

ORIGINAL ARTICLE

Overexpression of NRF2 is correlated with prognoses of patients with malignancies: A meta-analysis

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Keywords

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Abstract

Background: Previous published research has demonstrated that NRF2 expression is a poor prognostic factor for many malignancies. However, because of the small sample enrolled in a single study, it is difficult to draw valuable conclusions. Therefore, we hypothesized that NRF2 overexpression in cancer tissues may be associated with the prognoses of patients with solid malignancies, and conducted a systemic review and meta-analysis.

Methods: A comprehensive search of PubMed, Web of Science, Science Direct, Embase, and Ovid databases for relevant studies regarding the role of NRF2 expression in solid malignancies was conducted. Hazard ratios (HR) and 95% confidence intervals (CIs) were extracted from these studies to provide pooled estimates of the effect of NRF2 expression on patients' overall and disease-free survival.

Results: Nine studies met the criteria for analysis. Statistical analysis demonstrated that compared to patients with low NRF2 expression, patients with overexpression of NRF2 had poorer overall survival (HR 2.01, 95% CI 1.57–2.56; $P < 0.001$) and disease-free survival (HR 3.25, 95% CI 1.29–8.15; $P = 0.025$).

Conclusion: Published evidence of the role of NRF2 expression in survival of cancer patients is limited. This analysis supports the view that NRF2 overexpression is a poor prognostic factor for solid malignancies, thus optimizing treatment for patients with NRF2 overexpression may improve their overall survival.

Introduction

NRF2, which belongs to the basic leucine zipper family, is an important regulatory factor for maintaining the balance of oxidative stress under physical conditions.¹ NRF2 is widely spread in organs and could regulate some phase II metabolizing enzyme and antioxidant genes that contain antioxidant response elements in the promoter region, such as NAD(P)H-quinone oxidoreductase 1 (*NQO1*), glutathione S-transferases (*GSTs*), heme oxygenase-1 (*HO-1*), and other genes regulating the response to oxidative stress.² Currently, NRF2 demonstrates two effects in carcinogenesis: tumor suppression and tumor promotion. Temporary activation of NRF2 could protect normal cells from toxins or carcinogens, but sustained activation of NRF2 will upregulate the expression of downstream genes, providing a conducive environment for malignant cells and facilitating

cancer progression.^{3–5} NRF2 partially contributes to the regulation of tumor proliferation via the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway and the epidermal growth factor receptor- (EGFR)-MEK1/2-extracellular signal-related kinase axis. Moreover, NRF2 activation can directly increase the expression of anti-apoptotic genes *Bcl-2* and *Bcl-xL*, which suppress the apoptosis of tumor cells.⁶ The prognostic role of NRF2 expression in cancer has attracted attention; however, the literature displays conflicting results. Some studies have shown that high NRF2 expression is associated with poor prognosis in many types of cancer, including lung, breast, bladder, ovarian, and liver cancers,⁵ while other literature reported contradictory results or did not identify this association.⁷ Because of the limited number of patients enrolled in a single study, there is little reference value for further

research. We hypothesized that NRF2 expression may be associated with prognosis in patients with solid malignancies, and conducted a systemic review and meta-analysis.

Methods

Data sources and search strategy

PubMed, Web of Science, Science Direct, Embase, and the Ovid database were searched for English articles published up to March 2017 using the search terms “NRF2” OR “NFE2L2” OR “Nrf2” OR “nrf2” AND “immunohistochemistry” AND “neoplasms” OR “carcinoma” OR “sarcoma” OR “cancer.”

Study endpoints

The primary and secondary endpoints of this meta-analysis were the effect of NRF2 expression on overall survival

(OS) and disease-free survival (DFS), respectively, in patients with malignant tumors.

Inclusion criteria

Two authors independently screen the titles and identified abstracts eligible for the study. Both authors decided which studies would be included. Inclusion criteria were studies: (i) reporting clinical characteristics of cases with tumor tissues, as well as pathological types and sample sizes; (ii) describing the evaluation methods for NRF2 expression with immunohistochemistry; (iii) with quantification of NRF2 expression; and (iv) that provided hazard ratios (HRs) and 95% confidence intervals (CI) of OS and DFS.

Data extraction

Two authors independently extracted the following information: (i) author names, country, and publication year;

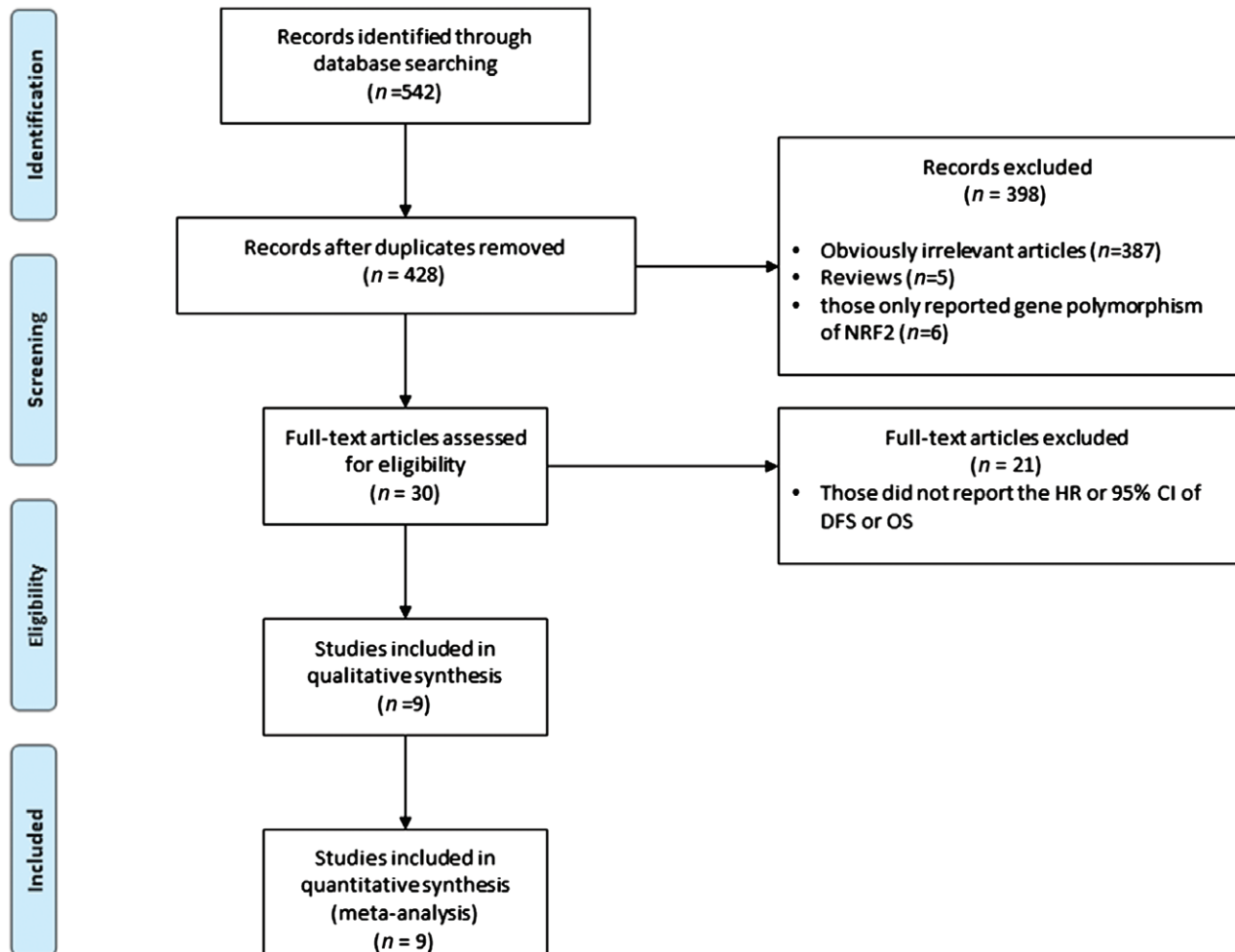


Figure 1 The Preferred Reporting Systems for Systematic Review and Meta-Analysis flow chart of the screened, excluded, and analyzed publications. CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

Table 1 Characteristics of the included studies

Study	Publication year	Country	Disease type	Cases (n)	NRF2 low expression (n)	NRF2 over-expression (n)	OS		DFS		NOS†
							HR (95%CI)	HR (95% CI)			
Cho et al. ⁸	2017	Korea	Ovarian cancer	100	85	15	1.282 (0.281–5.858)	1.022 (0.444–2.355)			9
Zheng et al. ⁹	2015	China	Gastric adenocarcinoma	175	67	108	1.37 (0.66–2.87)	NA			6
Raatikainen et al. ¹⁰	2014	Finland	Prostate cancer	204(C)‡	97	107	1.664 (1.056–2.621)	NA			7
Onodera et al. ¹¹	2014	Japan	Invasive breast cancer	205(n)§	134	71	2.462 (1.162–5.216)	NA			8
Kawasaki et al. ¹²	2014	Japan	Esophageal squamous cell carcinoma	106	59	47	NA	5.36 (1.76–16.34)			9
Ji et al. ¹³	2013	China	Glioblastoma	68	35	33	1.778 (1.043–3.029)	NA			8
Hu et al. ¹⁴	2013	China	Gastric cancer	186	82	104	3.9 (0.94–16.17)	4.3 (1.17–15.75)			8
Yang et al. ¹⁵	2011	China	Non-small cell lung cancer	60	26	34	3.33 (1.53–7.24)	5.75 (2.14–15.45)			9
Wang et al. ¹⁶	2010	China	Gallbladder cancer	59	14	45	3 (1.08–8.35)	NA			7

†NOS, Newcastle-Ottawa Scale; ‡(C), Cyttoplasmic expression of NRF2; §(n), Nuclear expression of NRF2; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; NA, not available; OS, overall survival.

(ii) the type of disease and number of cases; (iii) the number of cases with NRF2 overexpression and low expression; and (iv) HR value and 95% CI of OS and DFS. Disagreement between authors was resolved by consensus or with assistance from a third party. The quality of the studies in this meta-analysis was assessed using the Newcastle-Ottawa Scale (NOS); papers with scores ≥6 were defined as high quality.

Statistical analysis

All calculations were performed using stata 12.0 software (Stata Corp., College Station, TX, USA). The study endpoints were demonstrated by OS, DFS, and their HR and 95% CI. Heterogeneity among the included studies was assessed by Q test and I² statistic evaluation. If I² ≤ 50%, the fixed effect model was used; if I² > 50%, the random effect model was applied. Egger’s method was used to evaluate publication bias in the literature.

Results

Literature search

The literature search process is demonstrated in Figure 1. A total of 542 relevant studies were initially collected according to retrieval strategies and data collection methods, among which 428 remained after discarding duplications. After reading all titles and abstracts, 398 articles were excluded: (i) obviously irrelevant content; (ii) reviews; or (iii) studies only reporting NRF2 gene polymorphism. The remaining 30 articles were screened by carefully reading the whole text to further exclude those that did not report HR or 95% CI of OS or DFS. Finally, nine articles were included in this systemic review, including five studies that only reported OS, one that only reported DFS, and three with both OS and DFS rates.

Study characteristics

The study characteristics by research group, including publication year, author, country, sample size, number of cases with NRF2 low expression/overexpression, and OS or DFS are detailed in Table 1. The nine studies in the analysis were published from 2010 to 2017, with a total of 1040 cancer patients, and pathological types including prostate, gastric, breast, ovarian, glioma, non-small cell lung, bladder, and esophageal squamous cell cancers.

Publication bias test

Risk analysis of publication bias regarding the relationship between NRF2 expression and patients’ OS was assessed

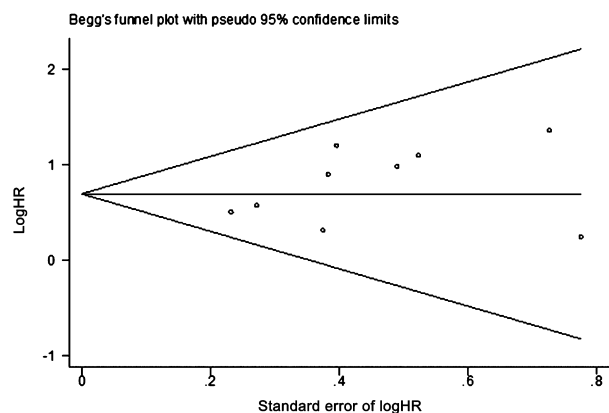


Figure 2 Risk analysis of publication bias risk regarding the relationship between NRF2 expression and overall survival OS (Egger's test). CI, confidence interval; HR, hazard ratio.

using an Egger's test (Fig 2). No significant publication bias existed, indicating that levels of heterogeneity and bias were acceptable.

Overall survival

Eight studies reported OS differences according to different NRF2 expression levels ($n = 934$). The heterogeneity test

showed $I^2 = 0$, $P = 0.656$, therefore, the fixed effect model was used for analysis. The result indicated that patients with NRF2 overexpression had poorer OS (HR = 2.01, 95% CI 1.57–2.56; $P < 0.001$) (Fig 3).

Disease-free survival

Four studies showed DFS differences according to different NRF2 expression levels ($n = 452$). The heterogeneity test of the included studies showed $I^2 = 67.9\%$, $P = 0.025$, therefore the random effect model was used for analysis. The result demonstrated that patients with NRF2 overexpression had poorer DFS (HR = 3.25, 95% CI 1.29–8.15; $P = 0.025$) (Fig 4).

Discussion

At present, the clinical significance of NRF2 in malignant tumors is gradually being recognized, and the detection of NRF2 expression in tumor tissues is hoped to offer benefit as a prognostic or predicative factor.¹⁷ However, only a few of the studies obtained in our literature search yielded conclusive results, with even fewer involving esophageal cancer and esophageal squamous cell carcinoma, which affected further research hypothesis. Therefore, we hypothesized

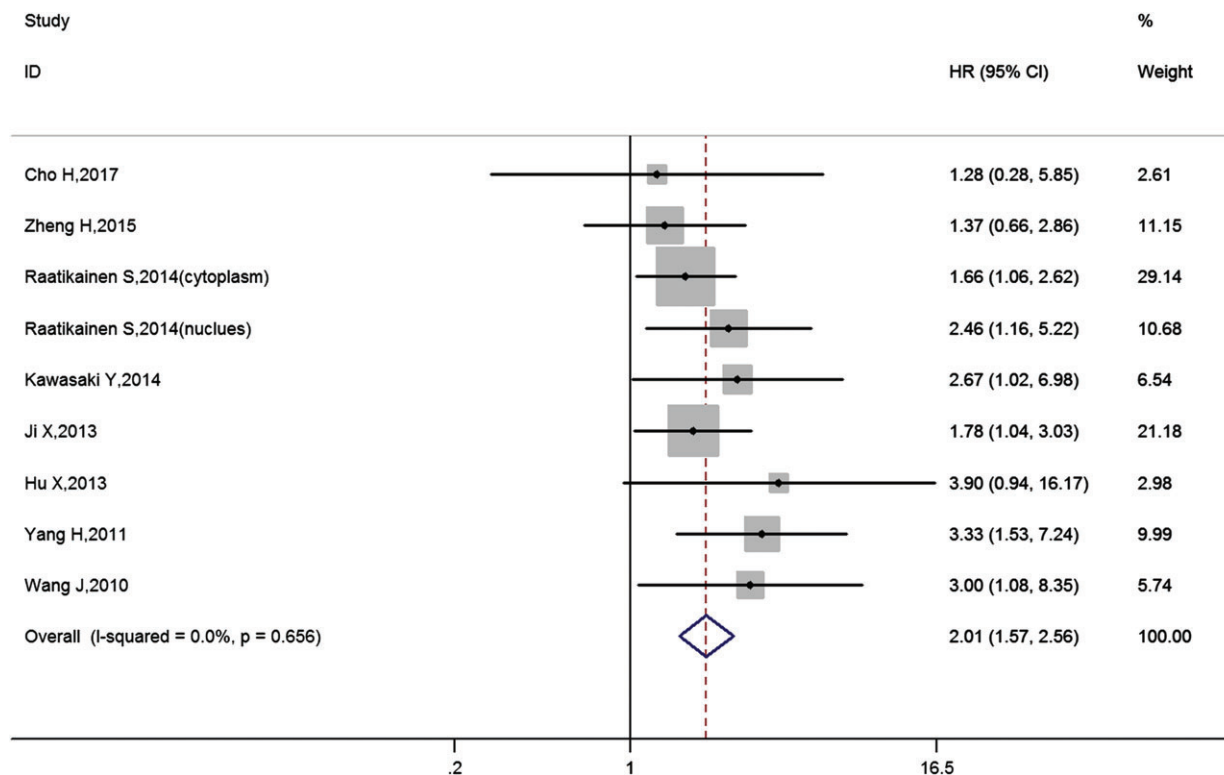


Figure 3 Forest plot of the association between NRF2 expression and overall survival. The hazard ratios (HRs) and 95% confidence intervals (CIs) are shown (fixed effect model analysis).

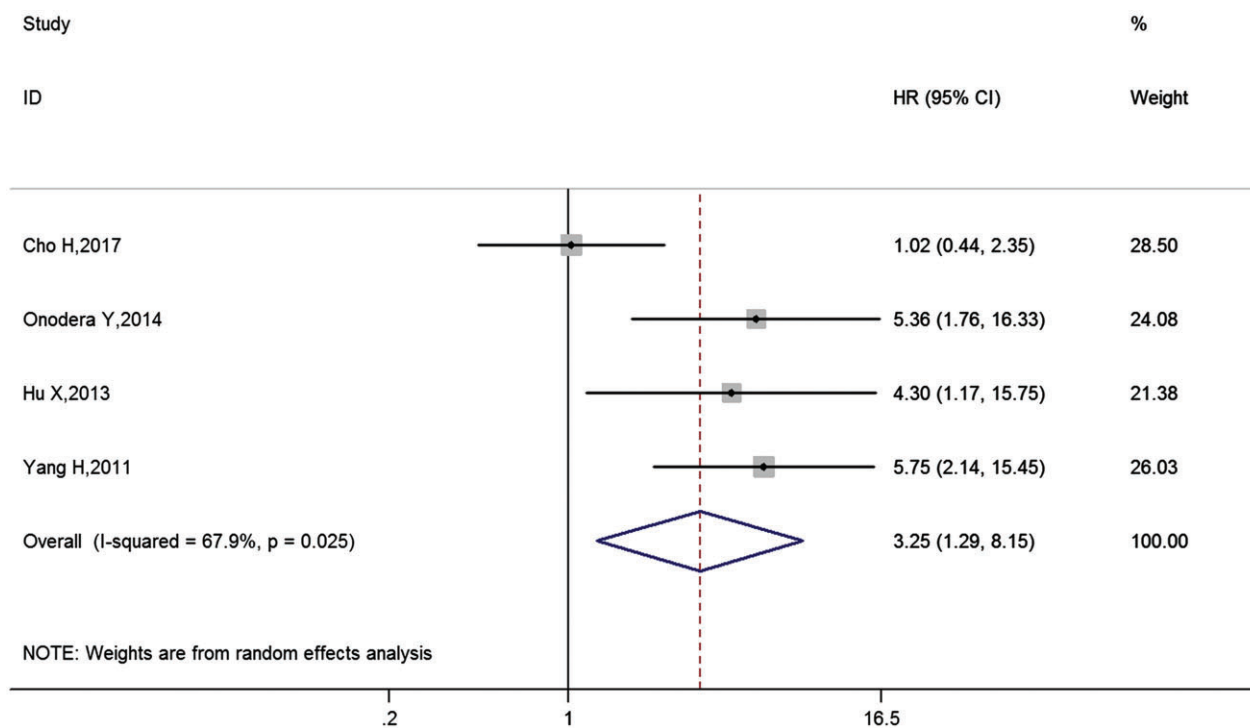


Figure 4 Forest plot of the association between NRF2 expression and disease-free survival. The hazard ratios (HRs) and 95% confidence intervals (CIs) are shown (random effect model analysis).

that NRF2 overexpression in tumor tissues is correlated with malignant biological tumor behavior. However, because of the small number of relevant studies, no conclusive results were obtained from previous literature. As a result, we examined the relationship between NRF2 expression and prognosis in malignant tumors.

Nine clinical studies published in English peer reviewed journals were included. The results demonstrated that patients with NRF2 overexpression had poorer survival, irrespective of OS and DFS, compared to patients with low NRF2 expression. This result suggests that the biological behavior of tumors with NRF2 overexpression is more aggressive. Therefore, it is reasonable to assume that NRF2 expression level could be used as a prognostic factor to help identify a subgroup of patients at high risk, further providing clues for specified molecular typing. Because cancers derived from different tissues share some common features of malignant biological behavior, it is rational to suppose that prognostic effects of this biomarker are comparable between different cancers. Thus, this study explored whether NRF2 overexpression is significantly associated with poor cancer prognosis in spite of the tissue origin. Although this study involved a variety of cancer types that present clinical heterogeneity, the prognostic value of NRF2 expression in these cancers is homogeneous.

Oxidative stress refers to a situation where the body is stimulated by a variety of adverse factors, both in vitro and in vivo. When the balance between oxidation and antioxidants is broken, oxidative stress can reduce apoptosis or promote the abnormal proliferation of some cells, ultimately inducing the development of some diseases.^{18,19} Presently, Kelch-like ECH-associated protein 1 (KEAP1)-NRF2 is the most common antioxidant stress signal pathway, mainly consisting of KEAP1 and NRF2, and could significantly induce the endogenous antioxidant response of the body. KEAP1 and NRF2 are located in the cytoplasm, and bond with each other steadily under physiological conditions. The ubiquitin proteasome pathway continuously degrades NRF2 protein; therefore, little NRF2 expression in cells occurs. When cells are stimulated by pathogenic factors, such as carcinogenic factors, KEAP1 structures, such as cysteine residues, could be modified, which further lead to the conformational change of the binding site to NRF2 and ultimately results in the dissociation of NRF2 and KEAP1. When the corresponding signal transduction pathway is inactivated and ubiquitination is blocked, the NRF2 protein is not degraded. Furthermore, free NRF2 is transferred from the cytoplasm to the nucleus, and binds with the antioxidant response element in the detoxification phase II enzyme promoter region, which initiates downstream transcription factors, including *NQO1*

and *GSTP1* to protect the body from injury arising from pathogenic factors.^{20–22} In recent years, some studies have carefully explored the relationship between the KEAP1-NRF2 pathway and tumorigenesis.²³ Temporary activation of NRF2 could protect normal cells from toxins or carcinogens, however, sustained activation of NRF2 will upregulate the expression of downstream genes, thus providing a conducive environment for malignant cells and facilitating cancer progression.^{9,10} Previous research has demonstrated that abnormal activation of NRF2 is associated with lung, breast, bladder, ovarian, pancreatic, and endometrial cancers and liver cancer metastasis.^{24–29} Contrarily, silencing of NRF2 in tumor cells would lead to decreased cell migration and metastasis potential.

Limitations of the study

The sample sizes of the nine studies included in our meta-analysis were relatively small and used treatment strategies including chemotherapy and radiotherapy, which varied among studies. Studies in Chinese language were excluded; therefore, our conclusions are limited. However, the high quality of the included studies does reflect the significance of NRF2 expression to patient prognosis to some extent.

In sum, NRF2 overexpression is a negative prognostic factor for many malignancies, representing a subgroup of patients who experience more severe biological behavior. Future research should focus on improving treatment efficacy for such patients. Optimizing the treatment of patients with NRF2 overexpression may improve OS.

Acknowledgments

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Disclosure

No authors report any conflict of interest.

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