RESEARCH ARTICLE

Nrf2 Overexpression Predicts Prognosis and 5-FU Resistance in Gastric Cancer

Xiu-Feng Hu, Jun Yao, She-Gan Gao, Xin-Shuai Wang, Xiu-Qing Peng, Yan-Tong Yang, Xiao-Shan Feng*

Abstract

Objective: NF-E2-related factor 2 (Nrf2) is activated in several human malignancies. However, the role of Nrf2 in gastric cancer (GC) remains incompletely understood. In this study, we therefore analyzed associations of Nrf2 expression status with clinical features and chemotherapeutic resistance in GC. Materials and Methods: A total of 186 samples from GC patients who underwent gastrectomy were used for prognostic assessment. A further 142 samples from GC cases who received first-line combination chemotherapy were applied for investigation of chemoresistance. The Nrf2 expression was evaluated by immunohistochemistry in GC samples, and its relationship with clinicopathological parameters and chemotherapy sensitivity was analyzed. The effect of Nrf2 gene silencing on chemotherapy resistance was also examined by cell viability assay in vivo. Results: Of the 186 patients with GC, 104/186 (55.9%) showed high expression for Nrf2. The overexpression of Nrf2 was an independent predictor of overall survival [OS, hazard ratio (HR) 3.9; *P*=0.011] and disease-free survival (DFS, HR 4.3; *P*=0.002). The gene silencing of Nrf2 reduced resistance to cell death induced by 5-FU in GC cell lines. Conclusion: Our data show that Nrf2 is an independent prognostic factor in GC. Furthermore, Nrf2 confers resistance to chemotherapeutic drug 5-FU in GC cells. Taken together, Nrf2 is a potential prognostic marker and predictive for 5-FU resistance in GC.

Keywords: Gastric cancer - Nrf2 - drug resistance - prognosis

Asian Pac J Cancer Prev, **14 (9)**, 5231-5235

Introduction

Gastric cancer (GC) is one of the most common malignancies in the digestive tract (Huang et al., 2012). Despite rapid advances in diagnosis and treatment, GC is the second leading cause of cancer death worldwide (Ferlay et al., 2010). The prognosis of GC patients with advanced disease remains poor and combination chemotherapy remains the main treatment options (Wagner et al., 2010). Recently, it has been reported that doublet and triplet regimens, especially taxane-based regimens, are helpful in the treatment of advanced or recurrent GC (Ajani et al., 2007; Yamamoto et al., 2009). Therefore, identification of potential markers for predicting prognosis and the chemotherapy sensitivity is critical for improving therapeutic effects for patients with GC.

NF-E2-related factor 2 (Nrf2) is a member of the transcription factors, and past data have shown confers protection against carcinogensis, however, new emerging data has revealed the "dark" side of Nrf2 (Lau et al., 2008). In established cancer, Nrf2 can protect cancer cells from the oxidative damage induced by chemotherapeutic drugs and radiation (Zhang et al., 2010). In lung tumors, strong expression of Nrf2 was associated with a poorer survival (Merikallio et al., 2012). High Nrf2 expression predicts chemoresistance and tumor progression in patients with advanced-stage non-small-cell lung cancer (Yang et al., 2011). Nrf2 knockdown inhibits tumor growth and increases efficacy of chemotherapy in cervical cancer (Ma et al., 2012). Nrf2 mutation confers malignant potential and resistance to chemoradiation therapy in advanced esophageal squamous cancer (Shibata et al., 2011). These studies suggest that Nrf2 may be involved in the progression and drug resistance in tumor biology. However, it remains unclear whether Nrf2 participates in the tumor progression and drug resistance in GC.

In this study, we examined the expression of Nrf2 protein in GC specimens and assessed the correlations between Nrf2 expression and clinicopathological characteristics. In addition, we investigated the relationship between expression of Nrf2 and drug resistance to chemotherapeutic agents in GC patients treated with chemotherapy and GC cell lines.

Materials and Methods

Patients and samples

This study was approved by the Ethics Committee

*Department of Oncology, Cancer Research Institute, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang, Henan, China *For correspondence: xiaoshan_feng@126.com*

of Henan University of Science and Technology. Prior informed consent was obtained from each patient regarding the use of biopsy and specimens.

For prognostic investigation, GC tissues were obtained from 186 patients who underwent gastrectomy at the Department of Oncology, Cancer Research Institute, the First Affiliated Hospital of Henan University of Science and Technology, from Jan. 2003 to Jan. 2009. All tumors were histologically diagnosed as gastric adenocarcinoma by two independent pathologists and were subjected to immunohistochemistry. None of these patients had received treatment such as chemotherapy and/or radiotherapy before operation. The median follow-up period was 36 months (range 3.0-108 months).

For chemoresistance investigation, 142 GC specimens were obtained via biopsy under endoscopy from gastric cancer patients who underwent chemotherapy and were subjected to immunohistochemistry.

Chemotherapeutic regimens and treatment responses

Seventy-three of 142 patients received combination chemotherapy of docetaxel, cisplatin, and 5-fluorouracil (5-FU) (DFP) before surgery. The remaining 69 patients were treated with combination chemotherapy of S-1 plus cisplatin (SP). In DFP therapy, treatment included docetaxel (60 mg/m^2 on day 1), 5-FU (350 mg/m^2/day continuously intravenous administration on days 1-5), and cisplatin (10 mg/m^2/day on days 1-5), which was repeated twice every 28 days before evaluating treatment responses using computed tomography and gastric endoscopy. In SP therapy, the treatment consisted of S-1 (80 mg/m^2/day on days 1-21) and cisplatin (60 mg/m^2 on day 8), which was repeated twice every 35 days before evaluating therapeutic responses.

Tumor response was assessed by computed tomography after two cycles of either treatment according to the Response Evaluation Criteria in Solid Tumors criteria (Therasse et al., 2000). Complete response (CR) was defined as the absence of all evidence of cancer for more than 4 weeks. Partial response (PR) was defined as more than a 50% reduction in the sum of the products of the perpendicular diameters of all lesions without any evidence of new regions or progression of any lesions. Stable disease (SD) was defined as less than a 50% reduction or less than a 25% increase in the sum of the products of the perpendicular diameters of all lesions, without any evidence of new lesions. Progressive disease (PD) was defined as more than a 25% increase in more than one region or the presence of new regions.

Immunohistochemical (IHC) analysis

Nrf2 expression was evaluated by IHC staining according to a previously described method (Jala et al., 2013). Tissue slides were deparaffinized in xylene and then rehydrated through graded ethanol. Tissue slides were incubated overnight with Nrf2 antibody (dilution 1:1,000, Santa Cruz) at 4°C. Sites of antibody binding were visualized with the ABC peroxidase detection system (Vector Laboratories). Finally, sections were counterstained with 0.1% hematoxylin. The percentage of cancer cells with positive staining was evaluated.

IHC staining was evaluated by two independent pathologists, who were blinded to the clinical data. In the present study, the scoring pattern for Nrf2 staining was as follows: score 0, negative staining for all cells; score 1+, weakly positive for cytosolic staining in <10% of cells; score 2+, moderate to strong positive staining covering between 10 to 50% of cells and score 3+, strongly positive staining including >50% cells. For statistical purposes,IHC scores were grouped into two groups, low expression (0 and 1+) and high expression/overexpresion (2+ and 3+).

Cell lines and cultures

Human gastric epithelial cells (HGEC) and human GC cell lines including HGC-27, AGS, MKN28, and MGC803, were obtained from the American Type Culture Collection and kept in our laboratory. All cell lines were maintained in Dulbecco's Modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37°C.

RNA extraction and quantitative RT-PCR

Total cellular RNA was extracted from each cell line with Trizol Reagent (Invitrogen) according to the manufacturer's protocol. The primer sequences for quantitative RT-PCR (qPCR) amplification were as follows, Nrf2: 5'-TCCAGTCAG AAACCAGTGGAT-3'(forward) and 5'-GAATGTCTGCGCCAAAAGCTG-3'(reverse), GAPDH was used as an internal control: 5'-ACAACTTTGGTATCGTGGAAGG-3' (forward) and 5'-GCCATCACGCCACAGTTTC-3' (reverse). The emission intensity of SYBR Green was detected in a LightCycler (Roche Diagnostics). The Nrf2 expression was adjusted by GAPDH in each sample.

Nrf2 small interfering RNA

Cells were cultured up to 80% confluence and transfected with 5 nmol/L of Nrf2 small interfering RNA (siNrf2) or negative control scrambled RNA (Life Technologies) with the siPORT Amine Agent (Life Technologies) as per the manufacturer's protocol. After transfection, cells were cultured for 48h and samples were collected at 24 and 48 h and were analyzed by qRT-PCR, Western blotting and MTT assay.

Western blot

GC tissues or adherent cells were washed with PBS and lysed in RIPA buffer (Qiagen), and the supernatant was collected. Equal amounts of cell extracts were fractionated by SDS-PAGE (Bio-Rad Laboratories) and transferred onto membranes (Millipore). After blockade with milk, membranes were incubated overnight at 4°C with primary antibodies and with secondary antibodies for 1 hour at room temperature. The following antibodies were used in this study: anti-GAPDH (1:1,000; Abcam) and anti-Nrf2 (1:1,000; Abcam). Enhanced chemiluminscence substrate was used to detect the signal.

Cell viability assay and chemotherapeutic agents

The MTT assay was used to assess cell viability. Cells were seeded onto 96-well plates at a concentration of

Table 1. Association of Nrf2 Expression with Clinicopathological Features in Gastric Cancer

Variable	Entire group	Nrf2 expression		*p* value
	(n=186)	Low (n=82)	High (n=104)	
Age				0.653
< 70 years	101	44	73	
≥ 70 years	85	38	31	
Gender				0.119
Male	107	42	70	
Female	79	40	34	
Tumor size				0.013*
≥ 5 cm	69	45	62	
< 5 cm	117	37	42	
Lymphatic invasion				0.009*
No	51	31	21	
Yes	135	51	83	
Venous invasion				0.016*
No	81	39	65	
Yes	105	43	39	
Differentiation status				0.86
Well	12	21	20	
Moderate	109	34	49	
Poor and others	65	27	35	
TNM stage				0.005*
I-II	53	32	25	
III-IV	133	50	79	

TNM, T, tumor; N, lymph node; M, distant metastasis; *p<0.05

5×10^3 per well and incubated overnight under the standard culture conditions; they were exposed to each of docetaxel, cisplatin, and 5-FU (Shanghai Zhongxi Biopharm Ltd., China) at various concentrations. After 10 μl of MTT solution was added to each well, the plates were incubated for 4 h at 37°C. The absorbance was measured at 570 nm, with 655 nm as the reference wavelength. All experiments were performed in triplicates. Docetaxel was dissolved in DMSO. 5-FU and cisplatin were dissolved in RPMI 1640.

Statistical analysis

Statistical analysis was performed with SPSS16.0. The relationship between Nrf2 expression and various clinicopathological parameters was assessed by the Chi-square test. Overall survival (OS) and disease-free survival (DFS) were evaluated using the Kaplan-Meier method and log-rank test. All parameters that were found to be significant on univariate analysis by the Cox proportional hazard model entered into multivariate survival analysis. P<0.05 were considered significant.

Results

Nrf2 expression and its association with clinicopathological features in GC

Immunohistochemistry was performed in 186 GC samples. As shown in Figure 1A, Nrf2 protein was mainly located in the cytoplasm. Nrf2 was significantly overexpressed (2-3 score) in 55.9% (104/186) GC cases. In contrast, none of adjacent noncancerous tissues showed Nrf2 overexpression. Further, using Western blot in GC, we confirmed the higher expression of Nrf2 in GC samples than adjacent noncancerous tissues (Figure 1B).

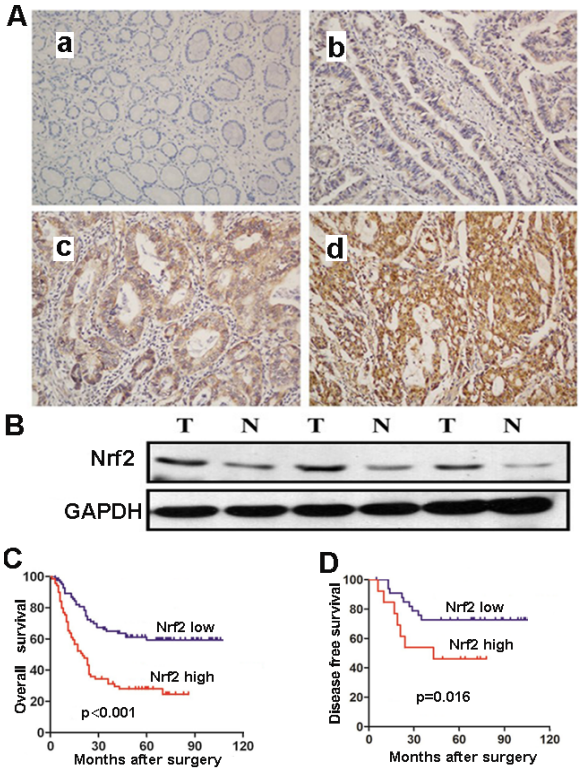

Figure 1. Prognostic Significances of Nrf2 Overexpression in 186 Gastric Cancer (GC) Patients. (A) Representative photos of Nrf2 expression in GC patients. a. Negative staining of Nrf2 in adjacent normal tissues; b.Weak staining of Nrf2 in GC tissues. c. Moderate staining of Nrf2 in GC tissues; d. Strong staining of Nrf2 in GC tissues (amplification×100). (B) Western blot images of GC and corresponding adjacent normal tissues. (C) Kaplan-Meier analysis of overall survival (OS) based on Nrf2 expression in all 186 GC patients. (D) Kaplan-Meier analysis of disease-free survival (DFS) based on Nrf2 expression in all 186 GC patients

The correlation between Nrf2 expression and clinicopathological characteristics was shown in Table 1. Nrf2 overexpression was positively correlated with tumor size (P=0.013), lymph node invasion (P=0.009), venous invasion (P=0.016) and TNM stage (P=0.005). In addition, Nrf2 expression correlated significantly with correlated significantly with poor OS and DFS (Figure 1C, D).

Prognostic significance of Nrf2 expression in GC

Cox's proportional hazard model was further used to examine the various correlations between various parameters and survival of patients (Table 2). Univariate analysis showed that TNM stage, lymph node invasion and Nrf2 expression correlated with OS. However, multivariate analysis identified Nrf2 expression as the only significantly independent prognostic factor for OS of GC patients (HR=3.9). Associations between various clinicopathological parameters and DFS were also analyzed by Cox's proportional hazard model (Table 2). Univariate analysis showed that TNM stage, vessel invasion, lymph node invasion, and Nrf2 expression significantly correlated with DFS. Multivariate analysis identified TNM stage and Nrf2 expression as independent prognostic predictors for DFS of GC patients (HR=3.3 and 4.3, respectively).

Table 2. Univariate and Multivariate Analyses of Overall and Disease-free Survival

Variable	Univariate analysis			Multivariate analysis		
	HR	95 % CI	*p*	HR	95 % CI	*p*
Overall survival						
Age (<70/≥70 years)	1.8	0.7–4.6	0.162			
Gender (F/M)	2.1	0.8–6.8	0.196			
Tumor size						
TNM	7.8	2.3–24.5	0.001	2.6	0.1-3.4	0. 87
Vessel invasion	1.9	0.8–4.5	0.134			
Lymph node invasion	4.2	1.3–16	0.019	0.42	0..02-4.7	0.546
Differentiation status (well, moderate/poor)	1.7	0.7–4.0	0.278			
Nrf2 (low/high)	4.8	1.5–30.2	0.012	3.9	1.1-18.9	0.011
Disease-free survival						
Age (<70/≥70 years)	1.4	0.6–3.4	0.354			
Gender (F/M)	2	0.8–6.1	0.129			
Tumor size						
TNM	10.2	3.4–20.8	<0.001	3.3	1.2-42.7	0.042
Vessel invasion	2.6	1.2–5.9	0.017	1.4	0.5-3.7	0.43
Lymph node invasion	5.4	1.4–34.2	0.003	0.65	0.1-5.9	0.69
Differentiation status (well, moderate/poor)	1.3	0.7–3.2	0.419			
Nrf2 (low/high)	3.9	1.5–26.9	0.008	4.3	1.2-16.1	0.002

Table 3. Association of Nrf2 Expression with Therapeutic Responses

	Nrf2 expression in cases with DFP		Nrf2 expression in cases with SP	
Response	Low (n =22)	High (n =51)	Low (n=25)	High (n =44)
CR	0	0	0	1
PR	17	16	10	25
SD	5	27	10	12
PD	0	8	5	6

DFP combination chemotherapy with docetaxel, 5-fluorouracil (5-FU), and cisplatin; SP combination chemotherapy with an oral-intake 5-FU derivative, S-1 plus cisplatin Complete response (CR); Partial response (PR); Stable disease (SD); Progressive disease (PD)

Nrf2 expression and response to chemotherapy

We also investigated the association between clinical response to chemotherapy and Nrf2 expression (Table 3). Among the 73 specimens treated with DFP, 51 (69.9%) were diagnosed as overexpression and 22 (30.1%) as low expression for Nrf2 by immunohistochemistry. The response rate to DFP therapy in 51 patients with Nrf2 overexpression was 31.4% (16/51), while the response rate to DFP therapy in 22 patients with low Nrf2 expression was 77.3% (17/22). There was a significant negative correlation between clinical response to DFP therapy and Nrf2 expression (p=0.014). Among the 69 specimens treated with SP therapy, 44 (63.8%) were diagnosed as overexpression and 25 (36.2%) as low expression for Nrf2 by immunohistochemistry. The response rate to SP therapy in 44 patients with high Nrf2 expression was 56.8% (25/44) and in 25 patients with low Nrf2 expression was 40% (10/25). There was no relationship between clinical response to SP therapy and Nrf2 expression (*p*=0.392).

Knockdown of Nrf2 reduced the resistance to 5-FU

First, we detected the Nrf2 protein expression in HGEC and four human GC cell lines including HGC-27, AGS, MKN28, and MGC803. We found that the protein expression of Nrf2 was higher in each of the four GC cell lines than that in the control (HGEC) cells, and the highest expression was observed in MGC803, as indicated by Western blot (Figure 2A).

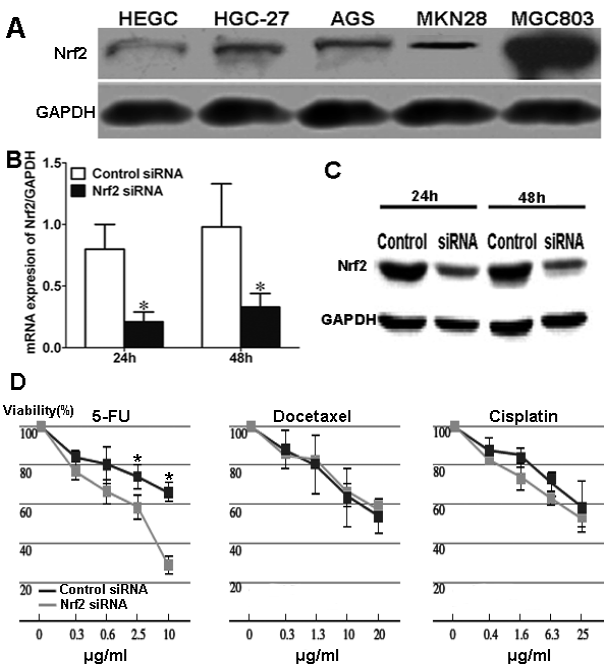

Figure 2. Knockdown of Nrf2 Recovers Chemosensitivity for 5-FU in Gastric Cancer (GC) Cells. (A) Western blot analysis of Nrf2 expression in all 4 GC cell lines. (B) qRT-PCR analysis of Nrf2 expression after siRNA transfection. (C) Western blot analysis of Nrf2 expression after siRNA transfection. D.MTT assay with MGC803 cells transfected with siNrf2 exposed to 5-FU, docetaxel and cisplatin

Next, we chose the MGC803 cells, with the highest Nrf2 expression, for the gene silencing experiment. qRT-PCR and Western blot analysis indicated that Nrf2 siRNA reduced the expression of Nrf2 at mRNA and protein levels, compared with that of control siRNA (Figure 2B, C). We examined chemosensitivity to docetaxel, cisplatin, and 5-FU by the MTT assay using Nrf2 siRNA-treated MGC803 cells for 48 h and control siRNA-treated cells. The MTT assay showed that chemosensitivity to 5-FU was significantly increased in MGC803 cells by Nrf2 siRNA (Figure 2D, IC50; 21 vs. 2.9 μg/ml), while resistance to cisplatin and docetaxel remained unchanged.

Discussion

Nrf2 shows dual roles in cancer, but more emerging data support its role as a protooncogene (Shelton et al., 2013). In the present study, we found Nrf2 was significantly overexpressed in the GC samples and GC cell lines. And we examined the clinical significance of Nrf2 overexpression in human GC. As a result, Nrf2 overexpression was an independent prognostic factor for OS and DFS in human GC. Furthermore, we also found enhanced Nrf2 expression was associated with 5-FU resistance in GC patients and cell lines. Our data also showed that downregulation of Nrf2 improved the sensitivity of chemotherapy in GC cell lines. Taken together, this study indicates that Nrf2 will be a promising marker for predicting prognosis and chemoresistance of GC.

Intriguingly, multiple human cancers frequently exhibit overexpresion levels of Nrf2. Overexpression of Nrf2 resulted in enhanced resistance of cancer cells to chemotherapeutic agents and downregulation of the Nrf2 rendered cancer cells more susceptible to these drugs in human lung carcinoma, breast adenocarcinoma and neuroblastoma cells (Wang et al., 2008). Activation of Nrf2 confers radioresistance in non-small-cell lung cancer cells (Singh et al., 2010). Inhibition of Nrf2 in DU-145 cells enhanced sensitivity to chemotherapeutic drugs and radiation-induced cell death, and greatly suppressed in vitro and in vivo tumor growth of DU-145 prostate cancer cells (Zhang et al., 2010). Cancer cells acquire malignant properties by hijacking the Keap1-Nrf2 system (Yoichiro et al., 2012). Indeed, the prognosis of Nrf2-positve patients with lung cancer is significantly poor (Solis et al., 2010; Inoue et al., 2012). Consistently, our data show that enhanced Nrf2 expression predicts in GC. Furthermore, Nrf2 confers 5-FU resistance in GC patients and cell lines. These data indicate that dysfunction of Nrf2 can be both a cause of GC and chemotherapy resistance in GC. Nevertheless, detailed mechanisms remain unclear and merit further investigation.

The present novel finding in may provide therapeutic target for the sensitivity of GC and could be applied to treat chemotherapy resistance in GC patients. Future investigations are needed to further support the pathogenic function of Nrf2 in GC prognosis and drug resistance. These investigations may help with the development of personalized treatment for patients who have abnormal levels of Nrf2.

Acknowledgements

The author(s) declare that they have no competing interests.

References

Ajani JA, Moiseyenko VM, Tjulandin S, et al (2007). Clinical benefit with docetaxel plus fluorouracil and cisplatin compared with cisplatin and fluorouracil in a phase III trial of advanced gastric or gastroesophageal cancer adenocarcinoma: the V-325 Study Group. *J Clin Oncol*, **25**, 3205-9.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Huang JY, Xu YY, Sun Z, et al (2012). Comparison different methods of intraoperative and intraperitoneal chemotherapy for patients with gastric cancer: a meta-analysis. *Asian Pac J Cancer Prev*, **13**, 4379-85.

Inoue D, Suzuki T, Mitsuishi Y, et al (2012). Accumulation of p62/SQSTM1 is associated with poor prognosis in patients with lung adenocarcinoma. *Cancer Sci*, **103**, 760-6.

Jala VR, Radde BN, Haribabu B, Klinge CM (2012). Enhanced expression of G-protein coupled estrogen receptor (GPER/GPR30) in lung cancer. *BMC Cancer*, **12**, 624.

Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD (2008). Dual roles of Nrf2 in cancer. *Pharmacol Res*, **58**, 262-70.

Ma X, Zhang J, Liu S, et al (2012). Nrf2 knockdown by shRNA inhibits tumor growth and increases efficacy of chemotherapy in cervical cancer. *Cancer Chemother Pharmacol*, **69**, 485-94.

Merikallio H, Pääkkö P, Kinnula VL, Harju T, Soini Y (2012). Nuclear factor erythroid-derived 2-like 2 (Nrf2) and DJ1 are prognostic factors in lung cancer. Hum Pathol, 43, 577-84.

Shelton P, Jaiswal AK (2013). The transcription factor NF-E2-related factor 2 (Nrf2): a protooncogene? *FASEB J*, **27**, 414-23.

Shibata T, Kokubu A, Saito S, et al (2011). NRF2 mutation confers malignant potential and resistance to chemoradiation therapy in advanced esophageal squamous cancer. *Neoplasia*, **13**, 864-73.

Singh A, Bodas M, Wakabayashi N, Bunz F, Biswal S (2010). Gain of Nrf2 function in non-small-cell lung cancer cells confers radioresistance. *Antioxid Redox Signal*, **13**, 1627-37.

Solis LM, Behrens C, Dong W, et al (2010). Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. *Clin Cancer Res*, **6**, 3743-53.

Therasse P, Arbuck SG, Eisenhauer EA, et al (2000). New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*, **92**, 205-16.

Wagner AD, GrotheW, Haerting J, et al (2006). Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. *J Clin Oncol*, **24**, 2903-9.

Wang XJ, Sun Z, Villeneuve NF, et al (2008). Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis*, **29**, 1235-43.

Yamamoto K, Fujiwara Y, Nishida T, et al (2009). Induction chemotherapy with docetaxel, 5-FU and CDDP (DFP) for advanced gastric cancer. *Anticancer Res*, **29**, 4211-5.

Yang H, Wang W, Zhang Y, et al (2011). The role of NF-E2-related factor 2 in predicting chemoresistance and prognosis in advanced non-small-cell lung cancer. *Clin Lung Cancer*, **12**, 166-71.

Yoichiro Mitsuishi, Hozumi Motohashi, Masayuki Yamamoto (2012). The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. *Front Oncol*, **2**, 200.

Zhang DD (2010). The Nrf2-Keap1-ARE signaling pathway: The regulation and dual function of Nrf2 in cancer. *Antioxid Redox Signal*, **13**, 1623-6.

Zhang P, Singh A, Yegnasubramanian S, et al (2010). Loss of Kelch-like ECH- associated protein 1 function in prostate cancer cells causes chemoresistance and radioresistance and promotes tumor growth. *Mol Cancer Ther*, **9**, 336-46.