Pretherapy nuclear factor-κB status, chemoradiation resistance, and metastatic progression in esophageal carcinoma

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Abstract

Background: **Transcriptional factor nuclear factor-κB (NF-κB) seems to be associated with aggressive clinical biology (chemoradiation resistance and metastatic progression) of esophageal cancer. We hypothesized that activated NF-κB would define clinical biology irrespective of the type of chemotherapy or sequence administered.** ***Methods:*** **Pretherapy and/or posttherapy cancer specimens were examined for activated NF-κB and correlated with pathologic response to chemoradiation, metastatic potential, overall survival, disease-free survival, and type of chemotherapy or sequence used.** ***Findings:*** **Eighty patients undergoing chemotherapy and concurrent radiation were studied. Activated NF-κB prior to any therapy was associated with the lack of complete pathologic response (pathCR, $P = 0.006$). Forty-five (78%) of 58 patients achieving <pathCR had activated NF-κB in pretherapy and/or posttherapy cancer specimens versus 2 (9%) of 22 patients with pathCR ($P = 0.001$). Twenty-four (51%) of 47 patients with activated NF-κB in cancer developed metastases versus 7 (21%) of 22 patients with negative NF-κB in cancer ($P = 0.01$). At a median follow-up of 32 months, 25 (53%) of 47 patients with activated NF-κB cancer had died versus 3 (9%) of 33 patients with negative NF-κB cancer. NF-κB activation was the only independent predictor of disease-free survival ($P = 0.01$) and overall survival ($P = 0.007$) in a multivariate model. The class of chemotherapy or its sequence had no effect on NF-κB expression or patient outcome.** ***Conclusions:*** **Our data are the first to show that pretreatment-activated NF-κB significantly correlates with clinical biology of esophageal cancer, and most importantly, with pathCR. To therapeutically exploit NF-κB-regulated genes and their pathways, further research is warranted. [Mol Cancer Ther 2006;5(11):2844–50]**

Introduction

The prognosis of esophageal (or gastroesophageal junction) carcinoma remains extremely poor in spite of combined modality approaches, with a 5-year survival rate of <20% (1, 2). Preoperative therapy, particularly preoperative chemoradiation, is commonly recommended to operable patients with localized cancer, although its role remains controversial (3–6). There is also no consensus on a specific group of chemotherapy agents to be combined with radiation. The survival improvement is mostly observed in patients who have no residual cancer cells in the resected esophagus (pathologic complete response or pathCR) compared with patients with chemoradiation-resistant cancer (<pathCR; refs. 7–11). The fraction of patients with pathCR has remained ~25% irrespective of the type of chemoradiation or institutions (12). Currently, patients with stage II or III cancer are often treated with an empirically chosen strategy of preoperative chemoradiation, but the outcomes vary greatly and unpredictably. Moreover, this approach is associated with substantial morbidity and benefits only a few patients (11, 13, 14). Because clinical variables prior to therapy are unable to predict prognosis or help decide which component of combined modality would be effective, it is imperative that we focus on cancer biology and the patients' genetics to derive answers. There are no reliable molecular markers that define the clinical biology of esophageal cancer. The availability of such markers will propel us in the arena of individualization of therapy and we might be able to develop new strategies that might encompass the preservation of the esophagus and the discovery of new therapeutic targets.

Nuclear factor-κB (NF-κB) is a sequence-specific transcription factor that responds to multiple cellular signaling pathways through the transcriptional regulation of target genes involved in cell survival (15, 16). NF-κB is active in the nucleus and is maintained in an inactive state by

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sequestration in a cytoplasmic complex with IκBα. In physiologic conditions, NF-κB activation is a tightly regulated and rapid process, which is initiated by stimuli such as inflammatory cytokines, viruses, carcinogens, and DNA-damaging agents (16).

Aberrant (i.e., constitutive) NF-κB activation has been associated with inflammatory diseases and cancer (15). Through the activation of survival pathways, it suppresses apoptosis when cancer cells are exposed to radiotherapy or chemotherapy, thus contributing to resistance (17–24). Concomitantly, through the enhancement of migratory (e.g., Cox-2, CAM adhesion proteins), invasive (e.g., matrix metalloproteinases), and proangiogenic (e.g., VEGF and Cox-2) properties, NF-κB contributes to metastatic progression (15). Through gene expression profiling using an Affymetrix (Santa Clara, CA) platform in conjunction with Ingenuity Pathway Analysis on esophageal cancer (25), we found that multiple signaling pathways converging on NF-κB activation were significantly up-regulated in chemoradiation-resistant cancers. This led us to examine the frequency of activated NF-κB and its correlation with clinical biology (chemoradiation resistance and metastatic progression) in 43 patients treated with the same chemoradiation regimen (26). Activated NF-κB was associated with chemoradiation resistance and metastatic potential. We recognized two shortcomings of our previous experience: the small number of patients studied and the correlation between the status of NF-κB in the untreated samples and clinical outcome was not significant, meaning that it would not allow the development of novel strategies before therapy was initiated. To validate this hypothesis, we nearly doubled the number of patients in this analysis and included unselected patients who underwent preoperative chemoradiation.

Materials and Methods

Patient Selection and Evaluation

Operable patients with localized histologically confirmed adenocarcinoma or squamous cell carcinoma of the thoracic esophagus and gastroesophageal junction were eligible. Patients were evaluated by chest radiograph, chest and abdomen computerized tomography, upper gastrointestinal double-contrast barium radiographs, esophago-gastroduodenoscopy with endoscopic ultrasonography, and when available, a positron emission tomography. Patients with T_2 to T_3 with any N, patients with M_{1a} cancer, and patients with T_1N_1 carcinoma were considered eligible. All patients signed a written informed consent. Patients with T_1N_0, or T_4 lesions or with metastatic cancer were excluded.

Treatment

A mixed group of patients was analyzed. Fifty patients were treated on clinical trials previously described (27, 28); 30 of the patients analyzed were not on a clinical trial but were treated at the University of Texas M.D. Anderson Cancer Center.

Step 1: Induction Chemotherapy. Induction chemotherapy was administered to 53 patients. Of these, 42 were enrolled in a clinical trial combining docetaxel, irinotecan, and 5-fluorouracil (27), 8 patients were treated in a platinol-based induction therapy trial (28), and 3 patients were treated with a taxane-based combination. If there was no cancer progression observed, patients received a second cycle of induction chemotherapy.

Step 2: Preoperative Chemoradiotherapy. Patients received up to 50.4 Gy of radiotherapy in 28 fractions. The baseline chemotherapy for all patients was 5-fluorouracil but all patients received additional drugs; most commonly, a platinol, taxane, or camptothecin. Concurrent chemotherapy combination was given for 5 weeks (Monday–Friday) of the first 5 radiation weeks.

Step 3: Surgery. Five to 6 weeks after the completion of chemoradiation, a complete restaging was done. Patients proceeded to surgery if they had no distant metastatic cancer and were physiologically fit. A transthoracic (Ivor-Lewis or total three-field) or transhiatal approach was used, including mediastinal and celiac lymphadenectomy in all cases. Each resected specimen was examined in an elaborate manner and was re-verified by one experienced gastrointestinal pathologist, without knowledge of patient outcome. The pathologic response was determined in the resected esophagus and assigned to one of two categories: no residual carcinoma (pathCR) or the presence of cancer cells (<pathCR). Patients having any residual cancer in the resected specimen were considered chemoradiation-resistant for the purpose of this analysis.

Patient Follow-up. Patients were assessed at 3, 6, 9, and 12 months, then every 6 months for 2 years, then every year or until death. Local-regional recurrence was defined as recurrence within the surgical field or mediastinal nodes. Metastatic cancer was defined as evidence of cancer outside the regional area, or death from unknown causes within 3 years of study.

Tissue Specimens

All tissue specimens were obtained through an approved protocol by the M.D. Anderson Institutional Review Board and after informed consent from patients. All tissue sections were matched to routine H&E stained slides used to evaluate for the presence of cancer by one pathologist (T-T. Wu). Cancer tissue specimens from 80 patients were analyzed in this study. Seventy-five pretreatment cancer biopsies (pretreatment unstained cancer specimens were unavailable in five patients), and 56 posttreatment cancer specimens were included in the analysis.

Immunohistochemistry and Protein Expression

Immunohistochemical staining for activated NF-κB was done on 4 μm formalin-fixed sections with the G96-337 monoclonal antibody (2 mg/mL; BD PharMingen, Palo Alto, CA; ref. 26). Staining procedure, positive and negative controls were previously described. Only nuclear immunoreactivity was considered positive for NF-κB. The intensity of NF-κB nuclear staining was evaluated on a three-point semiquantitative scale as follows: 0, no staining; 1, weak to moderate; 2, strong staining. The extent of cancer cells with positive NF-κB was expressed as the fraction of labeled cells (i.e., staining levels 1 and 2) in

Table 1. Clinical and histopathologic characteristics of 80 patients with esophageal carcinoma

Characteristics	No. of patients (%), $N = 80$
Gender	
Male	72 (90)
Female	8 (10)
Median age (years ± SD, range)	59 ± 9.44 (35–76)
Histologic type	
Adenocarcinoma	74 (92.5)
Squamous cell carcinoma	6 (7.5)
Primary sites	
Middle	9 (11.2)
Distal	35 (43.8)
Gastroesophageal junction	36 (45.0)
Pretreatment endoscopy ultrasound stage	
T stage	
T_2	10 (12.5)
T_3	70 (87.5)
N stage	
N_0	27 (33.7)
N_1	53 (66.3)
M stage	
M_0	77 (96.3)
M_1	3 (3.7)
Pretreatment clinical stage	
IIA	27 (33.8)
IIB	4 (5)
III	46 (57.5)
IVA	3 (3.7)

the cancer fields. Cases showing labeling index of ≥5% were regarded as positive for the purpose of the analysis. This cutoff was based on the NF-κB median +2 SD nuclear labeling index value of all pretreatment cancer specimens. All cancer fields present in the tissue sections were analyzed for NF-κB positivity. All 75 preoperative biopsies were evaluable for NF-κB expression and were classified as nuclear NF-κB-positive or -negative. Three investigators (J. Izzo, U. Malhotra, and T-T. Wu) independently determined NF-κB positivity. In three discrepant specimens (one pretreatment biopsy and two surgical resections) a final opinion was made based on consensus by all three investigators after double blind recounting. The scoring of NF-κB nuclear labeling indices had minimal variability between the three investigators, ranging between 0.13% and 0.68%.

Statistical Methods

Fisher's exact test, and Wilcoxon rank sum test were done to determine associations between categorical variables, such as NF-κB protein, clinicopathologic variables, and clinical outcome.

Survival analyses were done for overall survival (OS) and disease-free survival (DFS) time. OS was defined as the time from registration into the trial to death. When the date of death was not available, then the last follow-up date was used instead. Data from patients that had not died by the time of analysis were censored. DFS was defined as the time from surgical resection to disease recurrence, or until the last follow-up date if the data of disease recurrence or death was not available. Data from patients that were alive without disease at the time of analysis were censored. An association between NF-κB and OS or DFS was tested by comparing the Kaplan-Meier survival curves with log-rank tests used to test differences in survival distribution. After stepwise selection to determine which covariate was a significant predictor of DFS and OS, multivariate Cox proportional hazards models were fit, yielding hazard ratio estimates for NF-κB, pathologic response, age, and postoperative N status. All statistical analyses were two-sided and done at a 0.05 significance level. The SAS software package 6.12 was used for computations (SAS Institute, Inc., Cary, NC).

Results

Patient Characteristics

Table 1 illustrates the patient characteristics. The median age of 80 patients was 59 years (range, 35–76). Most

Table 2. Preoperative regimen and NF-κB expression

Preoperative regimen	NF-κB pretreatment*		NF-κB pretreatment or posttreatment†		NF-κB preconversion → postconversion‡	P§
	Positive	Negative	Positive	Negative	Conversion	
Chemotherapy alone ($n = 1$)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	
Concomitant chemoradiation ($n = 26$)	10 (40.0)	15 (60.0)	15 (57.7)	11 (42.3)	5 (19.2)	1.0
Neoadjuvant chemotherapy followed by chemoradiation ($n = 53$)	19 (38.7)	30 (61.2)	31 (58.5)	22 (41.5)	10 (18.8)	

NOTE: Values in parentheses represent percentages of the available cases.
*Analysis of 75 available pretreatment tissue specimens.
†Analysis of 80 pretreatment or posttreatment tissue specimens.
‡Number of cases with pretreatment negative and posttreatment positive NF-κB.
§Fisher's test comparing NF-κB positivity for concomitant chemoradiation versus neoadjuvant chemotherapy followed by chemoradiation.

patients were men (90%) and adenocarcinoma was the predominant histology (93%). Clinical stage included: stage IIA in 27%, IIB in 4%, III in 46%, and IVA in 3%. All patients underwent surgical resection following chemoradiation. Table 2 lists other treatment details.

Chemotherapy agents included: taxanes in 68 patients (85%); platinols in 25 patients (31%); fluoropyrimidine in all 80 patients (100%); and topoisomerase-1 inhibitors in 53 patients (66.2%). PathCR was observed in 22 patients (27%), the remaining 58 (73%) having <pathCR.

The median follow-up time was 32 months (range, 6–104). The median time to local-regional and metastatic progression was 28 months (range, 2–53 months). The median survival time was 46 months (range, 30–63 months), with a 5-year OS rate of 40%.

Immunocytochemical Detection of Nuclear NF-κB

The expression levels of activated NF-κB were examined by immunohistochemistry in the pretreatment cancer specimens of 75 of the 80 (94%) patients and in the resected specimens of 56 patients resistant to chemoradiotherapy (i.e., <pathCR).

Activated NF-κB (defined as a nuclear labeling index ≥5%) was observed in 29 (36%) of the 75 pretreatment cancer biopsies, and in 43 (77%) of the 56 cases with residual cancer in the resected specimen. Figure 1A (*top* and *bottom*) illustrates examples of NF-κB-negative and -positive cancers.

In 51 patients with pretreatment and posttreatment cancer specimens, 24 (47%) had NF-κB-positive cancer before and after treatment; 17 (33%) became positive after treatment, and 10 (20%) were negative pretreatment and posttreatment. None of the pretreatment-positive specimens became negative posttreatment.

Forty-seven of 80 patients (59%) had at least one NF-κB-positive specimen (pretreatment or posttreatment). No significant association was found between NF-κB nuclear positivity and pretreatment clinicopathologic characteristics, including clinical stage and location of the primary site. Moreover, NF-κB positivity was not associated with the class or combination of chemotherapy drugs, or sequence (i.e., concurrent chemoradiotherapy or induction chemotherapy followed by chemoradiation; Table 2).

Pathologic Response and NF-κB Status

Pretreatment cancer biopsies were available for all 22 pathCR patients and for 53 (91%) of 58 patients with <pathCR. NF-κB expression in the pretreatment cancer biopsies was significantly associated with the type of response to treatment. As shown in Fig. 1C, only 2 (9%) of the 22 patients achieving pathCR had NF-κB-positive cancer compared with 27 (51%) of the 53 patients achieving <pathCR ($P = 0.006$; Fisher's test).

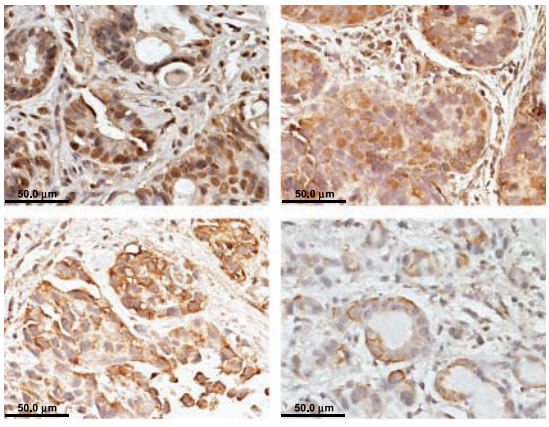
Significantly, lower pretreatment NF-κB protein expression levels (e.g., expressed as labeling index) predicted for pathCR [pretreatment LI median (interquartile range): pathCR = 0.001 (0–0.08); <pathCR = 0.01 (0–0.4); $P = 0.001$, Wilcoxon test]. However, no association was found between pretreatment NF-κB expression levels and the extent of residual cancer [pretreatment LI median (interquartile range): 1–10% = 0.01 (0–0.25); 11–50% = 0.1 (0–0.4); >50% = 0.02 (0–0.25); $P = 0.6$; Wilcoxon test].

Clinical Outcome and NF-κB Status

Neither the sequence of the preoperative treatment nor the class of chemotherapeutic drugs influenced patient OS. The median OS times were 58 months [95% confidence interval (CI), 45–64] and 40 months (95% CI, 15–64) for patients treated by induction chemotherapy followed by concurrent chemoradiation and concurrent chemoradiation, respectively ($P = 0.1$, log-rank test; Fig. 1A). Similarly, the OS times were not statistically different for patients treated by platinol versus taxanes or topoisomerase 1 inhibitors ($P = 0.06$ and $P = 0.18$, respectively; log-rank test; Fig. 1B).

Pretreatment-positive NF-κB cancer was statistically associated with shortened OS ($P = 0.009$, log-rank test; Fig. 2A). Sixteen (55%) of 29 patients with positive NF-κB cancer had died from cancer compared with 12 (26%) of the 46 patients with negative NF-κB. The 4-year OS rate for patients with NF-κB-positive cancer was 39% (95% CI, 26–52%) compared with 59% (95% CI, 44–71%) for patients with negative NF-κB.

A



B

Pathologic Response	No. of available specimens	NF-κB Positive	NF-κB Negative	P-value
Pre-treatment status				.006*
path CR	22	2 (9)	20 (91)	
<pathCR	53	27 (50.9)	26 (49.1)	
Pre- or post-treatment Status				.001*
path CR	22	2 (9)	20 (91)	
<pathCR	58	45 (77.6)	13 (23.2)	

Abbreviation: pathCR, complete pathologic response
*: Fisher's exact test comparing the pathCR versus <pathCR groups

Figure 1. Detection of nuclear NF-κB expression in esophageal cancers. **A,** immunohistochemical detection of NF-κB expression; *top,* nuclear NF-κB-positive pretreatment cancer biopsies; *bottom,* nuclear NF-κB-negative pretreatment cancer specimens. Note some cells with abundant cytoplasmic NF-κB expression. **B,** NF-κB nuclear expression and type of pathologic response.

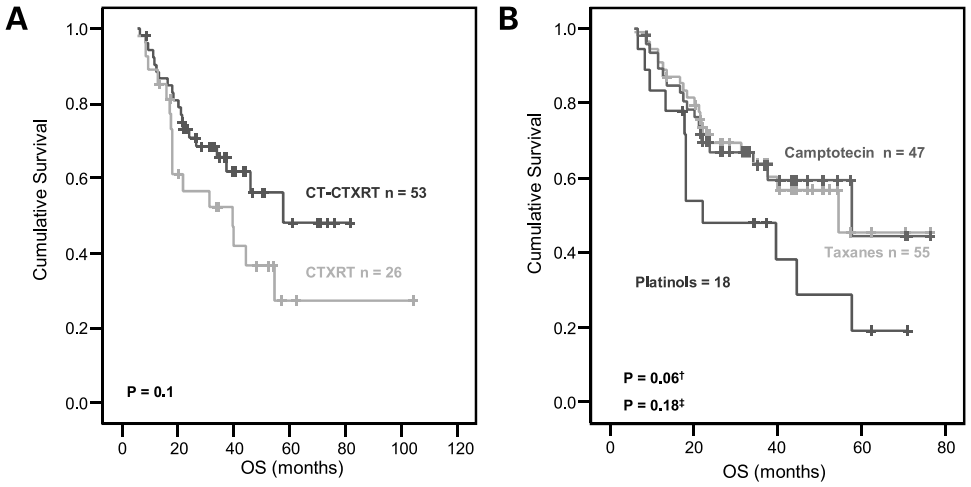


Figure 2. Kaplan-Meier curve for OS by preoperative treatment for patients with esophageal cancer. **A,** induction chemotherapy followed by concomitant chemoradiation (*CT-CTXRT*) versus concomitant chemoradiation (*CTXRT*). **B,** platinol agents versus taxanes (†) or camptothecins (‡); censored patients (+).

When considering either pretreatment or posttreatment NF-κB status, positive NF-κB was associated with shorter OS ($P = 0.0001$, log-rank test; Fig. 2B). Twenty-five (53%) of the 47 positive NF-κB patients had died of cancer compared with only 3 (9%) of the 33 patients with negative NF-κB cancer. The 4-year OS rate for patients with NF-κB-positive cancer was 31% (95% CI, 16–41%) compared with 78% (95% CI, 69–89%) for patients with negative NF-κB cancer.

The DFS of patients with pretreatment-positive NF-κB was significantly shortened ($P = 0.007$, log-rank test; Fig. 3). At the median follow-up of 32 months, 21 (72%) of the 29 patients with positive NF-κB cancer had developed a relapse compared with only 18 (39%) of the 43 patients with NF-κB-negative cancer. The 4-year DFS rate for patients with pretreatment NF-κB–positive cancer was 25% (95% CI, 16–43%) compared with 59% (95% CI, 43–72%) for patients with negative NF-κB cancer.

Likewise, pretreatment or posttreatment-positive NF-κB was associated with significantly shortened DFS ($P = 0.0002$, log-rank rest; Fig. 3). Thirty-two (70%) of the 47 patients with positive NF-κB cancer had developed a relapse compared with only 7 (21%) of the 33 patients with NF-κB-negative cancer. The 4-year DFS rate for patients with pretreatment NF-κB–positive cancer was 30% (95% CI, 18–42%) compared with 61% (95% CI, 48–71%) for patients with negative NF-κB cancer.

In multivariate models that included age, pretreatment clinical stage, location, lymph nodes metastasis, and pathCR versus <pathCR, pretreatment or posttreatment-positive NF-κB was the only significantly independent predictor of DFS ($P = 0.01$) and OS ($P = 0.007$). The hazard ratios of 0.28 for DFS and 0.19 for OS indicated that NF-κB-positive cancer patients were recurring and dying at 3.6 and 5.3 times the rate of NF-κB-negative patients (Table 3).

Recurrent Disease and NF-κB Status

Of the 47 patients with either pretreatment or posttreatment-positive NF-κB, 24 (51%) developed recurrent disease (21 [88%] had distant metastases and 3 [3%] had locoregional recurrence) compared with only 7 (21%) with negative NF-κB cancer (all distant metastases; $P = 0.01$; Fisher's test). Patients with NF-κB-positive cancer often had multiple concomitant metastatic sites. In contrast, patients with NF-κB-negative cancers rather had a single metastatic site. Of four pathCR patients who developed recurrent disease, two had a pretreatment-positive NF-κB cancer.

Discussion

The transcription factor NF-κB is a molecular master ''switch'' that activates cellular survival pathways and plays a critical role in cancer maintenance and progression (15). In response to various signaling pathways, induced by intracellular or/and extracellular stimuli, NF-κB inhibits programmed cell death, promotes proliferation, angiogenesis, and cell migration (15, 16). Thus, it is likely that NF-κB activation in cancer antagonizes the cytotoxic activity of chemotherapy and radiotherapy, contributing to resistance. Moreover, the concomitant activation of downstream pathways driving angiogenesis, cell migration, and invasion may enhance the aggressive phenotype of resistant cancers.

Chemoradiation resistance and development of distant metastases represent the clinical hallmark of >70% esophageal cancers undergoing multimodality therapy. Our previous study of a cohort of 43 patients, who were uniformly staged and treated similarly, showed that NF-κB was associated with clinical biology (chemoradiation resistance and metastatic progression); however, pretreatment NF-κB status did not significantly (only a trend) correlate with clinical biology (26). We, therefore, hypothesized that NF-κB status (pretreatment and pretreatment/posttreatment) would correlate with NF-κB in a larger cohort of patients and would do so irrespective of the type or sequence of chemotherapy used with radiation.

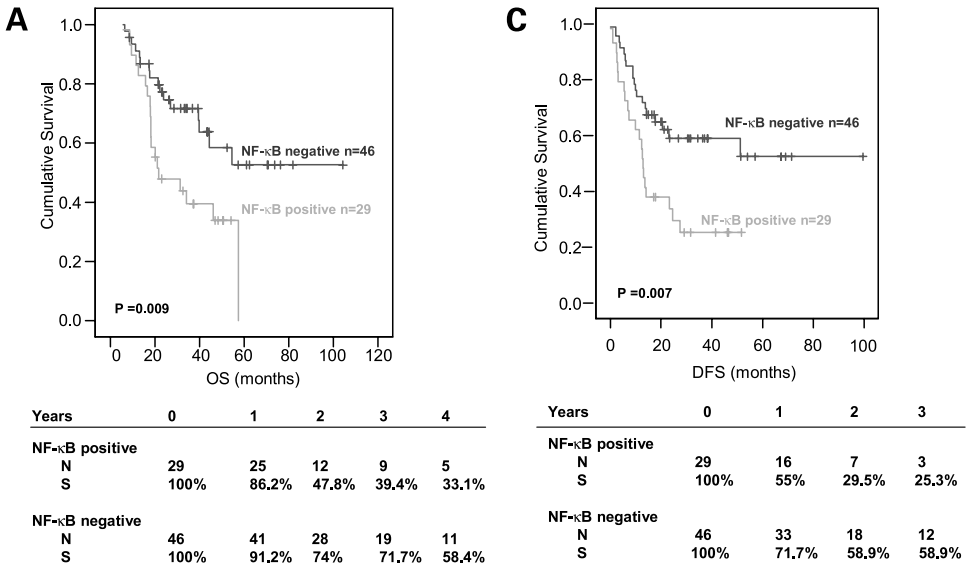
Our data, for the first time, establish that pretreatment NF-κB is significantly correlated with pathCR, OS, DFS, and metastatic progression. Our observation needs to be extended further and perhaps in concert with other molecular markers, one could exploit it to individual

therapy for patients with esophageal cancer. Individualization of therapy could conceivably eliminate the need for esophagectomy in some patients and help avoid ineffective and uniformly toxic chemoradiation in other patients. The other intriguing observation that emphasized the value of esophageal cancer biology is that the class of chemotherapy agent used or its sequence did not significantly affect OS or DFS.

Furthermore, the data are even stronger when one considers the NF-κB status before and after therapy; this may have a practical value. For example, NF-κB status of the residual cancer detected endoscopically prior to surgery may further aid in the decision to proceed with surgery. A strongly NF-κB-positive cancer in this situation would suggest a poor OS and debilitating surgery might not be warranted. We also observed that therapy induced activated NF-κB in 33% of pretreatment NF-κB-negative cancer. Although these findings could partially reflect tumor heterogeneity, this phenomenon underscores the potential exploitation of NF-κB-regulated genes and their pathways as therapeutic targets to overcome not only chemoradiation resistance but also metastatic progression; however, considerably more understanding of esophageal cancer biology would be required.

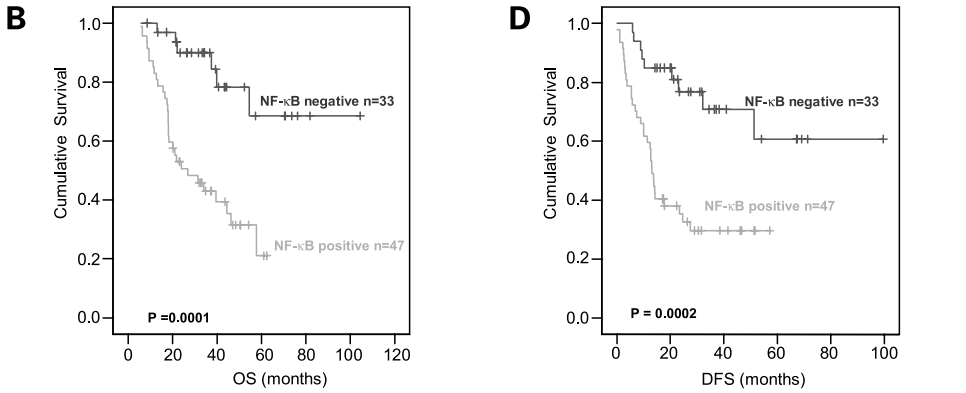
The mechanisms underlying constitutive and *de novo* NF-κB activation in cancer are not well understood. Multiple signaling pathways (e.g., extracellular, such as tumor necrosis factor-α and interleukin 1; and/or intracellular) may drive NF-κB activation concurrently depending on the biological context (16).

In conclusion, our data show that pretreatment NF-κB status correlates significantly with OS, DFS, and pathCR.



Years	0	1	2	3	4
NF-κB positive					
N	29	25	12	9	5
S	100%	86.2%	47.8%	39.4%	33.1%
NF-κB negative					
N	46	41	28	19	11
S	100%	91.2%	74%	71.7%	58.4%

Years	0	1	2	3
NF-κB positive				
N	29	16	7	3
S	100%	55%	29.5%	25.3%
NF-κB negative				
N	46	33	18	12
S	100%	71.7%	58.9%	58.9%



Years	0	1	2	3	4
NF-κB positive					
N	47	39	21	14	6
S	100%	83%	50.6%	43%	31.5%
NF-κB negative					
N	33	33	24	16	9
S	100%	100%	90%	90%	78.3%

Years	0	1	2	3
NF-κB positive				
N	46	28	13	7
S	100%	59.6%	35.3%	29.6%
NF-κB negative				
N	33	27	17	10
S	100%	84.8%	76.7%	70.8%

Figure 3. Kaplan-Meier curve for survival by NF-κB status. **A,** OS for patients with pretreatment NF-κB-positive cancer versus those with negative cancer. **B,** OS for patients with pretreatment or posttreatment NF-κB – positive cancer versus those with NF-κB-negative cancer. **C,** DFS of patients with pretreatment NF-κB – positive cancer versus those with pretreatment NF-κB – negative cancer. **D,** DFS between patients with pretreatment and/or posttreatment NF-κB-positive cancer and those with NF-κB-negative cancer. All statistical tests were two-sided. N and S indicate, respectively, the number of patients at risk and the Kaplan-Meier estimate of DFS at 0, 1, 2, and 3 y after registration (95% CI). Censored patients (+).

Table 3. Multivariate analysis of OS and DFS for patients with esophageal cancer

Survival	Variable	Hazard ratio	P*
Disease-free	NF-κB positive	0.28	0.01
	Clinical stage	1.58	0.22
	pResponse	1.07	0.9
	pLN	0.87	0.7
	Location	1.35	0.4
	Age	1.22	0.9
Overall	NF-κB positive	0.19	0.007
	Clinical stage	1.32	0.4
	pResponse	0.96	0.96
	pLN	0.63	0.22
	Location	1.2	0.4
	Age	1.2	0.9

*χ^2 analysis comparing the variables in the multiple regression model.

Pretreatment and/or posttreatment status further consolidates these findings. These observations are independent of type or sequence of chemotherapy used with radiation. Additionally, NF-κB status is an independent prognosticator of clinical biology of esophageal cancer. NF-κB, in concert with other molecular and genetic biomarkers, has the potential to contribute in the individualization of therapy for patients with localized esophageal carcinoma. However, considerably more understanding of molecular mechanisms would be required to accomplish the preservation of the esophagus by avoiding surgery and improving quality of life.

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