherapy and salvage cystectomy as the primary management of transitional cell carcinoma of the bladder. Results following the introduction of a CT planning technique. Clin Oncol (R Coll Radiol) 2002;14:141–7.
27. Quilty PM, Kerr GR, Duncan W. Prognostic indices for bladder cancer: an analysis of patients with transitional cell carcinoma of the bladder primarily treated by radical megavoltage X-ray therapy. Radiother Oncol 1986;7:311–21.
28. Ghoneim MA, el-Mekresh MM, el-Baz MA, el-Attar IA, Ashamallah A. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. J Urol 1997;158:393–9.
29. Qureshi KN, Griffiths TR, Robinson MC, et al. Combined p21WAF1/CIP1 and p53 overexpression predict improved survival in muscle-invasive bladder cancer treated by radical radiotherapy. Int J Radiat Oncol Biol Phys 2001;51:1234–40.
30. Garcia del Muro X, Condom E, Vigues F, et al. p53 and p21 Expression levels predict organ preservation and survival in invasive bladder carcinoma treated with a combined-modality approach. Cancer 2004;100:1859–67.

Clinical Cancer Research

APE1 and XRCC1 Protein Expression Levels Predict Cancer-Specific Survival Following Radical Radiotherapy in Bladder Cancer

Sei C. Sak, Patricia Harnden, Colin F. Johnston, et al.

Clin Cancer Res 2005;11:6205-6211.