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Expression of Nuclear Transcription Factor Kappa B in Locally Advanced Human Cervical Cancer Treated With Definitive Chemoradiation

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Abstract

Purpose—Nuclear factor kappa-B (NF- κ B), a transcriptional factor that has been shown to be constitutively active in cervical cancer, is part of an important pathway leading to treatment resistance in many tumor types. The purpose of our study was to determine whether expression of NF- κ B in pre-treatment specimens and specimens taken shortly after treatment initiation correlated with outcome in cervical cancer patients treated with definitive chemoradiation.

Experimental Design—Eighteen patients with locally advanced cervical cancer were enrolled on a study in which cervical biopsies were obtained before radiation therapy and 48 hours after treatment initiation. Matched biopsies from 16 of these patients were available and evaluated for the nuclear expression of NF- κ B protein using immunohistochemical staining.

Results—After median follow-up of 43 months, there were 9 total treatment failures. Nuclear staining for NF- κ B was positive in 3 of 16 (19%) pre-treatment biopsies and 5 of 16 (31%) post-radiation biopsies. Pre-treatment expression of NF- κ B nuclear staining correlated with increased rates of local-regional failure (100% vs. 23%, $p = .01$), distant failure (100% vs. 38%, $p = .055$), disease-specific mortality (100% vs. 31%, $p = .03$) and overall mortality (100% vs. 38%, $p = .055$). Best rates of local-regional failure (0%) and highest rates of overall survival (80%) were seen in the 5 patients where the expression of NF- κ B was negative in pre-treatment samples but changed to positive in the samples obtained 48 hours after treatment initiation.

Conclusion—Our data suggest that pre-treatment nuclear expression of NF- κ B may be associated with a poor outcome for cervical cancer patients treated with chemoradiation. Although

these data require validation in a larger group of patients, the results support the continued study of the relationship between NF- κ B and outcome in patients treated for carcinoma of the cervix.

Keywords

radiation; chemotherapy; cervical cancer; nuclear transcription factor kappa b

Introduction

Cervical cancer continues to be a major cause of morbidity and mortality worldwide, with an estimated incidence of over 11,000 new cases in the United States alone(1). Most patients with locally-advanced disease (IB2—IVA) achieve a durable response to definitive concurrent cisplatin-based chemoradiation therapy (2–4), however a majority of those who recur have a poor prognosis despite improving salvage therapies (5). Being able to predict which patients are likely to recur after standard therapy through tumor molecular profiling and biomarker studies is likely to improve overall outcome in these patients by helping to select those who may benefit from individualized targeted therapies.

NF- κ B is a transcription factor whose role in the pathogenesis of a wide variety of tumor types including cervical cancer has been well described (6–8). Cumulative evidence suggests that this protein functions as a mediator of cellular survival, inflammation, angiogenesis, and treatment resistance through regulating the transcription of over 200 target genes. NF- κ B has recently become a major target for cancer drug development as demonstrated by the many non-specific natural and synthetic compounds shown to exert their therapeutic effects at least in part through the inhibition of NF- κ B (9, 10). Furthermore recent pre-clinical and clinical studies that combine these agents with radiation and chemotherapy have shown promising results (9–11).

The role of NF- κ B as a potential prognostic biomarker has only recently been explored. In several cancers including breast, prostate, skin, lung, and pancreas, NF- κ B nuclear expression has correlated with poor clinical outcome (12–16). To date, no studies have evaluated the clinical prognostic significance of NF- κ B nuclear expression in human cervical cancer.

Our goal in the current study was to evaluate the predictive potential of NF- κ B through immunohistochemistry evaluation of prospectively collected paired cervical tumor biopsy samples obtained before and shortly after the initiation of definitive chemoradiation in patients with locally advanced cervical cancer.

Methods

This study was conducted as part of a prospective pilot study that aimed to evaluate gene expression profiling and potential molecular biomarkers in patients with locally advanced cervical cancer who were treated with definitive chemoradiation. Data from the gene expression profiling investigations have been published recently (17). The protocol was approved by the institutional review board at M. D. Anderson Cancer Center and all enrolled patients provided written informed consent to be a part of the protocol.

Patients with FIGO stage IB2 or IIA (with tumors > 4cm) and stage IIB or IIIB squamous cell carcinoma of the cervix were included. Patients with any previous treatment for cervical cancer were excluded. Treatment consisted of radiation therapy to a dose of 45 Gy to the pelvis in 180 cGy fractions using AP/PA fields and 15- or 18-MV photons, delivered concurrently with weekly cisplatin chemotherapy (usually 40 mg/m² with or without 5-fluorouracil). Patients with suspicious para-aortic lymph nodes on CT were biopsied and if positive, patients received extended field radiotherapy to encompass the para-aortic lymph nodes. No patients underwent para-aortic lymphadenectomy. This was followed by 2 low-dose rate intracavitary brachytherapy treatments (after-loading tandem and ovoid system) spaced 2 weeks apart. During the second intracavitary treatment, the final (usually the sixth) chemotherapy dose was given.

For the purpose of this study, biopsies of the cervical cancer were performed before and after approximately 48 hours from the start of treatment. At the time of the second biopsy, all patients had received 2 or 3 external-beam treatments and the first dose of chemotherapy.

Immunohistochemistry

Slides for immunohistochemistry staining were made from four-nm tissue sections taken from paraffin blocks that were mounted on charged slides. Slides were then deparaffinized and rehydrated with descending grades of ethyl alcohol. Immunohistochemistry staining was performed using the NF- κ B (Santa Cruz cat # sc109, diluted 1:100) antibody. All biopsy specimens taken before treatment and 48 hours after treatment initiation were analyzed.

A gynecologic pathologist (R.B.), who was blinded to treatment outcomes, scored the specimens according to the intensity of nuclear staining as compared to cytoplasmic staining (absent [0%], weak [< 20%], moderate [>20% < 50%], and strong [>50%]). This method is consistent with previously published reports that categorize NF- κ B staining according to percentage of positive staining (18–20). Positive and negative controls were used for each set of stained slides. Cases with absent or weak nuclear staining were labeled negative and those with moderate or strong staining were considered positive. Figure 1 shows an example of positive nuclear staining (a) and negative nuclear staining (b).

Statistical Analyses

A Chi-square or Fisher's exact test was used to analyze the relationships of nuclear expression of NF- κ B in the pre-treatment and post-treatment groups with clinical outcomes. All statistical analyses were performed using SPSS software version 12. Local-regional failure was defined as central, pelvic, or para-aortic recurrence.

Results

Clinical characteristics of patients in study

A total of 18 patients with cervical cancer were enrolled in the study and treated with chemoradiation between April 2001 and August 2002. Tumor specimens were inadequate in 2 patients therefore the analysis included 16 patients. The clinical characteristics and outcomes of these 16 patients are presented in Table 1. At a median follow-up of 43 months

(range 9–79 months), 9 of 16 patients (56%) had experienced either local or distant recurrence. Of these 9 patients with recurrent disease, 3 had pelvic recurrences; 2 with an isolated central recurrence and 1 with an isolated pelvic nodal recurrence. Four patients developed para-aortic recurrences outside their radiation fields (1 of whom developed central failure first) and 3 patients' recurrences were distant only. Six of the 7 patients with pelvic or para-aortic recurrences developed subsequent distant metastases. Patients with CT evidence of pelvic lymphadenopathy at presentation were more likely to develop subsequent local or distant failure than patients with negative initial pelvic scans (88% versus 25%, $p = 0.01$). Median time to first failure was 21 months (range 3–38 months). By last follow-up, 8 out of 16 patients (50%) were alive including 2 patients with disease (13%). Seven of the remaining 8 patients died of their disease at a median of 34 months after diagnosis.

Eight patients (50%) received concurrent weekly cisplatin and 8 (50%) received cisplatin/5-FU. Four patients (all who received 5-FU along with cisplatin) received less than the prescribed dose of chemotherapy due to toxicity (3 patients received only 3 cycles and 1 patient received 5 cycles with a 50% dose reduction). The median primary tumor size was 6.3 cm (range 3–8 cm). The type of chemotherapy regimen and tumor size did not correlate with any clinical endpoint. The median dose to Point A was 88.5 Gy (range 84.1–96.1 Gy).

Correlation of NF- κ B expression with clinical outcome

In the pre-treatment biopsy specimens, nuclear staining for NF- κ B was negative in 13 patients (81%) and positive in 3 patients (19%). In the post-treatment specimens, 11 (69%) were considered negative and 5 (31%) were positive. Selected characteristics as they relate to NF- κ B staining are presented in Table 2. In the pre-treatment specimens, NF- κ B positivity correlated with increased rates of distant metastases (100% vs. 31%, $p = .03$), local-regional-failure (100% vs. 23%, $p = .02$), and overall relapse (local and distant, 100% vs. 38%, $p = .055$). In the post-treatment specimens, local-regional failure occurred more often in patients with negative nuclear staining for NF- κ B (45% vs. 0%, $p = .07$). Neither pre-treatment nor post-treatment expression correlated with other variables including lymph node status at presentation or stage.

When comparing pre-treatment and post-treatment specimens, 5 patients (31%) had a change to positive nuclear staining after initial negative staining, 3 patients (19%) had a change to negative nuclear staining after an initial positive nuclear stain, and 8 patients (50%) had no change in nuclear staining category. Changes in these expression patterns as they correlate with clinical outcomes are presented in Table 3. In the 5 patients who had a change to positive nuclear staining, there was a significantly lower rate of local-regional failure (0%) than in those without a change in staining (38%) or in those who had a change to negative nuclear staining (100%) ($p = .01$). Non-significant improvements in rates of distant metastases and relapse of any kind favored these 5 patients as well.

Discussion

To our knowledge this is the first report evaluating NF- κ B nuclear expression as a potential prognostic marker in locally advanced cervical cancer patients treated with definitive chemoradiation therapy. Our results suggest that pre-treatment expression of NF- κ B may

correlate with more aggressive tumor behavior as evidenced by significantly increased local-regional (100% vs. 23%) and distant failure rates (100% vs. 38%) in those with positive nuclear staining. Interestingly, the lowest rates of local and distant failure (0% and 20%, respectively) and highest rate of survival (100%) were seen in the subgroup of patients whose matched biopsy specimens demonstrated a change in NF- κ B expression from negative in the pre-treatment sample to positive in the specimen taken 48 hours after treatment initiation.

Finding molecular markers that are able to effectively predict treatment failure after chemoradiation will allow a more individualized approach to cervical cancer by selecting patients who may benefit from targeted therapies. NF- κ B is a necessary component for normal cellular response to stress and immunity, but abnormally elevated NF- κ B signaling in tumors has been shown to be an important contributor to cellular resistance to chemotherapy and radiation (6, 9, 10, 21). It does so in part through the transcription of target genes that lead to increased cellular survival (c-Myc, Cyclin D1), inhibition of apoptosis (cIAP, Bcl-2) and overall reduction in cell death in response to treatment. This is further evidenced by the potentiation of chemotherapy- and radiation-induced apoptosis and treatment effect by the targeted inhibition of NF- κ B (6, 9, 10, 22).

The aberrant constitutive activation of NF- κ B in cervical cancer has been shown by two independent reports (7, 8). One group used 106 paraffin-embedded cervical tissue specimens of different histologic grades to show that NF- κ B expression increased progressively with more invasive tumors (7). A subsequent report confirmed the progressive DNA-binding activity of NF- κ B with higher grade cervical lesions (8). Taken together, this data supports the hypothesis that NF- κ B may be a valuable target in cervical cancer as in other tumor types.

The role of NF- κ B as a potential prognostic biomarker in tumors is less clear. Only a handful of reports have demonstrated that NF- κ B expression correlates with poor clinical outcome in a variety of other tumor types. This has been postulated to be the result of enhanced NF- κ B-mediated transcription of genes related to tumor progression and treatment resistance. In a previous study of breast cancer specimens at our institution, the nuclear expression of NF- κ B as assessed by immunohistochemistry correlated with the expression of Bcl-2 and Bax, leading to the conclusion that the NF- κ B/Bcl-2 pathway may be associated with a poor response to neoadjuvant chemotherapy (12). In 42 prostate cancer patients with a positive margin after radical prostatectomy, NF- κ B nuclear expression predicted strongly for biochemical recurrence on multivariate analysis (13). Furthermore, the in vitro and in vivo study of melanoma demonstrated that NF- κ B nuclear expression inversely correlated with disease-specific survival and was an independent adverse prognostic factor (14). Finally, two recent reports in non-small cell lung cancer and pancreatic cancer have demonstrated that NF- κ B nuclear expression independently predicts for poor outcome (15, 16).

Results from the current study require validation in a larger sample size. While enhanced expression of NF- κ B in tumors prior to the start of chemoradiation may indicate aggressive tumor biology and greater resistance to treatment, our finding of significantly worse clinical

outcomes among those with increased nuclear expression of NF- κ B in pre-treatment samples were based on relatively few patients and total events. Furthermore, the technique of NF- κ B staining and the subsequent scoring of individual slides are subject to uncertainty. For example, the finding of worse outcomes in patients whose tumors changed from positive NF- κ B expression in pre-treatment specimens to negative expression in specimens taken 2 days after treatment initiation is somewhat counterintuitive (see table 3) given that the targeted inhibition of NF- κ B expression in cervical cancer (among other tumor types) leads to enhanced radiosensitivity and increased cancer cell death (23). A mechanism for this interesting finding may be related to the pulsatile and temporal nature of NF- κ B activation in response to stimuli such as radiation (24), the effects of which may be best illustrated by evaluating additional biopsies taken at defined intervals throughout the course of treatment. Finally, while our report did not report HPV status, future series evaluating the role of NF- κ B in cervical cancer would benefit from such correlation in light of recent data supporting a major role of NF- κ B in HPV-mediated carcinogenesis (25, 26).

In conclusion, our data suggests that the pre-treatment expression of NF- κ B may be associated with a poor outcome in locally advanced cervical cancer patients treated with definitive chemoradiation therapy and additional studies aimed at validating this finding in independent data sets are warranted. Furthermore, changes in nuclear expression of NF- κ B shortly after treatment initiation may hold prognostic value. Taken together, our results support the continued study of NF- κ B as a potential prognostic biomarker in cervical cancer.

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a.

b.

Figure 1.

a. Example of positive nuclear staining of NF- κ B.

b. Example of negative nuclear staining of NF- κ B.

Clinical characteristics and outcomes

Table 1

Patient	Age	Follow-up (mo)	FIGO stage	LN status ¹	Grade	Relapse ²	Survival	NF-κB Pre ³	NF-κB Post ⁴
1	47	59	IIIA	Pelvic	3	none	Alive	-	-
2	56	16	IIB	Pelvic + PA	2	distant	Alive	-	-
3	40	74	IB2	none	2	none	Alive	-	-
4	36	50	IIB	none	2	none	Dead	-	-
5	53	43	IB2	Pelvic		pelvic	Dead	-	-
6	54	56	IIB	none	2	distant	Alive	-	+
7	49	9	IIB	none	3	para-aortic	Dead	+	-
8	44	76	IIB	none	2	none	Alive	-	+
9	54	79	IIB	none		none	Alive	-	+
10	61	41	IIIB	Pelvic	3	central	Dead	+	-
11	66	34	IIIB	Pelvic + PA	2	distant	Dead	-	+
12	46	32	IIB	Pelvic	3	para-aortic	Dead	-	-
13	30	19	IB2	Pelvic	3	para-aortic	Dead	+	-
14	33	73	IB2	none	2	none	Alive	-	-
15	40	43	IIB	none		none	Alive	-	+
16	28	34	IB2	Pelvic	3	central	Dead	-	-

FIGO, International Federation of Gynecology and Obstetrics

¹CT evidence of pelvic and/or para-aortic (PA) lymph node involvement prior to chemoradiation²Site of first relapse³NF-κB expression before treatment initiation⁴NF-κB expression 2 days after treatment initiation

Table 2

NF-κB staining and clinical outcomes

Characteristic	Pre-treatment			Post-treatment initiation		
	NF-κB positive ¹	NF-κB negative ²	p-value	NF-κB positive ¹	NF-κB negative ²	p-value
Overall Survival						
Alive	0	8	.06	4	4	.11
Dead	3	5		1	7	
Disease-specific Survival						
Alive	0	9	.03	4	5	.20
Dead	3	4		1	6	
Distant Metastases						
Yes	3	5	.06	2	6	.59
No	0	8		3	5	
Local-regional Failure ³						
Yes	3	3	.01	0	6	.04
No	0	10		5	5	

¹ positive includes moderate or strong nuclear staining
² negative includes absent or weak nuclear staining
³ local-regional includes central, pelvic, and para-aortic relapses

Table 3

NF-κB staining pattern changes and clinical outcome

Characteristic	Change to positive ¹	Change to negative ²	No change ³	p-value
Survival				
Alive	4	0	4	.09
Dead	1	3	4	
Disease-specific Survival				
Alive	4	0	5	.08
Dead	1	3	3	
Distant Metastases				
Yes	2	3	3	.16
No	3	0	5	
Local-regional failure ⁴				
Yes	0	3	3	.02
No	5	0	5	

¹ negative in pre-radiation specimen and subsequently positive in specimen taken 48 hours after treatment initiation² positive in pre-radiation specimen and subsequently negative in specimen taken 48 hours after treatment initiation³ no change in positivity or negativity between the patient specimens⁴ local-regional includes central, pelvic, and para-aortic relapses