Clinical Biology of Esophageal Adenocarcinoma after Surgery Is Influenced by Nuclear Factor-κB Expression

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Abstract

Background: **The expression of transcriptional factor nuclear factor κB (NF-κB) in untreated esophageal cancer specimens from patients who receive preoperative chemoradiation is associated with aggressive clinical biology. We hypothesized that nuclear NF-κB would define clinical biology even when surgery is used as primary therapy.**
Methods: **Consecutive patients who did not receive any preoperative therapy were selected. Surgical cancer specimens were examined for nuclear NF-κB and correlated with overall survival (OS) and disease-free survival (DFS).**
Results: **One hundred twenty-three patients (stage I, 9%; stage II, 24%; stage III, 53%; stage IV, 15%) with adenocarcinoma who underwent surgery as primary therapy were analyzed. Most patients were men (90%) and the median age was 63 years. For all 123 patients, the median DFS was 21 months and the median OS was 28 months. Nuclear NF-κB was associated with shortened DFS (P = 0.001) in 123 patients but also in stage II (P = 0.03) and stage III (P = 0.04). Nuclear NF-κB was associated with shortened OS (P = 0.002) in 123 patients and in stage II (P = 0.04) and showed trend in stage III (P = 0.17). Numbers are too small for stages I and IV. In multivariate models, nuclear NF-κB was an independent predictor for both DFS and OS (P = 0.005 and P = 0.01).**
Conclusions: **Our data are the first to show that NF-κB status significantly correlates with DFS and OS for patients with esophageal adenocarcinoma undergoing surgery as primary therapy. NF-κB is an independent prognosticator of outcome, even for individual stages (e.g., stages II and III). More comprehensive molecular studies could help the design of strategies to individualize therapy of esophageal adenocarcinoma.** (Cancer Epidemiol Biomarkers Prev 2007;16(6):1200–5)

Introduction

Over the past 30 years, the incidence of esophageal or gastroesophageal (E/GEJ) adenocarcinoma has dramatically increased in the United States and other Western Countries (1). Once considered a rare diagnosis (incidence rate of 1.6 per 100,000 in 1973), E/GEJ adenocarcinoma has recorded, in the U.S. population alone, an incidence rate of 6.5 per 100,000 in 2002, which represents a mean annual increase of 9.6% (1). The prognosis of E/GEJ remains extremely poor in spite of combined modality approaches and the 5-year survival rate of <20% (2, 3). Preoperative therapy, particularly preoperative chemoradiation, is commonly recommended to operable patients with localized cancer, although its role remains controversial (4-7). Surgery as primary therapy is also practiced and remains an option for patients who do not qualify or decline preoperative therapy. However, the outcome from surgery as primary therapy remains poor (8) and carries considerable morbidity. A main challenge in the therapeutic management of patients with localized E/GEJ carcinoma is that we are unable to predict the outcome from any therapy (surgery, preoperative chemoradiation, or definitive chemoradiation) we give to patients. Our current knowledge of molecular determinants of E/GEJ cancer is suboptimal and confounded by the considerable biological heterogeneity within the tumors reflecting the various molecular events underlying cancer progression. Clinical parameters before therapy are unable to predict prognosis or help decide which component of combined modality would be effective (9-11). Thus, it is imperative that we focus on cancer biology and patients' genetics to derive answers permitting to improve the clinical management of E/GEJ cancer patients. Availability of reliable biomarkers will propel us in the arena of individualization of therapy, allowing reduced morbidity and toxicity with concomitant improved effectiveness of therapy.

Nuclear factor κB (NF-κB), a sequence specific transcription factor, is a gatekeeper of multiple cellular processes involved in cell survival (12, 13). In physiologic conditions, NF-κB is present in the cytoplasm as an inactive heterodimer consisting of p50, p65, and IκBα subunits. In response to extracellular and intracellular stimuli (i.e., inflammatory cytokines, viruses, carcinogens, and DNA damaging agents), NF-κB becomes active through the degradation of the inhibitory subunit IκBα followed by the nuclear translocation of the p65-p50 heterodimer (13). Once in the nuclear compartment, NF-κB binds to specific chromatin sequences to initiate the transcription of target genes.

Inappropriately or constitutive activated (i.e., sustained nuclear expression), NF-κB has been associated with the development and maintenance of inflammatory diseases, such as lung fibrosis, asthma, and glomerulonephritis, and of neoplasia (12, 13). Through the activation of survival pathways, NF-κB prevents apoptosis in response to stress or insult such as exposure to radiotherapy or chemotherapy, thus contributing to cancer resistance (14-24). In parallel, by enhancing migratory (e.g., Cox-2 and CAM adhesion proteins), invasive (e.g., matrix metalloproteinases), and proangiogenic (e.g., vascular endothelial growth factor and Cox-2) cell properties, NF-κB contributes to cancer metastastic progression and resistance to therapy as well (25, 26). In a previous

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study of gene expression profiling, using Affymetrix platform in conjunction with Ingenuity Pathway Analysis in pretreatment tissue biopsies of E/GEJ cancers, we found that multiple signaling pathways converging on NF-κB activation were significantly up-regulated in chemoradiation-resistant cancers (27). We also showed that in patients who are undergoing preoperative chemoradiation, nuclear NF-κB was independently associated with poor disease-free survival (DFS) or overall survival (OS; refs. 28, 29). In our studies, we observed two distinct NF-κB patterns whereas some E/GEJ cancers presented intrinsic (i.e., pretreatment) NF-κB activation, whereas in others the activation seemed to follow the exposure to chemoradiation treatment. In both cases, nuclear NF-κB seemed to define a similar clinical aggressive phenotype of E/GEJ cancers. This observation led to the hypothesis that nuclear NF-κB has an effect on clinical biology of E/GEJ cancer and that it potentially represents a biological classifier also for patients who do not receive preoperative chemoradiation (have surgery as primary therapy). To address this question, we studied nuclear NF-κB expression in a large number of patients who, for diverse reasons, did not receive preoperative therapy and had surgery as the primary therapy for esophageal cancer.

Patients, Materials, and Methods

Patients. One hundred twenty-three patients were included in the study. All patients underwent esophagectomy as primary treatment of esophageal adenocarcinoma between January 1986 and December 1997. All patients were clinically staged using barium swallow radiography and computed tomography of chest, abdomen, and esophagoscopy (modern staging tools were used in the more recent cohort). For surgery, transthoracic or transhiatal approaches were used. Pathologic restaging was done according to the American Joint Committee on Cancer criteria (30). Postsurgical surveillance was done every 3 months during the first year, thereafter every 6 months for 2 additional years, and then yearly. This study was approved by the Institutional Review Board.

Tissue Specimens. All tissue specimens were obtained retrospectively from the surgical specimens. H&E-stained slides of all specimens were re-reviewed and confirmed by one gastrointestinal pathologist (T.T.W.). Subsequently, formalin-fixed paraffin-embedded blocks were cut into 4-μm-thick sections and adjacent sections were used for biomarker assessment by immunohistochemistry.

Immunohistochemical Determination of NF-κB Protein. Immunohistochemical analysis for NF-κB was done with a mouse monoclonal antibody (G96-337, 2 μg/mL; BD PharMingen) as previously described (28, 29). This antibody recognizes specifically only the p65/RelA protein, without cross-reaction with other Rel family members. Positive controls consisted in formalin-fixed paraffin-embedded cell pellets of the SKGT-4 esophageal cell line (Dr. Schrump, National Cancer Institute, Bethesda, MD) placed on the same slide as the tumor tissue. Negative controls were included in each immunohistochemistry batch and consisted in a slide of formalin-fixed paraffin-embedded SKGT-4 cells immunolabeled with the omission of the primary antibody. Only nuclear immunoreactivity was considered positive for NF-κB; however, the presence of cytoplasmic staining was also recorded. The levels of nuclear NF-κB protein expression staining were 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 the cancer fields. Aberrant expression of NF-κB protein was defined as a nuclear labeling index (LI) ≥5% based on the distribution of expression observed in the examined cancers and similar cutoffs previously chosen by our group (28, 29). Cancer cases showing aberrant NF-κB nuclear expression were defined as positive for the purpose of the analysis. All cancer fields present in the tissue sections were analyzed for NF-κB expression and NF-κB positivity was spatially evaluated with regard to vascular, nervous, and connective/soft tissue structures. Three investigators (J.I., U.M., and T.T.W.) independently examined NF-κB staining and determined positivity; in case of discrepancy, a final opinion was made based on consensus by all three investigators after double blind recounting. The scoring of NF-κB nuclear LIs had minimal variability between the three investigators, ranging between 0.16% and 0.47%.

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 OS and DFS time. Perioperative mortality, defined as death within 30 days postsurgery, was excluded from the survival analyses because the end point of the study was long-term survival and not short-term morbidity due to surgery. OS was defined as the time from surgical resection until 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 were 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 using the log-rank test to assess differences in survival distribution. After stepwise selection to determine which covariate (i.e., gender, age, histology parameters, tumor location, clinical stage, postoperative N status, perineural or vascular invasion, and surgical technique) was a significant predictor of DFS and OS, multivariate Cox proportional hazards models were fit to yield hazard ratio estimates. 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.).

Results

Patient Characteristics. Table 1 illustrates the patient characteristics. Of the 123 patients examined, the majority (90%) were men and the median age was 63 years (range, 28-83 years). All patients had adenocarcinoma, mostly poorly (49%) and moderately (44%) differentiated. Clinical stages included stage I in 9%, stage II in 24.4% (IIA, 15.4%; IIB, 8.9%), stage III in 52.8%, and stage IV in 13.8%. All patients underwent surgical resection without receiving any preoperative therapy and only four patients received 5-fluorouracil–based postoperative adjuvant chemotherapy.

The median follow-up time was 16 months (range, 2-214 months). The median time to locoregional and metastatic progression was 21 months [95% confidence interval (95% CI), 4-37 months]. The median OS time was 28 months (95% CI, 20-36 months).

Nuclear NF-κB Expression and Tumor Phenotypes. Aberrant nuclear NF-κB protein expression (defined as LI ≥5%) was observed in 79 of 123 (64%) cancer specimens (Table 1). Forty-four (35.7%) of the 123 cases had no nuclear NF-κB staining detectable in the tumors, 36 (29.3%) had weak to moderate staining, and 43 (35%) had strong nuclear immunolocalization of NF-κB protein. Positive cancers presented a wide range of NF-κB LI (median, 0.2; range, 0.05-0.9); however, 67 of the 79 (84.8%) positive cancers harbored nuclear NF-κB in <40% of

Table 1. Patient clinicopathologic characteristics and NF-κB nuclear expression

Characteristics	Total (n = 123)	NF-κB positive (n = 79), n (%)	NF-κB negative (n = 44), n (%)	P
Sex				
Male	111	69 (62.1)	42 (37.9)	0.20*
Female	12	10 (83.4)	2 (16.6)	
Race				
Caucasian	116	75 (61.0)	41 (39.0)	
African American	3	2 (66.6)	1 (33.4)	0.69*
Asian	2	2 (100.0)	0 (0.00)	
Hispanic	2	0 (0.00)	2 (100.0)	
Histology				
WD	9	6 (66.6)	3 (33.4)	
MD	54	34 (63.0)	21 (37.0)	0.78 †
PD	60	39 (65.0)	21 (35.0)	
Tumor stage (Unio Internationale Contra Cancrum)				
I	11	3 (27.2)	8 (72.8)	
IIA	18	11 (61.0)	8 (39.0)	0.03 ‡
IIB	11	8 (72.7)	3 (27.3)	
III	65	48 (73.8)	17 (26.2)	
IVA	12	4 (33.3)	8 (66.7)	
IVB	6	6 (100.0)	0 (0.00)	
Esophagectomy				
Transthoracic	56	36 (64.3)	20 (35.7)	
Transhiatal	67	43 (64.2)	24 (35.8)	1.00*
Residual tumor				
R0	98	55 (56.0)	43 (44.0)	
R1	25	24 (96)	1 (0.4)	0.001*

Abbreviations: WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated or undifferentiated.
*Two-sided Fisher's exact test comparing the NF-κB–positive and NF-κB–negative groups.
†Cochran-Armitage trend test comparing the NF-κB–positive and NF-κB–negative groups.
‡Pearson correlation.

the tumor cells. Of interest, the observed staining pattern was heterogeneously distributed within the tumors, with NF-κB–positive cells predominantly observed in the peripheral and invasive fronts of the cancer fields. Concomitant strong cytoplasmic NF-κB staining was observed in 52 of the 79 (65.8%) positive cancers, mostly spatially associated with the aberrant nuclear expression. However, in a few cases, the strong cytoplasmic expression was observed scattered throughout the whole tumor. Similar distribution patterns were observed in 25 of the 44 (56.8%) negative cancers that presented with intense NF-κB cytoplasmic localization. As shown in Table 1, nuclear NF-κB expression was not associated with the degree of tumor differentiation; however, it was significantly correlated with higher tumor stage (P = 0.03). The presence of nuclear NF-κB was significantly associated with aggressive pathologic tumor characteristics, and this was independently of clinical tumor staging (Table 2). Of the 79 NF-κB positive cancers, 58 (85.3%) showed tumor invasion into adjacent structures compared with only 11 (29.7%) in the NF-κB–negative cancer group (P < 0.0001). Importantly, NF-κB–associated invasion was preferentially observed in perineural and vascular structure rather than in the connective and soft tissues. Forty-five of the 79 (66.1%) NF-κB–positive cancers had perineural and vascular invasions compared with 12 of the 44 (32.4%) NF-κB–negative tumors (P = 0.009), whereas connective/soft tissue tumor invasion was observed in similar proportion in both groups (P = 0.681; Table 2). The pathologic aggressive cancer features were associated with clinical aggressive phenotype and poor outcome. Twenty-four (30%) of the NF-κB–positive cancers had postsurgical positive margins, all of which showed presence of cancer cells in the perineural or vascular compartments, compared with only 1 (2%) of the NF-κB–negative cancers. Additionally, NF-κB–positive cancer patients developed significantly more distant recurrences (31 of 79) compared with the NF-κB–negative group (6 of 44; P = 0.002; Table 2). There was no association found between degree of NF-κB positivity (i.e., percent LI) and occurrence of either perineural/vascular invasion or distant metastases.

NF-κB Expression and Clinical Outcome. On univariate analysis, positive NF-κB was significantly associated with shortened DFS (P = 0.001, log-rank test; Fig. 1A). At the median follow-up time of 16 months, 35 of the 79 (44%) patients with nuclear NF-κB–positive cancers developed a relapse compared with 10 of the 44 (23%) patients with NF-κB–negative tumor. The 3-year DFS rate for patients with aberrant nuclear NF-κB expression was 32% (95% CI, 26-38%) compared with 65% (95% CI, 57-74%) for patients with NF-κB–negative cancers. The effect of NF-κB status on DFS for each pathologic cancer stage was examined. Strikingly, positive NF-κB expression adversely affected the DFS of stage II (P = 0.038, log-rank test; Fig. 1B; Table 3) and stage III (P = 0.047, log-rank test; Fig. 1C; Table 3). There was a slight but nonsignificant trend for the small number of patients in stage IV category (P = 0.528; Fig. 1D; Table 3). The number of patients with stage I cancer was too small for this type of analysis. The 3-year DFS rate for patients with NF-κB–positive cancer in stages II and III were 40% (95% CI, 25-55%) and 25% (95% CI, 17-32%), respectively, compared with 78% (95% CI, 64-92%) and 55% (95% CI, 39-71%) for patients whose cancers were without NF-κB expression.

Similarly, nuclear expression of NF-κB was associated with significantly shortened OS (P = 0.002, log-rank test; Fig. 2A). At the median follow-up time of 16 months, 24 of the 79 (30%) patients with cancer positive for NF-κB expression had died compared with only 7 of 44 (16%) patients with cancer without NF-κB expression. The 3-year OS rate for NF-κB–positive cancer patients was 30% (95% CI, 24-37%) compared with 63% (95% CI, 54-72%) for patients with NF-κB–negative cancer. The OS of NF-κB–positive cancer stage II patients was considerably shortened compared with NF-κB–negative cancer

Table 2. NF-κB status, pathologic invasion, and local and distant recurrences in stage I, II, and III esophageal cancer patients

	NF-κB positive (n = 70)				NF-κB negative (n = 36)				P*
	Stage I (n = 3)	Stage II (n = 19)	Stage III (n = 48)	All (n = 70)	Stage I (n = 8)	Stage II (n = 12)	Stage III (n = 17)	All (n = 36)	
Any invasion	1 (33.3)	13 (68.4)	45 (93.5)	59 (84.2)	1 (12.5)	3 (25.0)	6 (35.3)	10 (27.7)	<0.0001
PN/vascular	3 (100.0)	11 (57.8)	32 (66.6)	46 (65.7)	2 (25.0)	3 (25.0)	6 (35.3)	11 (30.5)	0.0009
CN/soft	3 (100.0)	10 (52.6)	17 (35.4)	30 (42.8)	2 (25.0)	3 (25.0)	8 (47.0)	13 (36.1)	0.537
LC Rec	0 (0)	1 (5.8)	8 (16.6)	9 (11.4)	1 (12.5)	1 (8.3)	2 (11.7)	4 (9)	1.000
Metastases	0 (0)	10 (52.6)	22 (45.8)	32 (45.7)	0 (0)	2 (16.6)	3 (17.6)	5 (13.8)	0.001

Abbreviations: PN, perineural; CN, connective; LC Rec, locoregional recurrence.
*Two-sided Fisher's exact test comparing NF-κB–positive and NF-κB–negative cases.

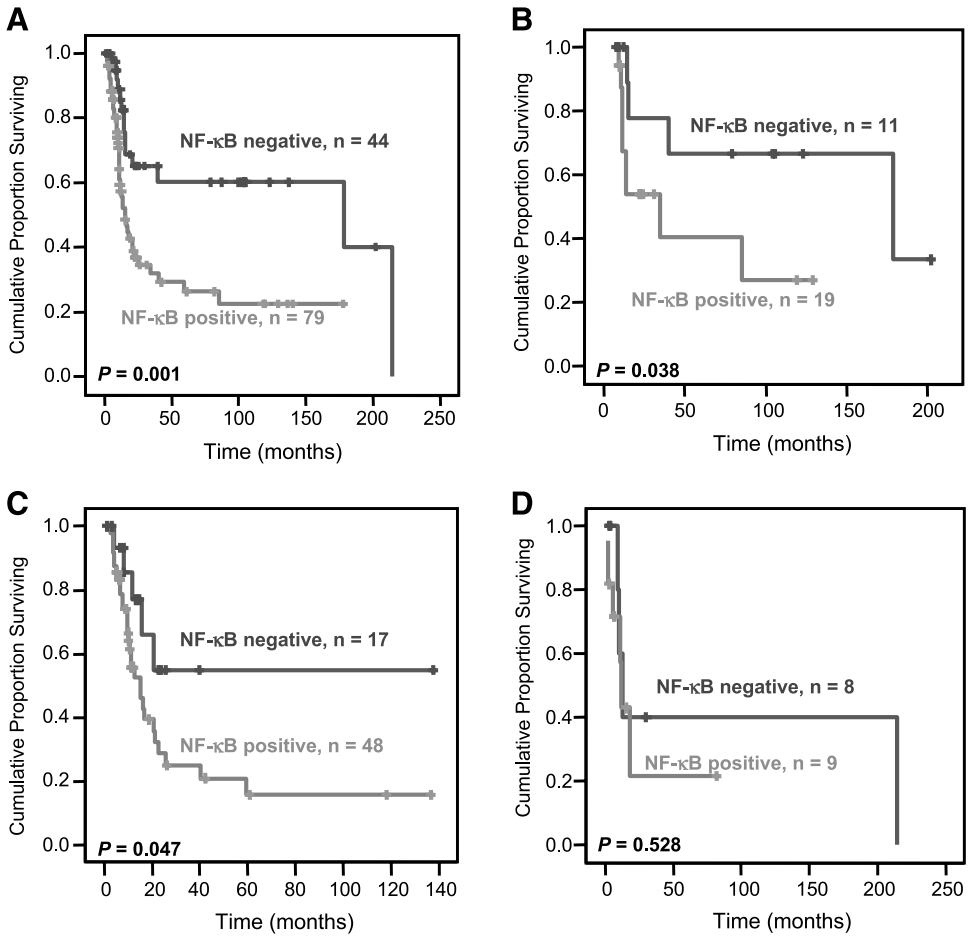

Figure 1. Kaplan-Meier curve for DFS by NF-κB status for E/GEJ cancer patients. **A.** DFS for 123 patients, all clinical stages included. **B.** DFS for 29 stage II patients. **C.** DFS for 65 stage III patients. **D.** DFS for 18 stage IV patients. |, censored patients.

patients within the same stage ($P = 0.044$, log-rank test; Fig. 2B; Table 3). The 3-year OS rates for stage II and stage III patients with NF-κB–positive cancer were respectively reduced to 45% (95% CI, 31-59%) and 23% (95% CI, 16-31%) compared with 78% (95% CI, 64-92%) and 37% (95% CI, 20-59%) for patients with cancer not expressing NF-κB.

Of interest, the adverse effect of NF-κB on both DFS and OS was independent from the degree of NF-κB positivity found in the tumors.

In the multivariate models that included age at surgery, gender, location, histology grade, pathologic stage, R0 or R1, and surgical technique, positive NF-κB was an independent predictor of both DFS and OS (Table 4). Positive NF-κB carried the highest risk of relapse (hazard ratio, 2.52; 95% CI, 1.31-5.69; $P = 0.002$) and of dying (hazard ratio, 2.25; 95% CI, 1.178-4.30; $P = 0.014$; Table 4).

Table 3. Median DFS and OS per NF-κB status in esophageal cancer patients

Survival	Stage	Time (95% CI), mo		P*
		NF-κB positive	NF-κB negative	
DFS	I	NR	NR	0.562
	II	34.7 (9.0-60.3)	178.2 (0.0-380.1)	0.038
	III	15.1 (9.0-21.2)	NR	0.047
	IV	11.8 (9.8-13.7)	12.9 (6.0-19.7)	0.528
OS	I	NR	NR	0.563
	II	34.6 (0.45-68.8)	178.2 (0.0-376.8)	0.044
	III	22.7 (13.4-31,9)	32.6 (16.6-48.6)	0.173
	IV	15.5 (10.1-20.8)	12.8 (6.0-19.7)	0.710

Abbreviation: NR, not reached.
*Log-rank test comparing NF-κB–positive and NF-κB–negative cases.

Discussion

Even when esophageal cancers are grouped into one clinical or pathologic stage, the clinical outcomes are frequently unpredictable. This clinical heterogeneity is dictated by the biological diversity of E/GEJ cancers. Lack of understanding of molecular heterogeneity of cancer has led to the empirical approaches in therapy of esophageal carcinoma. Thus, all patients with stage II carcinoma are treated either with surgery (8) or with chemoradiation. The survival rate of patients even with stage II cancer is <35% (8). It would be of considerable advantage if one could identify patients who will have a very poor prognosis in spite of surgery. Currently, there is a lack of reliable molecular markers that can discriminate outcome of patients who have localized esophageal carcinoma and receive surgery as primary therapy. If one were to advance the goal of rational individualization of therapy for patients with carcinoma of the esophagus, a considerable molecular biological investigation would be needed.

Our experience with NF-κB as a potential discriminator of esophageal cancer clinical biology has been limited to only those patients who had received preoperative chemoradiation. In this setting, NF-κB was correlated with chemoradiation resistance and aggressive biology (28), and the type of combinations of chemotherapy did not influence the effect of NF-κB status (29). However, it is not clear if NF-κb status has an influence on the outcome of patients with localized esophageal cancer who undergo surgery as primary therapy. The data presented in this report are the first to show that a biomarker–like NF-κB is associated with poor outcome (DFS and OS) but, most intriguingly, the NF-κB status discriminated clinical biology for stage II cancer patients (for DFS and OS) and stage III cancer patients (for DFS, and a strong

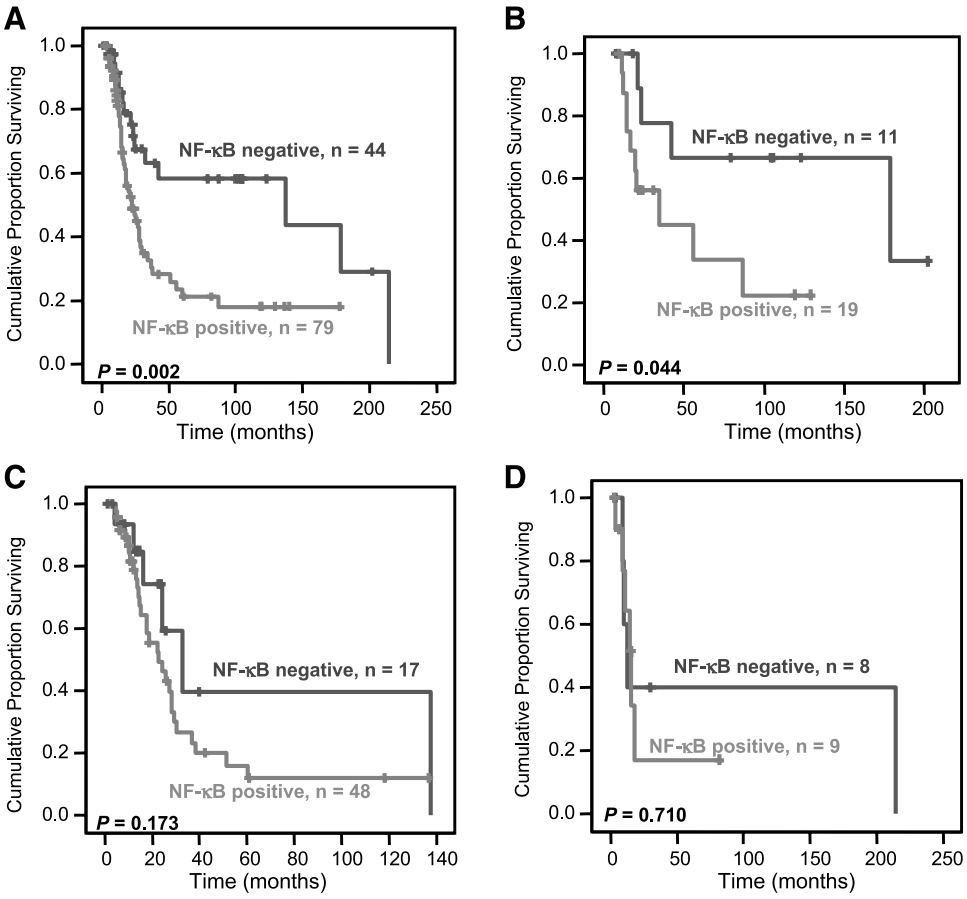

Figure 2. Kaplan-Meier curve for OS by NF-κB status for E/GEJ cancer patients. **A.** OS for 123 patients, all clinical stages included. **B.** OS for 29 stage II patients. **C.** OS for 65 stage III patients. **D.** OS for 18 stage IV patients. |, censored patients.

nonsignificant trend for OS). Even among stage IV patients, whose numbers are very small, there was a nonsignificant but interesting trend.

Another intriguing aspect of our data is found in the relationship of NF-κB status with clinical staging or with invasive properties of cancers. As previously reported by Abdel-Latif et al. (31), we found a correlation between clinical stage and aberrant NF-κB; however, and somewhat surprisingly, the degree of NF-κB expression, as measured by nuclear LIs, was not correlated with tumor size. Similarly, the presence of altered NF-κB was associated with perineural/vascular invasion independently from the number of cancer cells carrying abnormal NF-κB. These observations undescore the likelihoood that the alteration of the NF-κB pathway in only a few cancer cells is sufficient to fuel the emergence of more aggressive cell clones. This mechanistic hypothesis may explain the profound adverse effect of NF-κB found in stage II E/GEJ cancer patients.

These data also suggest that interventions aiming to interfere with the activation of NF-κB could be developed and integrated into the therapeutic management of E/GEJ cancer patients. However, the development of successful strategies is critically dependent on the understanding of the molecular underpinning of NF-κB activation. Evidence from several research groups supports the hypothesis that the chronic inflammatory conditions found in the progression of E/GEJ cancer are closely associated with NF-κB activation (32). A number of molecular targets potentially driving NF-κB activation have been identified, including the proinflammatory cytokines interleukin 8, interleukin 1β, and tumor necrosis factor α (33-36). However, greater understanding of the NF-κB circuitry in esophageal cancer biology would be necessary to optimally define and exploit the window of therapeutic opportunity.

Clearly, the data presented in this article are insufficient to make any firm conclusions and need further confirmation and validation in larger cohorts. A single biomarker is highly unlikely to help characterize the biological behavior of a cancer. In addition, a multitude of biomarkers will have to be defined before they are put to test in a prospective setting where clinical decision might be influenced by the knowledge of pretreatment biomarker profile. Further understanding of molecular biology of esophageal cancer will also potentially yield exploitable therapeutic targets.

In conclusion, the expression status of NF-κB in esophageal cancer specimens of patients undergoing surgery as primary therapy is associated with DFS and OS. In addition to being an

Table 4. Multivariate analysis of DFS and OS for E/GEJ cancer patients

Survival	Variable	Hazard ratio (95% CI)	P*
DFS	NF-κB positive	2.52 (1.31-5.69)	0.002
	Invasion	2.34 (1.11-6.30)	0.024
	Pathologic stage	1.84 (1.24-2.72)	0.480
	Location	0.42 (0.22-0.82)	0.912
	Age	0.99 (0.32-1.03)	0.833
	Gender	0.70 (0.27-1.80)	0.450
	Margins	0.53 (0.30-0.94)	0.031
OS	NF-κB positive	2.25 (1.17-4.30)	0.014
	Invasion	2.55 (1.01-6.41)	0.046
	Pathologic stage	1.85 (1.25-2.73)	0.002
	Location	0.51 (0.20-0.91)	1.02
	Age	0.63 (0.34-1.11)	0.11
	Gender	0.65 (0.25-1.66)	0.37
	Margins	0.95 (0.50-1.7)	0.89

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

independent prognosticator of DFS and OS, NF-κB seems to discriminate the outcome of patients with stage II (DFS and OS) and stage II (DFS with a strong trend for OS) cancer.

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