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ORIGINAL ARTICLE

Nrf2 and Keap1 abnormalities in esophageal squamous cell carcinoma and association with the effect of chemoradiotherapy

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Abstract

Background: The Keap1-Nrf2 pathway is a key antioxidant and redox signaling cascade. Pathway abnormalities enhance the reactive oxygen species scavenging ability of cancer cells; thus the pathway is involved in carcinogenesis and resistance to chemoradiotherapy (CRT). This retrospective study was conducted to examine the status of the Keap1-Nrf2 pathway in locally advanced esophageal squamous cell carcinoma (ESCC) and to analyze its prognostic value in patients receiving CRT.

Methods: Nrf2 and Keap1 expression were immunohistochemically examined in 152 ESCC and 31 normal esophageal mucosae. All ESCC specimens were obtained from patients with locally advanced ESCC who underwent CRT.

Results: Strong staining of nuclear and cytoplasmic Nrf2 and limited or absent Keap1 expression was uncommon in normal tissues, but frequently observed in ESCC. Interaction between Nrf2 and Keap1 in normal mucosae is negatively correlated, while in tumors there is no negative correlation, indicating that there is little to no interaction between Nrf2 and Keap1 in ESCC. Positive Nrf2 expression in the nucleus was of diagnostic value for predicting ESCC from normal esophageal mucosae, and was significantly associated with poorer clinical response and poor progression-free survival after CRT. The value of Keap1 expression for diagnosis and predicting CRT outcomes was marginal. These different influences of Keap1 and Nrf2 on ESCC indicated that the signaling of this pathway was disturbed and displayed a Keap1-independent pattern.

Conclusion: Aberrant signaling via the Keap1-Nrf2 pathway was common in ESCC and was associated with response and survival after CRT.

Introduction

Esophageal carcinoma is one of the most common malignancies, with almost 500 000 new cases of the disease diagnosed worldwide every year.[1] Esophageal squamous cell carcinoma (ESCC) is the major histological type and prevails in developing countries, causing over 200 000 deaths in China annually.[2,3] For patients with locally advanced ESCC, definitive chemoradiotherapy (CRT) has been used as an effective treatment and significantly prolongs survival compared with radiotherapy alone.[4,5] However, although CRT can initially achieve a considerable response at the cost of severe toxicity, most patients suffer recurrence within three years. Nevertheless, the underlying mechanisms as to how ESCC can resist CRT are not yet known.

Reactive oxygen species (ROS) play a dual role in cancer. Not only are they implicated in the genesis and progression of many cancers including ESCC, they are also involved in the antitumor mechanism of cytotoxic agents

and radiation.[6–18] Therefore, antioxidant and redox signaling has drawn increasing attention in cancer research and the Keap1-Nrf2 pathway is definitely one of the most important signaling cascades.

As a core transcription factor, Nrf2 is bound to Keap1 in cytoplasm and degrades in a proteasome-dependent manner under homeostatic conditions.[19] Oxidative stress results in conformational change of the Keap1–Nrf2 complex, allowing Nrf2 to be translocated into the nucleus and activating the transcription of target antioxidant and redox genes.[20–23] Aberrant signaling via the Keap1-Nrf2 pathway frequently occurs in cancer cells, leading to the over-activation of Nrf2 and elevation of ROS scavenging ability, facilitating the initiation and development of malignant cells that produce ROS during rapid proliferation.[24–30] On the other hand, ROS are also indispensable for the therapeutic effect of platinum complexes, fluorouracil, and X-ray, which means that Nrf2 over-activation could also help cancer cells survive chemotherapy and irradiation and subsequently relapse.[9,11–14,26,31]

The promoting role of aberrant Keap1-Nrf2 signaling in carcinogenesis and therapy resistance has been well demonstrated in vivo and animal models;[7,13,17,25,29,31,32] however, relevant clinical studies remain inadequate. Moreover, Keap1/Nrf2 expression in normal tissues has rarely been reported. In the present study, we examined the expression of Nrf2 and Keap1 in ESCC and normal esophageal mucosa and investigated their prognostic significance for predicting CRT response.

Methods

Patients and tissue samples

Patients with locally advanced ESCC (stage II and III according to the 2002 Union for International Cancer Control Tumor Node Metastasis [TNM] Staging System) diagnosed between January 2010 and February 2014 were enrolled in this study. The Ethics Committees at Shandong Cancer Hospital and Institute approved this study and informed consent was obtained from all participants.

Histological specimens of tumors were collected endoscopically and fixed in 10% formalin and embedded in paraffin wax. All patients underwent two cycles of cisplatin and 5-fluorouracil regimens (cisplatin 25 mg/m^2 3 days, 5-fluorouracil 450–500 mg/m^2 5 days) during radiotherapy at Shandong Cancer Hospital and institute (Jinan, China). Radiotherapy doses ranged from 60.4 to 70.0 Gy (median 65 Gy) to planning target volume delivered in 30–38 daily fractions (median 35). Additionally, we collected normal esophageal mucosae from patients who underwent esophagectomy for early stage ESCC. All normal tissues were collected in sites at least 5 cm from carcinoma tissues.

Follow-up and clinical data

Basic information including age, gender, smoking history, alcohol consumption history, tumor location, and TNM staging at the time of diagnosis was collected from medical records. All patients were regularly followed up with physical examinations every three months during the first two years after the last radiotherapy, and every six months thereafter until death or the closing date on 9 February 2017. Clinical response was assessed by standard clinical measurements, esophagography, and computed tomography examinations according to Response Evaluation Criteria in Solid Tumors. Overall survival (OS) was defined as the duration between diagnosis and death from any cause and was censored for survivors at the date of the last follow-up. Progression-free survival (PFS) was defined as the duration between diagnosis and the date of disease progression or death from any cause, and was censored at the date of the last visit for patients without progression.

Immunohistochemistry and evaluation of Nrf2 and Keap1 expression

All 4 μm thick ESCC and normal esophageal mucosae sections were cut from formalin fixed paraffin embedded (FFPE) blocks, deparaffinized in Bond Dewax Solution (Leica Microsystems, Wetzlar, Germany), and rehydrated by graded alcohol. Heat-induced antigen retrieval was achieved under high pressure for 20 minutes at 100°C using Bond Epitope Retrieval Solution 1 (Leica Microsystems). The sections were soaked in 3% hydrogen peroxide solution for 10 minutes to reduce endogenous peroxidase activity and were incubated afterward with primary antibodies against Nrf2 (ab31163, Abcam, Cambridge, UK) and Keap1 (10503-2-AP, Proteintech, Chicago, IL, USA) for two hours at room temperature. Post-primary immunoglobulin G linker reagent was applied for 10 minutes, and the slides were incubated with polymeric horseradish peroxidase immunoglobulin G reagent for 10 minutes to localize the primary antibodies. Diaminobenzidine-tetrahydrochloride was used as the substrate to detect antigen–antibody binding. Finally, hematoxylin was applied for five minutes to counterstain nuclei.

Two pathologists independently evaluated the intensity, percentage, and sublocalization of each section. Conflicting results were summarized thereafter and resolved by using a multi-headed microscope. Cytoplasmic Keap1 and nuclear and cytoplasmic Nrf2 were quantified using a four-value intensity score (0, 1+, 2+, or 3+) and the percentage (0–100%) of the extent of reactivity. The quick (Q) score was used to determine expression levels, which was obtained by multiplying the percentage of positive cells (P) by the intensity (I) ($Q = P \times I$; maximum = 300[33] The median values of

the Q scores were used as cutoff points to classify "negative or low expression" and "positive or high expression."

Statistical analysis

The differences between Nrf2 and Keap1 expression in normal esophageal mucosae and ESCC samples were assessed using a Mann–Whitney *U* test. Correlations of Nrf2 and Keap1 expression were evaluated using Spearman's correlation test and illustrated as scattered plots. The χ^2 test was performed to evaluate the association of categorical variables. Curves for OS and PFS were obtained using the Kaplan–Meier method, and log-rank tests were performed to analyze differences in survival rates. Hazard ratios and corresponding 95% confidence intervals (CIs) for outcomes were estimated via univariate and multivariate Cox proportion regression models. All two-sided *P* values > 0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Comparison of Nrf2 and Keap1 expression in normal esophageal mucosa and esophageal squamous cell carcinoma (ESCC)

A total of 152 ESCCs and 31 normal esophageal mucosae samples were included in this study. Immunohistochemical staining of normal esophageal mucosae and tumor specimens exhibited different patterns of Nrf2 and Keap1 expression (Fig 1). Cytoplasmic and nuclear staining of normal tissues revealed light to no Nrf2 expression. By contrast, Nrf2 expression in both the cytoplasm and nucleus was frequently observed in ESCC samples (Fig 1a). The medium Q scores of nuclear Nrf2 expression were 0 and 10 for the normal esophageal mucosa and ESCC, respectively. The medium Q scores of cytoplasmic Nrf2 were 0 for both kinds of specimens. While there was little difference between the median scores, Nrf2 expression was significantly more volatile in ESCC, reflecting inter-individual heterogeneity of tumors (Fig 1c). Generally, nuclear and cytoplasmic expression of Nrf2 in ESCC was stronger than in the normal tissue ($P < 0.001$). The difference in Keap1 expression between the normal esophageal mucosa and ECSS sample was also obvious (Fig 1b,c). Similarly, while the medium Q scores of Keap1 were 270 for both types of specimens, the Keap1 expression level fluctuated much more in ESCC ($P = 0.025$).

Furthermore, receiver operating characteristic analysis was conducted to evaluate the sensitivity and specificity of Nrf2 and Keap1 expression for predicting ESCC compared to normal tissues (Fig 2). Notably, high nuclear Nrf2 expression displayed considerable diagnostic significance with an area under the curve (AUC) of 0.829 (95% CI 0.771–0.887; $P<0.001$). High cytoplasmic Nrf2 expression displayed modest diagnostic significance with an AUC of 0.682 (95% CI 0.598–0.765; $P = 0.001$). The diagnostic value of low Keap1 expression was marginal with an AUC of 0.619 (95% CI 0.514–0.724; $P = 0.037$).

Correlation of Nrf2 and Keap1 protein expression

Spearman's correlation testing was performed to examine the relationships of Nrf2 and Keap1 expression in normal esophageal mucosa and ESCC (Fig 3). In normal tissues, Keap1 expression was negatively correlated to both cytoplasmic (rho = −0.344) and nuclear (rho = −0.495) Nrf2 expression. The relationship between cytoplasmic and nuclear Nrf2 expression exhibited no correlation. In ESCC, the relationship between Nrf2 and Keap1 expression disappeared, while nuclear Nrf2 expression was positively correlated to cytoplasmic Nrf2 expression (rho = 0.763).

Relationships between Nrf2 and Keap1 expression and clinicopathologic characteristics of ESCC

The relationships between Nrf2 and Keap1 expression and clinicopathologic characteristics of ESCC are summarized in Table 1. The median Q scores of nuclear and cytoplasmic Nrf2 in ESCC were 10 and 0, respectively, which were subsequently used as cutoff points. Seventy-eight cases (51.32%) were classified as negative nuclear expression, whereas 74 cases (48.68%) were classified as positive. No significant correlations between nuclear Nrf2 and clinicopathologic characteristics were observed. Cases were further grouped into negative (79, 51.97%) and positive (73, 48.03%) Nrf2 expression subgroups. Positive cytoplasmic expression was associated with a heavy smoking history, probably reflecting the enhanced oxygen stress induced by cigarettes.

The median Q score of Keap1 was 270. Using the median score as a cutoff point, 64 (42.11%) and 88 (57.89%) cases were classified as low and high Keap1 expression, respectively. No significant correlations between Keap1 expression and clinicopathologic characteristics were observed.

Relationship between Nrf2 and Keap1 expression and clinical response to CRT

A total of 121 patients (79.61%) achieved a complete response (CR) or partial response (PR), while 31 patients (20.39%) experienced stable disease (SD) or progressive

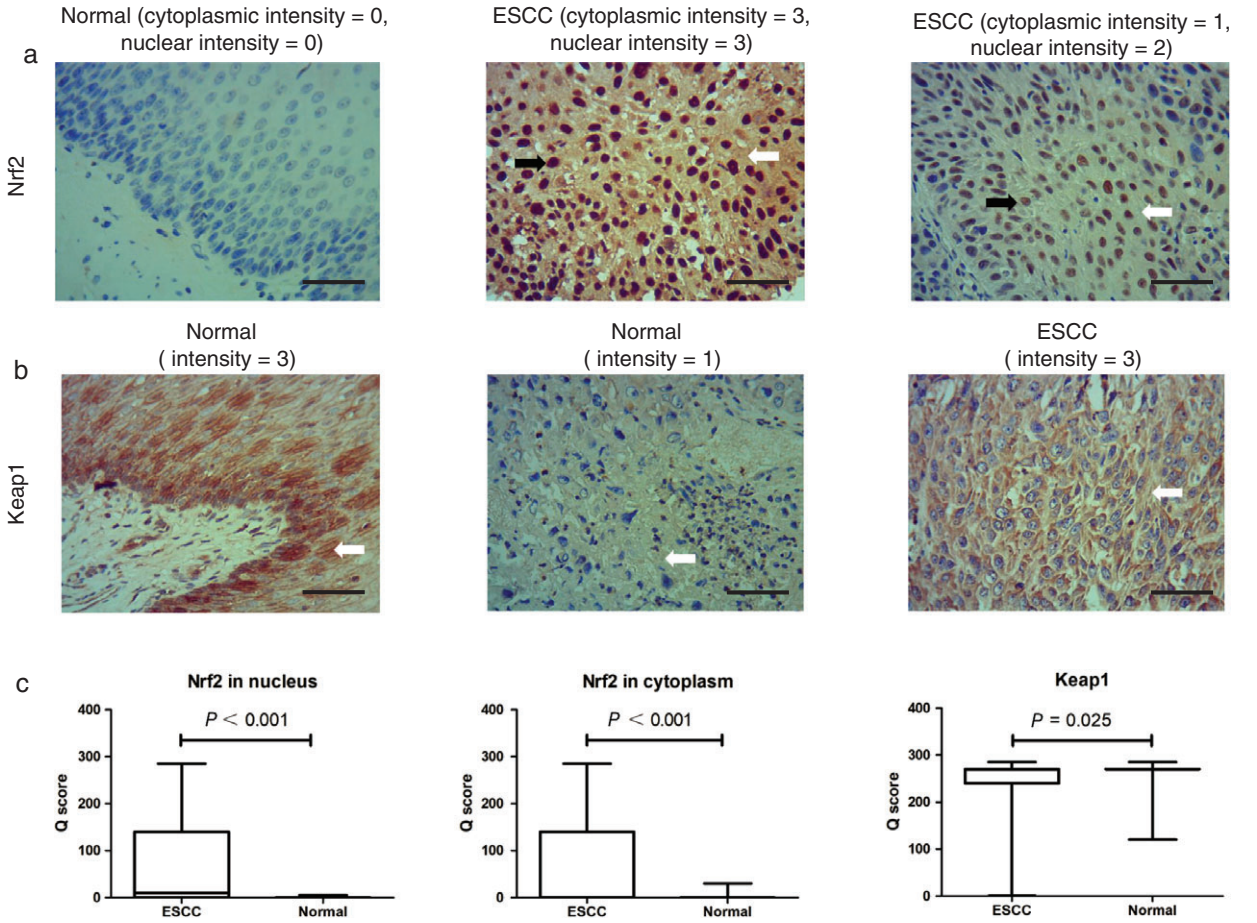

Figure 1 Nrf2 and Keap1 immunohistochemical stains in normal esophageal mucosae and esophageal squamous cell carcinoma (ESCC). (**a**) Representative cases of Nrf2 staining. Neither cytoplasmic nor nuclear expression of Nrf2 was common in normal esophageal mucosa (intensity = 0). ESCC samples displayed increased Nrf2 staining in both the cytoplasm and nucleus. (**b**) Representative cases of Keap1 staining. Keap1 expression was high in the normal esophageal mucosa (intensity = 3). ESCC showed various staining patterns of Keap1 and limited expression was common. (Original magnification = 400,Scale bar 50 μm). Black arrows indicate positive nuclear staining and white arrows indicate positive cytoplasmic staining. (**c**) Comparison of immunohistochemical Q scores of Nrf2 and Keap1 between ESCC and normal esophageal mucosae. The medium lines of boxes show the median value, the top and bottom lines of boxes represent the 75th and 25th percentiles, respectively; and the ends of whiskers represent the 10th and 90th percentiles.

disease (PD). Positive nuclear Nrf2 expression was associated with a significantly poorer response than negative Nrf2 expression (CR + PR: 71.62% vs. 87.18%; $P = 0.017$). By contrast, Keap1 and cytoplasmic Nrf2 expression were not associated with clinical response to CRT (Table 2).

Association between Nrf2 and Keap1 expression and survival after CRT

Kaplan–Meier survival analysis using log-rank tests indicated that positive nuclear Nrf2 expression was associated with poor PFS ($P = 0.010$) (Fig 4). Although the PFS curve of the high Keap1 expression group remained above the low Keap1 expression group, significance was not achieved ($P = 0.095$). By contrast, Nrf2 cytoplasmic expression and Keap1 expression were not associated with OS and only Nrf2 staining in the nucleus influenced OS by trend ($P = 0.075$). In univariate and multivariate Cox proportional hazard analyses of clinicopathologic characteristics for PFS, nuclear Nrf2 expression was validated as an independent prognostic factor, as well as age and N stage (Table 3).

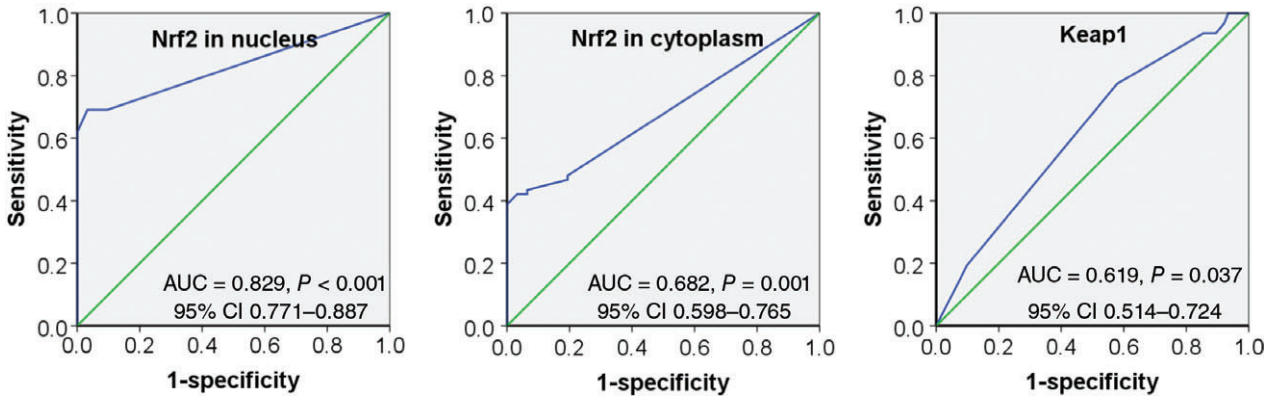

Figure 2 Receiver operating characteristic curve for prediction of esophageal squamous cell carcinoma using immunohistochemical Q scores of Nrf2 and Keap1. The area under the curves (AUC) of nuclear Nrf2, cytoplasmic Nrf2, and Keap1 Q scores were 0.829, 0.682, and 0.619, respectively. CI, confidence interval.

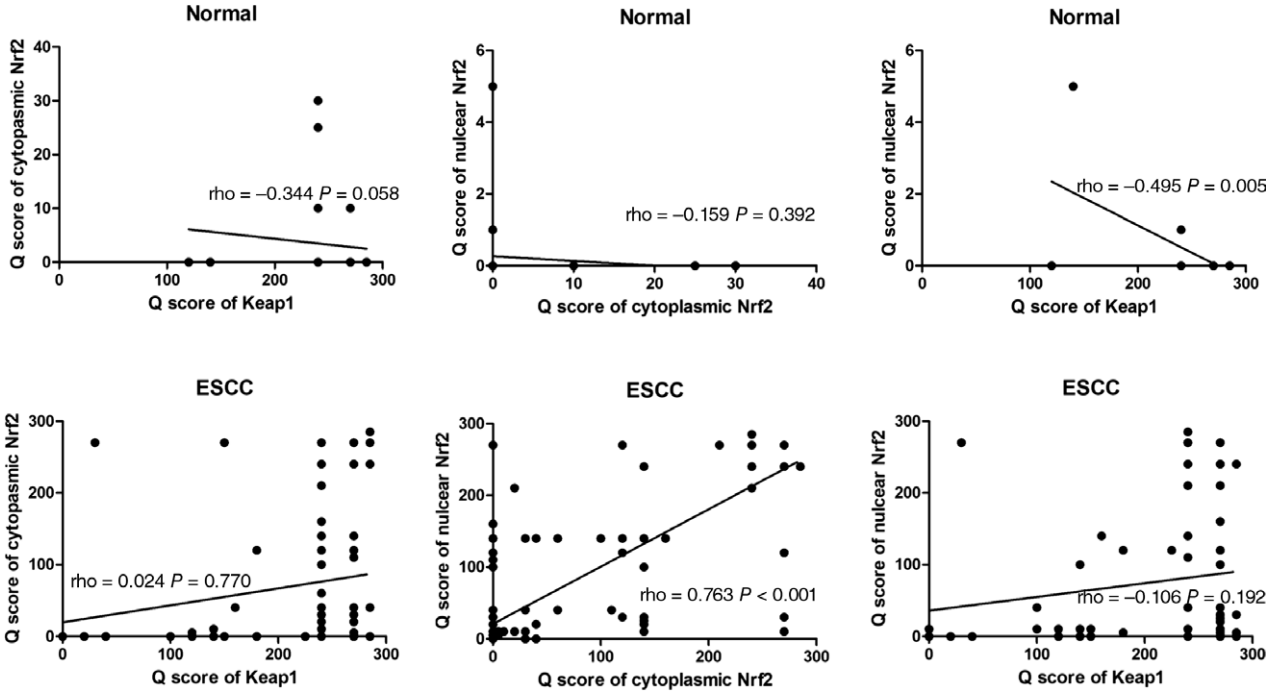

Figure 3 The relationships between Nrf2 and Keap1 expression are illustrated as scattered plots with linear regression lines. In normal esophageal mucosae, Keap1 was negatively correlated to both cytoplasmic and nuclear Nrf2, whereas there was no relationship between cytoplasmic and nuclear Nrf2. In ESCC, the relationship between Keap1 and Nrf2 in cytoplasm and nucleus disappeared, while cytoplasmic Nrf2 was positively correlated to nuclear Nrf2.

Discussion

In this study, Nrf2 and Keap1 protein expression differed between normal esophageal mucosa and ECSS samples. In normal esophageal mucosa, stable expression of Keap1 and little to no Nrf2 expression in the nucleus reflected the homeostatic condition of normal cells. By contrast, positive expression of Nrf2 and limited immunohistochemical staining of Keap1 were much more common in the ESCC samples, which implied that Keap1/Nrf2 signaling might be disturbed during the development or progression of ESCC. Moreover, the diagnostic significance of nuclear Nrf2 positive expression was well displayed in receiver operating characteristic analysis, indicating that excessive nuclear translocation of Nrf2 exclusively occurred in tumors. These findings are inconsistent with the results of previous studies of in vitro and mouse models, which found that Keap1 dysfunction and Nrf2 over-activation

Table 1 Relationship between Nrf2 and Keap1 expression and clinicopathologic characteristics in ESCCs

		Nuclear Nrf2			Cytoplasmic Nrf2			Keap1		
Characteristic	N	Negative	Positive	*P*	Negative	Positive	*P*	Low	High	*P*
All cases	152	78	74		79	73		64	88	
Age										
≤ 65	81	42	39	0.890	40	41	0.495	29	52	0.093
>65	71	36	35		39	32		35	36	
Gender										
Male	108	55	53	0.880	56	52	0.962	42	66	0.208
Female	44	23	21		23	21		22	22	
Smoking index										
<400	94	53	41	0.112	55	39	0.040*	39	55	0.845
≥ 400	58	25	33		24	34		25	33	
Alcohol intake										
Yes	59	30	29	0.927	26	33	0.363	27	32	0.467
No	93	48	45		43	40		37	56	
Location										
Upper	66	37	29	0.305	37	29	0.377	32	34	0.163
Lower	86	41	45		42	44		32	54	
T										
T2	18	9	9	0.921	9	9	0.642	8	10	0.962
T3	111	58	53		60	51		46	65	
T4	23	11	12		10	13		10	13	
N										
N0	32	16	16	0.867	20	12	0.180	14	18	0.832
N1	120	62	58		59	61		50	70	
Stage										
IIA	30	15	15	0.900	19	11	0.256	12	18	0.948
IIB	11	5	6		4	7		5	6	
III	111	58	53		56	55		47	64	

*$P < 0.05$. ESCC, esophageal squamous cell carcinoma.

Table 2 Correlation between Nrf2/Keap1 expression and clinical response

		Clinical response			
Protein	Expression	CR + PR	SD + PD	χ2	*P*
Nrf2 in nucleus	Negative (%)	68(87.18%)	10(12.82%)	5.661	0.017*
	Positive (%)	53(71.62%)	21(28.34%)		
Nrf2 in cytoplasm	Negative (%)	64(81.01%)	15(18.99%)	0.201	0.654
	Positive (%)	57(78.08%)	16(21.92%)		
Keap1	Low (%)	48(75.00%)	16(25.00%)	1.444	0.229
	High (%)	73(82.95%)	15(17.05%)		
Total		121(79.61%)	31(20.39%)		

*$P < 0.05$. CR, complete response; PD, progressive diseases; PR, partial response; SD, stable disease.

could facilitate normal cells to gain histological and molecular features of cancers.[17,32]

The different correlation patterns of Nrf2 and Keap1 protein expression in the normal esophageal mucosa and ECSS samples also present aberrant signaling of the Keap1/Nrf2 pathway. Keap1, the inhibitor of the pathway, also acts as an adaptor in the Cul3-based E3 ligase complex which ubiquitinates Nrf2 binding with Keap1.[19] Thus, in functional signaling, Nrf2 always degrades in a Keap1-dependent manner and Nrf2 expression should be negatively correlated with Keap1. Our Spearman test in normal esophageal mucosa confirmed this point. We also found that the relationship between Keap1 and nuclear Nrf2 is stronger than that of Keap1 and cytoplasmic Nrf2, probably because of the cytoplasmic anchoring effect of Keap1 on Nrf2. By contrast, in the ECSS samples, the negative correlation between the two proteins disappeared.

A reasonable explanation is the disrupted interaction between Keap1 and Nrf2, which could result from somatic mutation. While ECSS rarely harbored Keap1 mutations,

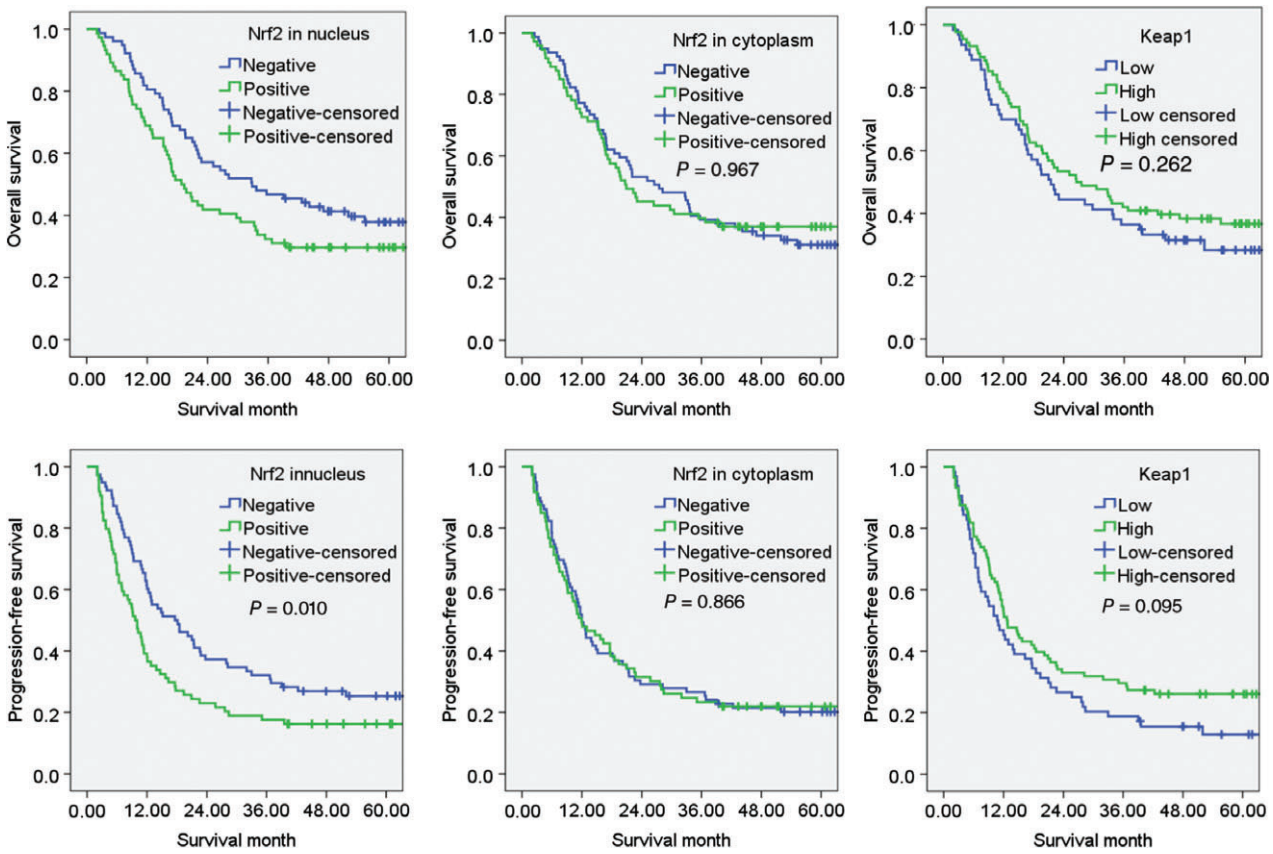

Figure 4 Kaplan–Meier curves for overall survival (OS) and progression-free survival (PFS) according to Nrf2 expression in the nucleus and cytoplasm and Keap1 expression in esophageal squamous cell carcinoma (ESCC) samples. Positive Nrf2 nuclear expression was significantly related to short PFS and predicted prolonged OS without significance. Negative, Positive, Negative-censored, Positive-censored; Negative, Positive, Negative-censored, Positive-censored; Negative, Positive, Negative-censored, Positive-censored; and Negative, Positive, Negative-censored, Positive-censored. Low Keap1 expression was associated with slightly improved PFS, but no significance was achieved. Low, High, Low-censored, High-censored; Low, High, Low-censored, High-censored.

Table 3 Cox regression analyses for progression-free survival

		Univariate analysis		Multivariate analysis	
Characteristic		HR (95% CI)	*P*	HR (95% CI)	*P*
Age	≤ 65 vs. > 65 years	0.692 (0.484–0.990)	0.044*	0.618 (0.420–0.909)	0.015*
Gender	Female vs. male	0.992 (0.665–1.481)	0.992	0.868 (0.559–1.348)	0.529
Location	Lower vs. upper	0.939 (0.655–1.345)	0.730	0.972 (0.673–1.405)	0.881
T	T4 vs. T2/T3	1.090 (0.667–1.781)	0.731	0.923 (0.561–1.519)	0.753
N	N1 vs. N0	1.905 (1.176–3.086)	0.009*	2.077 (1.278–3.374)	0.003*
Nrf2 in nucleus	Negative vs. positive	0.629 (0.439–0.900)	0.011*	0.606 (0.419–0.877)	0.008*
Keap1	Low vs. high	1.356 (0.946–1.942)	0.097	1.314 (0.903–1.910)	0.154

*$P < 0.05$. CI, confidence interval; HR, hazard ratio.

Nrf2 mutations were frequent, at a rate of 11.4–22%.[29,34] All mutations impaired the binding affinity of motifs, which are the binding sites with Keap1.[29,34] Hence, Keap1 lost function in Nrf2 mutated cells and Nrf2 was constitutively translocated into the nucleus. Unfortunately because every single endoscopically collected specimen was limited, DNA extraction and sequencing was unavailable. However, the positive nuclear Nrf2 staining rate of ECSS was 48.68%, much higher than Nrf2 mutation rates reported in previous studies, indicating the existence of other involved mechanisms. Indeed, recent studies have proven this point. Several disruptor proteins were identified, including p62 and PALB2, which can compete with Nrf2 to bind Keap1, thus resulting in Nrf2 over-activation.[35–39] *K-ras*, *B-raf*, and *Myc*

oncogene activation and PTEN anti-oncogene disruption could upregulate the transcription of Nrf2.[40–42] Furthermore, Keap1 promoter methylation and microRNA-targeting Keap1 have also been found in several cancers.[43–47] In summary, Nrf2 hyperactivity could be induced in diverse ways during the carcinogenic progress. Therefore, the positive correlation between cytoplasmic and nuclear Nrf2 immunostaining in ECSS could reflect Keap1-independent upregulation of Nrf2.

Chemoradiotherapy with 5-fluorouracil and cisplatin is the standard treatment for locally advanced ECSS.[4,5] ROS formation is indispensible in the mechanism underlying its therapeutic effect. About two-thirds of X-ray damage is caused by ROS generation via ionization of water molecules,[18] and both 5-fluorouracil and cisplatin induce apoptosis in a ROS-dependent fashion.[8,9,12] Therefore, in cancer cells with high Nrf2 activity, the cytotoxic efficacy of CRT should be heavily impaired as a result of antioxidant enzyme upregulation. A series of previous studies proved this viewpoint in cells and animal models. Tian *et al.* found that modification of Nrf2 and Keap1 expression changed cancer cell line sensitivity to platinum-based drugs.[31] Lee *et al.* demonstrated that the functional inhibition of Nrf2 led to radiosensitivity enhancement in cells and mice xenografts.[13] Other laboratory research has reached similar conclusions.[17,29] Moreover, although rare, Kawasaki *et al.* conducted a relevant clinical trial and found that Nrf2 expression was related to CRT outcomes in patients with ESCC.[30]

In regard to prognostic analysis, our findings were similar to those of Kawasaki *et al.* Using a much larger sample, we show that positive nuclear Nrf2 staining is associated with poor prognosis after CRT. The results demonstrate that CRT could induce a significantly higher objective response rate in patients with negative Nrf2 expression in the nucleus. In survival analysis, nuclear Nrf2 status only influenced a trend of OS after CRT and positive nuclear Nrf2 was significantly associated with poor PFS. Moreover, Nrf2 in the nucleus was identified as an independent prognostic factor of PFS. All of our results are consistent with those of previous studies and suggest that excessive nuclear translocation of Nrf2 indicates an impaired therapeutic effect of CRT.

We also analyzed the role of cytoplasmic Nrf2 and Keap1 expression in CRT for ESCC. In contrast to nuclear Nrf2, Nrf2 in the cytoplasm had no effect on response or survival rates after CRT. Perhaps high cytoplasmic expression of Nrf2 reflects downregulated degeneration and/or enhanced transcription of the protein, but not activation. Similarly, the prognostic value of Keap1 was marginal and its low expression indicated slightly poorer PFS, while high Keap1 expression indicated survival in lung squamous cell carcinoma.[26] The lack of Keap1 influence on CRT in ESCC is probably a result of Nrf2, which can be activated in diverse ways, many of which are independent of Keap1 regulation. The loss of a negative correlation between nuclear Nrf2 and Keap1 in ESCC specimens indicates this is the case.

We examined the differences in Nrf2 and Keap1 expression between normal esophageal mucosa and ECSS samples. The promoting role of aberrant Keap1-Nrf2 signaling in carcinogenesis was proven in a clinical setting. Positive Nrf2 expression in the nucleus was associated with poor prognosis of ESCC after CRT. The results of this study imply that hyperactivity of Nrf2 contributes to cancer genesis and resistance of CRT in ESCC. Therefore the Keap1/Nrf2 pathway should be a key target of novel therapy in future and deserves more attention.

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Disclosure

No authors report any conflict of interest.

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