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SYSTEMATIC REVIEW AND META-ANALYSIS

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NF-κB Expression and Outcomes in Solid Tumors

A Systematic Review and Meta-Analysis

Dang Wu, MD, PhD, Pin Wu, MD, PhD, Lufeng Zhao, MD, Lijian Huang, MD, Zhigang Zhang, MD, Shuai Zhao, MD, and Jian Huang, MD, PhD

Abstract: Nuclear factor-kappaB (NF-κB) is a key inflammatory transcription factor expressed frequently in tumors. Numerous studies have investigated the correlation between NF-κB expression and prognosis in solid tumors, but the conclusions are still in contradiction. Here, we conduct a meta-analysis to explore the overall association of NF-κB overexpression and survival in human solid tumors.

Pubmed and EBSCO databases were searched for studies evaluating expression of NF-κB (as measured by immunohistochemistry) and overall survival (OS) and disease-free survival (DFS) in solid tumors. Published data were extracted and computed into odds ratios (ORs) for death at 3, 5, and 10 years. Data were pooled using the Mantel–Haenszel random-effect model. All statistical tests were two-sided.

Forty-four studies with a total of 4418 patients were included in this meta-analysis. NF-κB overexpression was associated with worse OS at 3 years (OR = 3.40, 95% confidence interval [CI] = 2.41–4.79, $P < 0.00001$), 5 years (OR = 2.72, 95% CI = 1.92–3.85, $P < 0.00001$), and 10 years (OR = 2.63, 95% CI = 1.34–5.16, $P = 0.005$) of solid tumors. Results for 3- and 5-year DFS were similar. NF-κB expression was associated with poor 3-year OS in both Tumor, Lymph Node, Metastasis stage I-II (OR = 9.11, 95% CI = 2.90–28.68, $P = 0.0002$) and III-IV (OR = 2.59, 95% CI = 1.61–4.15, $P < 0.0001$). There is no correlation between cellular localization of NF-kB overexpression and OS of solid tumors. Among the tumor types, NF-κB was associated with worse 3 year-OS of colorectal cancer (OR = 2.70, 95% CI = 1.64–4.46, $P < 0.0001$), esophageal carcinoma (OR = 6.00, 95% CI = 3.29–10.94, $P < 0.0001$) and worse 5 year-OS of colorectal cancer (OR = 2.72, 95% CI = 1.92–3.85, $P < 0.00001$), esophageal carcinoma (OR = 5.96, 95% CI = 3.48–10.18, $P = 0.03$), and nonsmall cell lung cancer (OR = 1.69, 95% CI = 1.20–2.38, $P = 0.002$).

Expression of NF-κB is associated with worse survival in most solid tumors irrespective of NF-κB localization.

(*Medicine* 94(40):e1687)

Abbreviations: CI = confidence interval, DFS = disease-free survival, IHC = immunohistochemistry, NF-κB = nuclear factor-kappaB, NOS = Newcastle–Ottawa Scale, ORs = odds ratios, OS = overall survival.

INTRODUCTION

Cancer is an extremely complex fatal disease with accumulation of abnormal genetic/epigenetic and inflammatory alterations during multistep development.[1] Inflammatory transcriptional factors, such as signal transducer and activator of transcription 3 and nuclear factor-kappaB (NF-κB), are considered to play a pivotal role in tumor initiation and progression.[2,3] Accumulating evidence has demonstrated that persistent activation of inflammatory transcriptional factors facilitate tumor development through various mechanisms, such as providing proliferation and survival advantages, and promoting tumor-related inflammation.[4]

NF-κB, a transcriptional factor involved in multicellular biology response, was first discovered by Sen and Baltimore in 1986.[5] The mammalian NF-κB family consists of 5 protein members: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB, and c-Rel.[6] NF-kB transcriptional factor, as an inactive complex with inhibitory proteins called I-κB in the cytoplasm in normal resting cells,[7] can be activated to enter the nucleus where it regulates the expression of diverse genes encoding cytokines, growth factors, cell adhesion molecules, and apoptotic-related proteins.[8,9] Aberrant activation of NF-κB has been linked to inflammatory and autoimmune diseases, infection and cancer.[10] The first hint to a link between NF-κB and cancer has emerged with the discovery of RelA/p65, and the realization of the close kinship to c-Rel and its oncogenic avian homolog v-Rel.[11] Following studies reported that NF-κB was constitutively activated in various malignancies such as lymphoma,[12] gastrointestinal tumor,[13–15] genitourinary,[16,17] gynecological,[18,19] thoracic,[20] head, and neck tumors.[21,22] Notably, the presence of activated NF-κB in a tumor is not necessarily causal. NF-κB is involved in most if not all cellular processes in tumor evolution including inflammation, transformation, proliferation, angiogenesis, invasion, metastasis, chemoresistance, and radioresistance.[23] Given the tumor promoting role of NF-κB, targeting NF-κB for tumor prevention and therapy might be beneficial. A myriad of studies have investigated the correlation between NF-κB overexpression and prognosis in solid tumors. However, the prognostic

Editor: Mariusz Adamek.
Received: May 28, 2015; revised: September 1, 2015; accepted: September 4, 2015.
From the Department of Radiation Oncology (DW); Department of Surgical Oncology (SZ, JH); Department of Thoracic Surgery (PW, LZ, LH); Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, National Ministry of Education; Provincial Key Laboratory of Molecular Biology in Medical Sciences) (DW, PW, ZZ, SZ, JH); and Department of Gynaecology and Obstetrics, Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China (ZZ).
Correspondence: Jian Huang, Department of Surgical Oncology, Cancer Institute, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China (e-mail: drhuangjian@zju.edu.cn).
Supplemental Digital Content is available for this article.
DW and PW contributed equally to this work.
This work was supported by grants from the National Natural Science Foundation of China (81572800, PW), the Science and Technology Department of Zhejiang Province (2011c13034~1, JH), and Natural Science Foundation of Zhejiang Province (Z2100366, JH and LY15H160041, PW)
The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001687

value of NF-κB overexpression across different solid tumors is still in contradiction.

We therefore conducted an exhaustive meta-analysis combining evidence to evaluate the prognostic impact of NF-κB overexpression in solid tumors. We also evaluated whether the outcome differs between nuclear and cytoplasmic NF-κB expression and between different tumor types. This meta-analysis aimed to evaluate the role of NF-κB in relation to survival in solid tumors, thereby supporting more rational development of therapeutic strategies against NF-κB.

MATERIAL AND METHODS

This meta-analysis was conducted according to preferred reporting items for systematic reviews and meta-analyses statement.[24] This study based on summary and analysis of the results of previous studies, so the ethical approval was not necessary.

Identification and Selection of Studies

Pubmed and EBSCO were searched for studies evaluating the correlation between NF-κB expression and survival in solid tumors from 2004 to January 2015. The search terms ''NF-κB'' and ''neoplasms'' were used and only human studies of solid tumors were included. In addition, the entry ''NF-κB'' and the name of each specific solid tumor were used to screen additional studies. We identified a total of 9168 and 13,092 entries, respectively. The inclusion criteria for selecting articles were the measurement of NF-κB by immunohistochemistry (IHC), availability of survival data for at least 3 years, and publication in English. Studies evaluating gene expression of NF-κB measured by real-time polymerase chain reaction were excluded. We screened the citation lists of retrieved articles to ensure sensitivity of the search strategy. Interreviewer agreement was evaluated using Cohen kappa coefficient. Disagreement was resolved by discussion and a final consensus was achieved.

Endpoints of Interest

Overall survival (OS) at 3, 5, and 10 years were the primary endpoints. Where not available, data for disease-free survival (DFS) at 3 and 5 years were collected. Tumors were classified by NF-κB expression status using cut-off value as defined by each study.

Data Collection Process and Quality Assessment

Two authors (DW and PW) independently reviewed and extracted data using predefined data abstraction forms from each eligible studies. Extracted information included first author's name, publication year, country, type of cancer, number of patients, median age, gender, Tumor, Lymph Node, Metastasis (TNM) stage, time of follow-up, antibody used for the evaluation, technique used to quantify NF-κB, and cut-off value to determine NF-κB positivity. OS and DFS data were extracted from the text, tables, or Kaplan–Meier curves for both NF-κB negative and NF-κB positive group.

The studies included in this meta-analysis were cohort studies. The quality of each included cohort study was evaluated using Newcastle–Ottawa Scale (NOS) by 2 independent authors.[25] The studies with 6 scores or more were classified as high quality studies. A consensus NOS score for each item was achieved.

Data Synthesis

The relative frequency of 3-, 5-, and 10-year OS or DFS between NF-κB negative and NF-κB positive group was expressed as an odds ratio (OR) and its 95% confidence interval (CI). Sensitivity analyses were carried out for different analytical methods and cut-offs for defining NF-κB expression and NOS scores for quality assessment of included studies.

Statistical Analysis

Data were extracted, computed into ORs, and pooled using the Mantel–Haenszel random-effect model by RevMan 5.3 analysis software (Cochrane Collaboration, Copenhagen, Denmark). Heterogeneity was evaluated with the Cochran's Q and I^2 statistics. Statistical differences between subgroups were assessed using the methods described in *Cochrane Handbook for Systematic Reviews of Interventions*.[26] Meta-regression analysis was conducted using Stata 12.0 software (StataCorp LP, College Station, TX). All statistical tests were 2-sided, and *P* values less than.05 were defined statistically significant.

RESULTS

Search Results

Literature searches yield 22,260 records and the results are shown in Figure 1. The potentially relevant articles were screened for eligibility by duplication, language, abstract and article type, and 13,101 records were excluded. Next, 9114 citations were excluded for detailed evaluation and at last 44 studies with survival data were included.

Description of Studies

Characteristics of studies including OS and DFS data are shown in Table 1. Data were available for OS from 43 studies. Nine evaluated colorectal cancer,[27–34] 5 evaluated esophageal carcinoma,[15,35–38] 5 evaluated gastric cancer,[14,39–42] 5 evaluated ovarian cancer,[18,43–46] 4 evaluated lung cancer,[47–50] 2 studies evaluated cervical cancer,[19,51] 2 evaluated laryngeal squamous cell carcinoma,[52,53] 2 evaluated nasopharyngeal carcinoma,[54,55] and 1 each evaluated salivary glands carcinoma,[56] astrocytomas,[57] gallbladder carcinoma,[58] head and neck squamous cell carcinoma,[59] cholangiocarcinoma,[60] oral squamous cell carcinoma,[61] prostate cancer,[62] urothelial carcinoma,[63] and pancreatic cancer.[64] For DFS, 9 studies provided data at 3 years,[15,34,37,38,41,48,55,62,65] and 7 studies provided data at 5 years.[37–39,41,48,55,62] A total of 4418 patients were included in those studies.

Evaluation and Expression of NF-κB

A description of the antibodies, detection, and definition method of NF-κB used in the included studies is shown in Table S1, http://links.lww.com/MD/A445. Diverse antibodies were used for the evaluation of NF-κB expression. For NF-κB p50 antibody, 4 studies used clone sc114, 1 study used clone sc1190, and 1 study did not report the clone. For NF-κB p65 antibody, 4 studies used clone sc8008, 3 studies used clone G96-337, 6 studies used clone sc109, 1 study each used clone sc372, sc502, D14E12, and 24 studies did not report the clone. The cut-off for NF-κB positivity depended on the staining score and the detection method used. Among the groups determined as NF-κB positive, the median expression of NF-κB staining was 55.33%. NF-κB expression in solid tumors ranged 17.59% to 96.34%.

Association of NF-κB With OS

There were 41 studies reporting 3-year OS data. Results showed that NF-κB overexpression in tumor tissue was

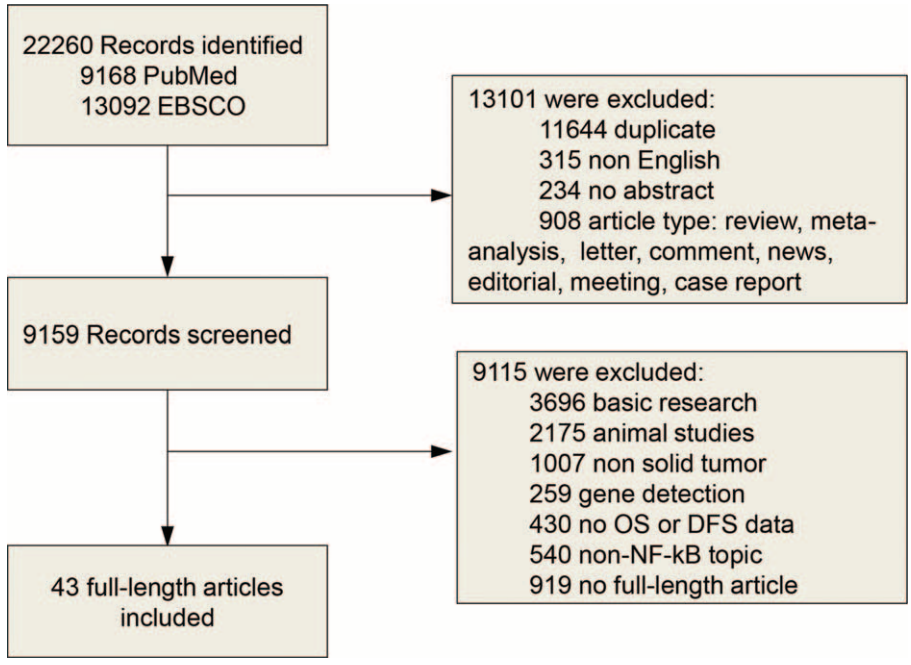

FIGURE 1. Flow diagram of study selection. NF-κB = nuclear factor-kappaB.

associated with unfavorable 3-year OS of solid tumors (OR = 3.40, 95% CI = 2.41–4.79, $P < 0.00001$) (Fig. 2). Significant heterogeneity among studies (Cochran Q $P < 0.00001$, $I^2 = 74\%$) was observed, so we conducted meta-regression analysis and subgroup meta-analysis to explore the possible sources of heterogeneity.

Eight studies provided 3-year OS for colorectal cancer, 5 studies for esophageal carcinoma, 5 studies for gastric cancer, 4 studies for lung cancer, and 5 studies for ovarian cancer. In the stratified analysis by type of cancer, NF-κB overexpression was associated with worse 3-year OS of colorectal cancer (OR = 2.70, 95% CI = 1.64–4.46, $P < 0.0001$) and esophageal carcinoma (OR = 6.00, 95% CI = 3.29–10.94, $P < 0.00001$) (Fig. 3). However, there was no significant association between NF-κB overexpression and 3-year OS of gastric cancer (OR = 3.21, 95% CI = 0.83–12.45, $P = 0.09$), nonsmall cell lung cancer (OR = 3.84, 95% CI = 0.72–20.49, $P = 0.12$), and ovarian cancer (OR = 1.69, 95% CI = 0.43–6.55, $P = 0.45$) (Figure S1, http://links.lww.com/MD/A445).

We also evaluated the correlation between NF-κB overexpression and the TNM stage of tumor. High expression level of NF-κB was significantly associated with TNM stage III-IV (OR = 0.5, 95% CI = 0.33–0.77, $P = 0.001$) (Figure S2, http://links.lww.com/MD/A445). Next, we conducted subgroup meta-analysis according to TNM stage. NF-κB expression was associated with poor 3-year OS in both TNM stage I-II (OR = 9.11, 95% CI = 2.90–28.68, $P = 0.0002$) and III-IV (OR = 2.59, 95% CI = 1.61–4.15, $P < 0.0001$) (Fig. 4).

The association of NF-κB expression and decreased 3-year OS seemed independent of NF-κB localization, with similar result for studies including only nuclear expression (OR = 3.75, 95% CI = 1.90–7.42, $P < 0.0001$), those including only cytoplasmic expression (OR = 3.97, 95% CI = 2.15–6.95, $P < 0.0001$) and those with unselected NF-κB expression (OR = 3.15, 95% CI = 1.82–5.45, $P < 0.0001$) (Figure S3, http://links.lww.com/MD/A445).

Meta-regression analysis showed that publication year, country, age, gender, and NOS score did not contribute to the heterogeneity (data not shown).

Thirty-two studies reported 5-year OS data. Analogous result was observed with 3-year OS data that NF-κB overexpression was significantly correlated with poor 5-year OS of solid tumors (OR = 2.72, 95% CI = 1.92–3.85, $P < 0.00001$) (Fig. 5). Heterogeneity among studies was also very high (Cochran Q $P < 0.00001$, $I^2 = 77\%$); therefore, we conducted subgroup meta-analysis according to type of cancer for heterogeneity exploration.

Five studies provided 5-year OS for colorectal cancer, 4 studies for esophageal carcinoma, 5 studies for gastric cancer, 4 studies for lung cancer, and 5 studies for ovarian cancer. NF-κB overexpression was associated with worse 5-year OS of colorectal cancer (OR = 2.40, 95% CI = 1.48–3.90, $P < 0.00001$), esophageal carcinoma (OR = 5.96, 95% CI = 3.48–10.18, $P < 0.00001$), and nonsmall cell lung cancer (OR = 1.69, 95% CI = 1.20–2.38, $P = 0.002$) (Fig. 6). However, there was no significant association between NF-κB overexpression and the 5-year OS of gastric cancer (OR = 3.48, 95% CI = 0.93–13.03, $P = 0.06$) and ovarian cancer (OR = 1.46, 95% CI = 0.41–5.21, $P = 0.56$) (Figure S4, http://links.lww.com/MD/A445).

Similar to 3-year OS results, the association of NF-κB expression and decreased 5-year OS seemed independent of NF-κB localization, with similar result for studies including only nuclear expression (OR = 2.61, 95% CI = 1.18–5.77, $P = 0.02$), those including only cytoplasmic expression (OR = 3.27, 95% CI = 1.82–5.89, $P < 0.0001$) and those with unselected NF-κB expression (OR = 2.58, 95% CI = 1.59–4.19, $P = 0.0001$) (Figure S5, http://links.lww.com/MD/A445).

Results from 8 studies showed that NF-κB overexpression was significantly associated with worse 10-year OS of solid tumors (OR = 2.63, 95% CI = 1.34–5.16, $P = 0.005$) (Fig. 7).

TABLE 1. Characteristics of Studies Including OS and DFS in the Meta-Analysis

References	Country	Type of Cancer	No.	Age, Median (Range)	Male/Female	Stage	Follow-Up, months (Range)	NF-κB (−/+) NO.	3-year OS (−/+)%	5-year OS (−/+)%	10-year OS (−/+)%	NOS Score
Studies including OS												
Abdel-Latif et al (2004)[35]	USA	Esophageal carcinoma	97	62.1 ± 12.8	80/17	I-III	NR	38/59	27.7/9.2	12.2/1.1	NR	7
Annunziata et al (2010)[18]	Norway	Ovarian cancer	31	NR	0/31	III-IV	Up to 108	13/18	92.4/43.1	83.6/37	NR	7
Balermpas et al (2013)[59]	Germany	Head and Neck Squamous Cell Carcinoma	101	61.2 (39.2–91.4)	80/21	III-IV	25 (2.3–63)	67/34	62.6/40.5	NR	NR	8
Berardi et al (2012)[27]	Italy	Rectal cancer	73	66 (36–85)	46/27	NR	28 (6.7–75.8)	38/35	91.1/46.1	NR	NR	8
Chu et al (2011)[28]	China	Colorectal cancer	260	60.1 ± 12.3	139/121	I-IV	55 (1–84)	133/127	88/70.9	66.2/35.4	NR	8
Darb-Esfahani et al (2010)[43]	Germany	Ovarian cancer	85	57 (32–85)	0/85	I-IV	NR	36/47	90.8/64.9	82.4/58.3	NR	7
Guo et al (2008)[44]	China	Ovarian cancer	68	50 (17–73)	0/68	I-IV	NR	17/51	22/61.2	20/46.1	NR	7
Hatata et al (2012)[36]	Japan	Esophageal carcinoma	69	NR	NR	II-III	NR	28/41	57.4/21.1	42.9/14.3	NR	6
Huang et al (2009)[52]	China	Laryngeal squamous cell carcinoma	78	60.5 (35–82)	70/8	I-IV	66 (6–92)	31/47	96.8/76.6	87.2/55.4	NR	8
Izzo et al (2007)[38]	USA	Esophageal carcinoma	123	63 (28–83)	111/12	I-IV	16 (35–79)	44/79	63.2/30.9	58.6/21.4	58.6/17.9	8
Izzo et al (2006)[15]	USA	Esophageal carcinoma	80	59 (35–76)	72/8	II-IV	32 (6–104)	33/47	90/42.9	68.4/21.1	NR	8
Izzo et al (2006)[37]	USA	Esophageal carcinoma	43	56 (35–72)	41/2	NR	23 (5–39)	22/21	95/39.3	NR	NR	8
Jiang et al (2011)[53]	China	Laryngeal squamous cell carcinoma	89	59 (33–85)	78/11	I-IV	65 (10–89)	32/57	93.8/72.8	87.1/49.3	NR	8
Jin et al (2008)[47]	China	Nonsmall cell lung cancer	88	NR	NR	I-II	51 (39–62)	47/41	100/100	67.8/49	NR	7
Kleinberg et al (2009)[45]	Norway	Ovarian cancer	68	NR	0/68	I-IV	38 (8–117)	23/45	65.4/35.4	17.4/7.6	NR	8
Korkolopoulou et al (2008)[57]	Greece	astrocytomas	82	55.23 (19–84)	49/33	II-IV	12 (3–104)	3/79	65.3/4.9	NR	NR	8
Kwon et al (2010)[29]	Korea	Colorectal cancer	148	60 (22–82)	NR	III	53.2	78/70	79.7/64.5	77.1/52.4	NR	7
Kwon et al (2012)[39]	Korea	Gastric cancer	115	NR	68/47	I-IV	66.6 (9.3–88.8)	66/49	95.4/34.8	90.8/32.7	NR	8
Lee et al (2005)[14]	Korea	Gastric cancer	290	54.8	196/94	I-IV	54 (1–72)	239/51	66.2/82.1	60.6/75.7	NR	8
Levidou et al (2007)[40]	Greece	Gastric cancer	93	65.48 (29–89)	53/40	I-IV	20 (3–101)	8/85	56/24.5	51.4/12.6	NR	8
Lewander et al (2012)-1[30]	Sweden	Colorectal cancer	203	NR	NR	I-IV	56 (0.06–288)	129/74	74.6/74.1	67.1/67.1	61.5/57.9	7
Lewander et al (2012)-2[30]	Sweden	Colorectal cancer	203	NR	NR	I-IV	56 (0.06–288)	73/130	83.4/69.4	76.4/60.7	67.9/56.5	7
Li et al (2009)-M1[a19]	China	Cervical cancer	79	42.8 ± 9.1	0/79	I-II	NR	29/50	100/79	NR	NR	7
Li et al (2009)-M2[b19]	China	Cervical cancer	79	42.8 ± 9.1	0/79	I-II	NR	14/65	100/83.3	NR	NR	7
Lin et al. (2012)[31]	China	Colorectal cancer	108	NR	NR	I-IV	>24	75/33	63.7/51.8	NR	NR	6
Nair et al (2014)[48]	USA	Nonsmall-cell lung cancer	334	NR	NR	I-IV	NR	143/191	61.5/50.3	30.8/23.6	NR	6
Nariai et al (2011)[61]	Japan	Oral squamous cell carcinoma	50	68.5 (43–89)	35/15	I-IV	61.2 (7–179)	24/26	86.8/45.9	81.4/41.7	71.9/30.2	8
O'Neil et al (2011)[32]	USA	Rectal cancer	63	58 (26–89)	NR	II-IV	NR	5/58	75.1/42.3	50.8/33.6	50.8/33.6	7
Okera et al (2011)[62]	USA	Prostate cancer	55	NR	55/0	NR	64.8	26/29	73/93.2	49.9/65.7	29.4/31.9	7
Pancione et al (2009)[33]	Italy	Colorectal cancer	72	NR	44/28	I-IV	56 ± 19.8	30/42	90/69	86.3/59.3	NR	8
Park et al (2014)[41]	Korea	Gastric cancer	154	51 (24–75)	105/49	II-IV	NR	86/68	73/50	60.9/42.3	NR	7
Sun et al (2012)[54]	China	Nasopharyngeal carcinoma	110	NR	NR	I-III	NR	49/61	71.8/72.8	51.9/55.1	NR	6
Voboril et al (2008)[34]	Czech Republic	Rectal cancer	25	67.2 (37–82)	20/5	I-IV	NR	20/5	94.4/44.6	NR	NR	7
Weichert et al (2007)-1[c64]	Germany	Pancreatic cancer	82	65 (39–80)	NR	NR	NR	45/37	34.8/7.9	NR	NR	6
Weichert et al (2007)-2[d64]	Germany	Pancreatic cancer	82	65 (39–80)	NR	NR	NR	40/42	36.6/9.3	NR	NR	6
Wu et al (2008)[58]	China	Gallbladder carcinoma	38	63 (39–83)	14/24	I-IV	23 (2–46)	16/22	43.4/22.5	NR	NR	8
Wu et al (2013)[51]	China	Cervical cancer	80	45 (33–68)	0/80	I-IV	>60	22/58	95.4/63.8	95.4/63.8	NR	8

References	Country	Type of Cancer	No.	Age, Median (Range)	Male/Female	Stage	Follow-Up, months (Range)	NF-κB (−/+) NO.	3-year OS (−/+)%	5-year OS (−/+)%	10-year OS (−/+)%	NOS Score
Yamanaka et al (2004)[42]	Japan	Gastric cancer	63	NR	45/18	I-IV	NR	42/21	66.2/35.9	66.2/30	NR	7
Yang et al (2011)[46]	USA	Ovarian cancer	324	NR	0/324	NR	NR	63/261	40.3/55.8	20.9/36.1	17.2/22.9	6
Yeh et al (2010)[63]	China	Urothelial carcinoma	90	64.5	37/33	NR	43.4	66/24	76.8/48.5	71.6/41.9	64.2/31.9	7
Zhang et al (2007)[49]	China	Nonsmall cell lung cancer	116	NR	77/39	I-II	55 (39–60)	60/56	100/98	59.6/44	NR	8
Zhang et al (2005)[56]	China	Adenoid Cystic Carcinoma of Salivary Glands	80	NR	34/46	NR	72 (24–168)	52/28	100/86.8	95.6/78.1	79.7/15	8
Zhang et al (2011)[55]	China	Nasopharyngeal carcinoma	42	NR	25/17	I-IV	48.4 (11–60)	15/27	100/59.3	93.5/44.2	NR	8
Zhang et al (2006)[50]	China	Nonsmall cell lung cancer	45	NR	29/16	NR	NR	15/30	86.8/30.6	30.6/30.6	NR	6
Zhou et al (2013)[60]	China	Intrahepatic Cholangiocarcinoma	33	NR	18/15	NR	28.1 (3.2–63.0)	16/17	12/11.1	NR	NR	7
Studies including DFS												
Izzo et al (2007)[38]	USA	Esophageal carcinoma	123	63 (28–83)	111/12	I-IV	16 (35–79)	44/79	65/32	60.3/26.6		8
Izzo et al (2006)[15]	USA	Esophageal carcinoma	80	59 (35– 76)	72/8	II-IV	32 (6–104)	33/47	70.7/29.6	60.5/29.6		8
Izzo et al (2006)[37]	USA	Esophageal carcinoma	43	56 (35–72)	41/2	NR	23 (5–39)	22/21	95/0	NR		8
Kwon et al (2012)[39]	Korea	Gastric cancer	115	NR	68/47	I-IV	66.6 (9.3–88.8)	66/49	NR	90.9/32.7		8
Nair et al (2014)[48]	USA	Nonsmall-cell lung cancer	322	NR	NR	I-IV	NR	140/182	34.3/22	13.6/5.5		6
Okera et al (2011)[62]	USA	Prostate cancer	55	NR	55/0	NR	64.8	26/29	27.2/31.1	27.2/13.6		7
Park et al (2014)[41]	Korea	Gastric cancer	154	51 (24–75)	105/49	II-IV	NR	86/68	72.8/49.7	64.9/46.3		7
Voboril et al (2008)[34]	Czech Republic	Rectal cancer	23	67.2 (37–82)	20/5	I-IV	NR	18/5	80.9/16.9	NR		7
Yoon et al (2009)[65]	Korea	Mucinous adenocarcinoma	15	64.7 (45–87)	3/12	NR	(12–60)	9/6	83.3/0	NR		8
Zhang et al (2011)[55]	China	Nasopharyngeal carcinoma	42	NR	25/17	I-IV	48.4 (11–60)	15/27	80.2/42.6	80.2/38.6		8

a. M1: Marker 1, NF-kB p65; b. M2: Marker 2, NF-kB p50; c. 1: nuclear expression; d. 2: cytoplasmic expression; e. NR: no report. DFS = disease-free survival, NF-κB = nuclear factor-kappaB, NOS = newcastle–Ottawa Scale, OS = overall survival.

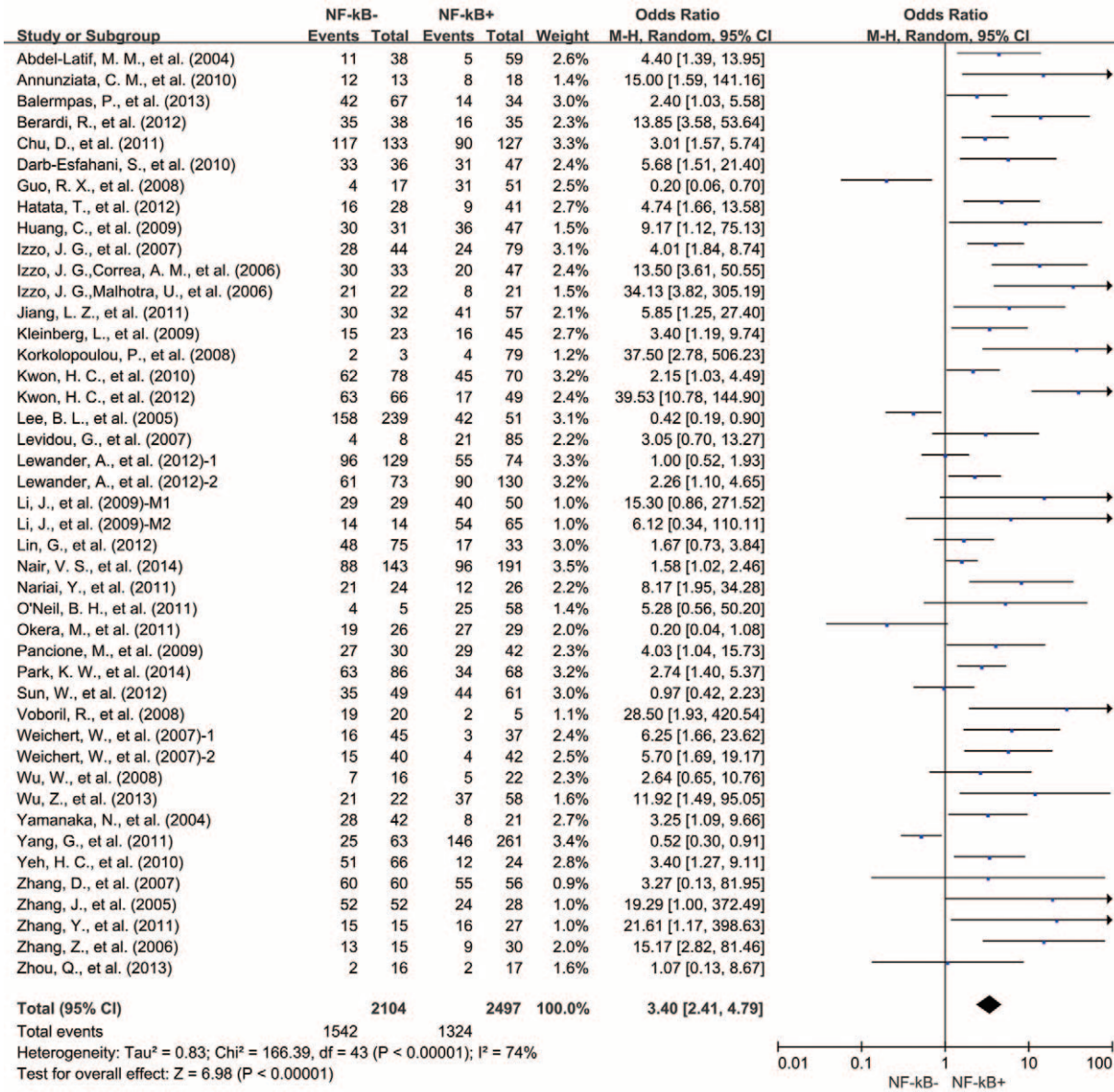

FIGURE 2. Three-year OS by NF-κB expression. M1 = Marker 1, NF-kB p65; M2 = Marker 2, NF-kB p50, NF-κB = nuclear factor-kappaB, OS = overall survival, 1 = nuclear expression; 2 = cytoplasmic expression.

Association of NF-κB With DFS

A total of 9 studies reported results for 3-year DFS. There was no statistically significant heterogeneity between studies (Cochran Q $P = 0.05$, $I^2 = 49\%$). NF-κB expression was associated with a statistically significant worse 3-year DFS (OR = 3.17, 95% CI = 1.87–5.38, $P < 0.0001$) (Fig. 8). Seven studies reported results for 5-year DFS. NF-κB expression was associated with an unfavorable 5-year DFS (OR = 4.65, 95% CI = 2.39–9.06, $P < 0.00001$) (Fig. 6). However, there was significant heterogeneity among studies (Cochran Q $P = 0.01$, $I^2 = 64\%$).

Sensitivity Analyses

Removal of the studies that was an outlier (score, IRS, >50% vs 1%–25% for other studies) or no report (NR) with regard to the cut-off of NF-κB overexpression by IHC did not influence results for 3- or 5-year OS (OR = 4.33, 95% CI = 2.84–6.60, $P < 0.00001$; OR = 2.81, 95% CI = 1.83–4.31, $P < 0.00001$, respectively), but changed the result for 10-year OS (OR = 1.69, 95% CI = 0.75–3.83, $P = 0.21$). Exclusion of these studies did not reduce heterogeneity for 3- or 5-year OS (Cochran Q $P < 0.00001$, $I^2 = 72\%$; Cochran Q $P < 0.00001$, $I^2 = 79\%$, respectively).

Removal of studies using marker p50 or no report to assess the expression of NF-κB by IHC (p50, NR vs p65 for other studies) did not substantially affect the association between NF-κB expression and worse 3-, 5-, or 10-year OS compared with no NF-κB expression (OR = 3.08, 95% CI = 2.14–4.43, $P < 0.00001$; OR = 2.65, 95% CI = 1.84–3.83, $P < 0.00001$, OR = 2.61, 95% CI = 1.28–5.33, $P = 0.008$, respectively). Exclusion of these studies did not reduce heterogeneity for

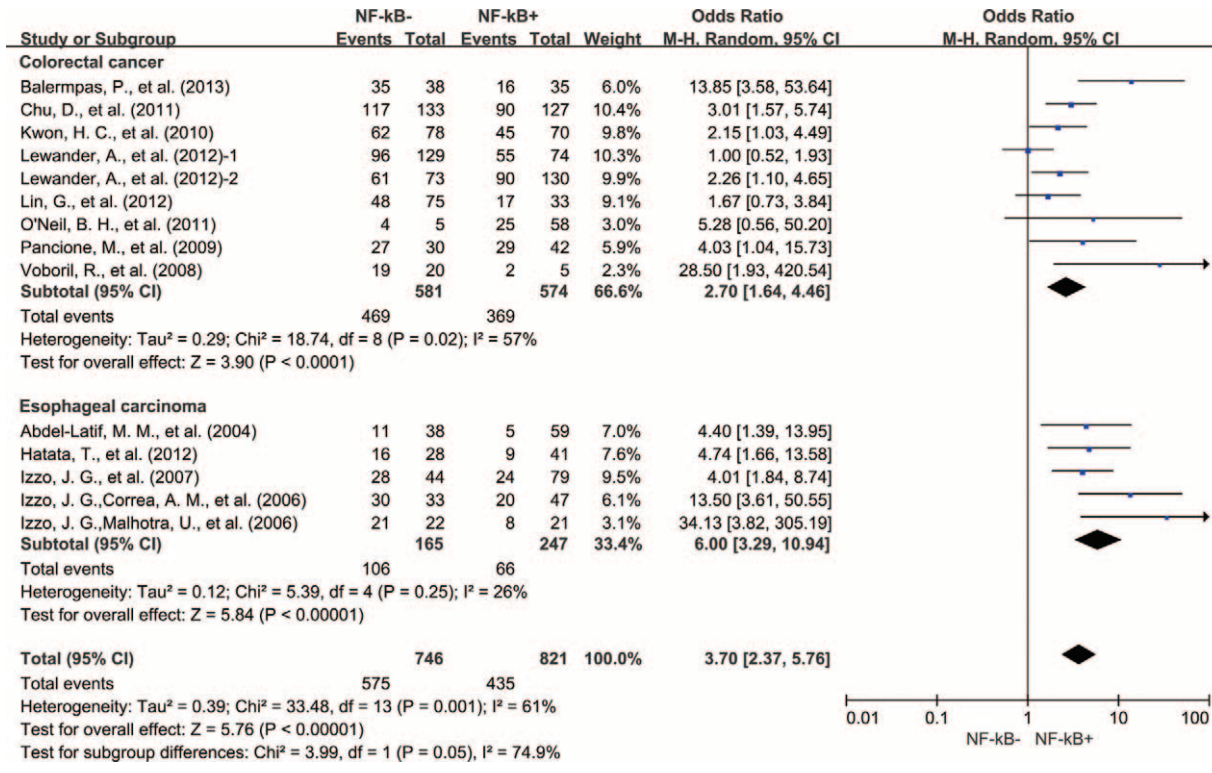

FIGURE 3. Subgroup analysis of 3-year OS by NF-κB expression in different tumor types. NF-κB = nuclear factor-kappaB, OS = overall survival, 1 = nuclear expression; 2 = cytoplasmic expression.

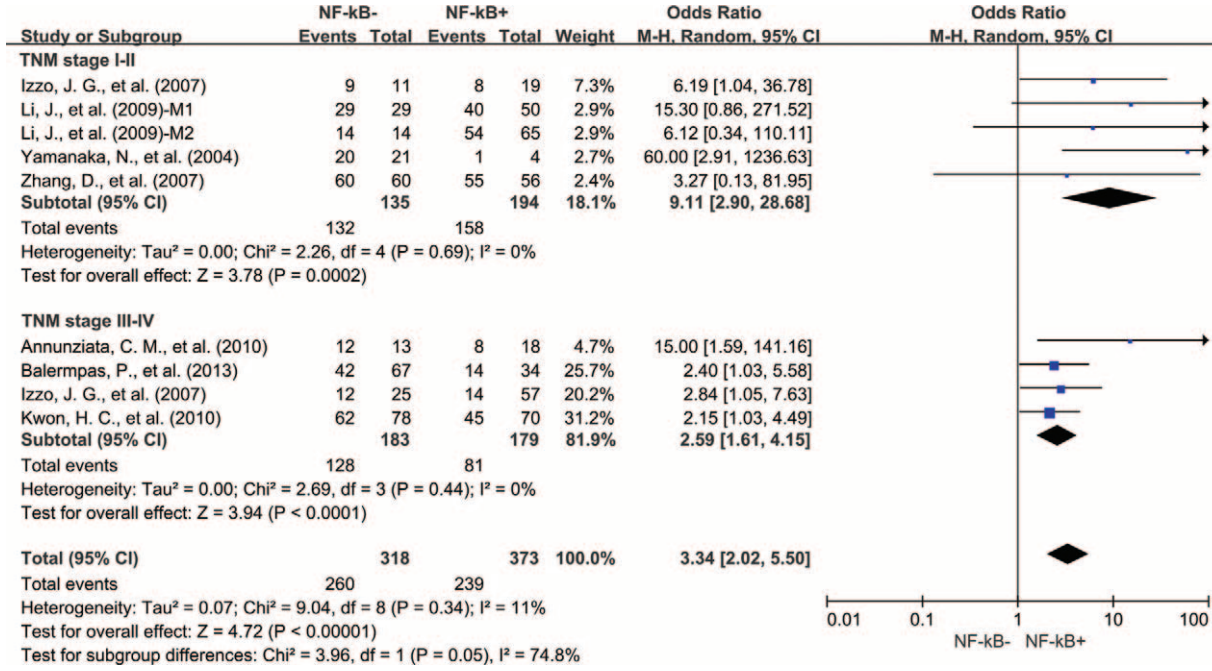

FIGURE 4. Subgroup analysis of 3-year OS by NF-κB expression in different TNM stages of tumor. NF-κB = nuclear factor-kappaB, OS = overall survival, M1 = Marker 1, NF-kB p65; M2 = Marker 2, NF-kB p50.

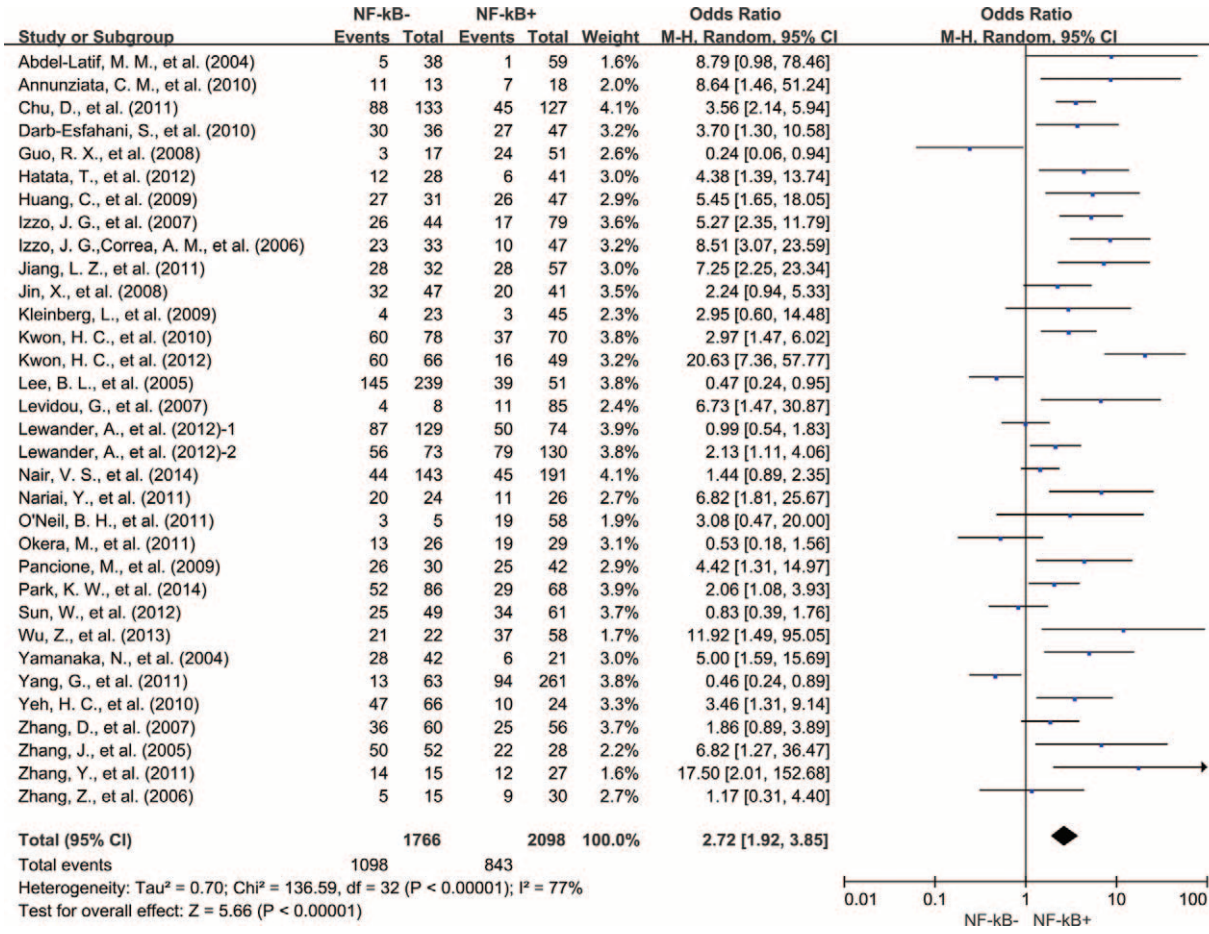

FIGURE 5. Five-year OS by NF-κB expression. NF-κB = nuclear factor-kappaB, OS = overall survival, 1 = nuclear expression; 2 = cytoplasmic expression.

3-, 5-, or 10-year OS (Cochran Q $P < 0.00001$, $I^2 = 77\%$; Cochran Q $P < 0.00001$, $I^2 = 79\%$, Cochran Q $P < 0.00001$, $I^2 = 82\%$, respectively).

Removal of studies with NOS score 6 did not influence results for 3-, 5-, or 10-year OS (OR = 2.94, 95% CI = 2.47–3.49, $P < 0.00001$; OR = 3.23, 95% CI = 2.23–4.67, $P < 0.00001$; OR = 3.17, 95% CI = 1.59–6.34, $P = 0.001$, respectively). Exclusion of these studies did not reduce heterogeneity for 3-, 5-, or 10-year OS (Cochran Q $P < 0.00001$, $I^2 = 70\%$; Cochran Q $P < 0.00001$, $I^2 = 73\%$, Cochran Q $P < 0.00001$, $I^2 = 73\%$, respectively).

Publication Bias

Funnel plot analysis and Egger test showed that there was no statistical evidence of publication bias in our meta-analysis (data not shown).

DISCUSSION

Multiple approaches for targeting NF-κB to treat cancer have been put forward. However, there remain unanswered questions about the effect of NF-κB on outcome and whether the outcome is consistent among different types of solid tumor. Our comprehensive meta-analysis of 4418 patients included in 44 different studies demonstrates that the expression of NF-κB is a marker of poor prognosis. This effect appeared independent of the TNM stage of tumor and NF-κB localization.

Experimental data from laboratories have established strong support for the critical role of NF-κB in tumor development and progression.[66] Therefore, targeting NF-κB is thought to be a potent node of pharmacological interference against tumors and acquires clinical benefit response. However, there is no evidence on the basis of the evidence-based medicine with regard to the association between NF-κB expression and worse survival of tumors to date. Our meta-analysis shows that NF-κB overexpression is associated with worse 3-, 5-, 10-year OS and 3-, 5-year DFS of solid tumors. Moreover, NF-κB expression is associated with poor survival in both TNM stage I-II and III-IV. These results suggest that NF-κB is a valuable biomarker for prognostic prediction for solid tumors.

Among the tumor types evaluated, the expression of NF-κB is associated with worse 3- and 5-year OS for colorectal cancer and esophageal carcinoma, while NF-κB is not significantly associated with either 3- or 5-year OS for gastric cancer and ovarian cancer. This suggests that the prognostic significance of NF-κB depends on the types of tumor. Our study may provide strong supporting evidence for blocking NF-κB as a target for clinical intervention of different solid tumors in future.

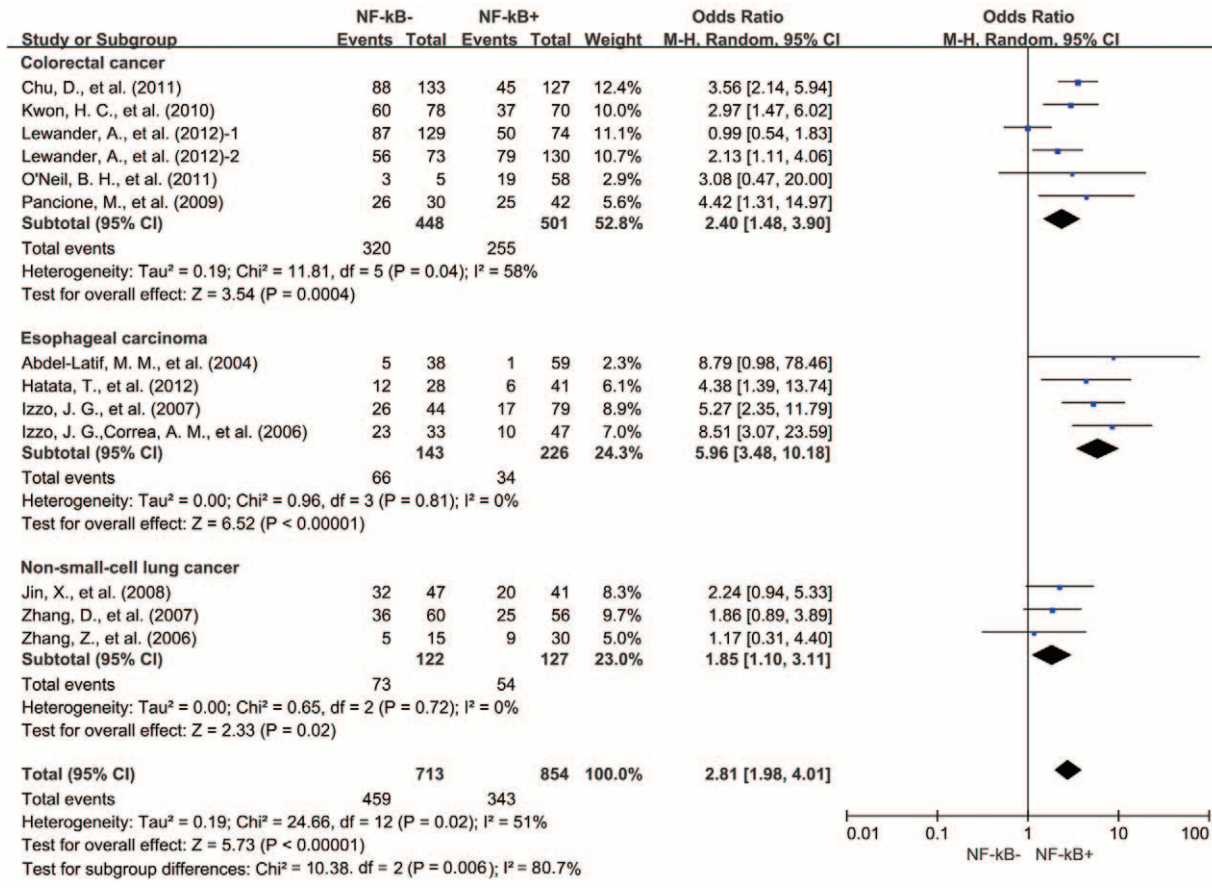

FIGURE 6. Subgroup analysis of 5-year OS by NF-κB expression in different tumor types. NF-κB = nuclear factor-kappaB, OS = overall survival, 1 = nuclear expression, 2 = cytoplasmic expression.

From a mechanistic perspective, nuclear expression is considered an active marker of NF-κB, whereas cytoplasmic localization of NF-κB is generally thought to indicate inactivation of the pathway.[67] However, 1 interesting finding of our study was that the expression of NF-κB, either nuclear or cytoplasmic, was correlated with unfavorable prognosis of solid tumors. Studies on kinetics of nuclear translocation of NF-κB proteins following activation demonstrated that only ~10% of the total Rel and p50 proteins, which were freed from IκB, was detected in the nucleus and most of the NF-κB proteins remained within the cytoplasm.[68] Moreover, Tam et al[69] found that cytoplasmic localization of p65 required the nuclear export receptor CRM1, indicating that cytoplasmic NF-κB p65 may be a secondary event due to coactivation of other biological factors. These experimental researches might explain that cytoplasmic localization of NF-κB also has its clinical significance in tumor outcome.

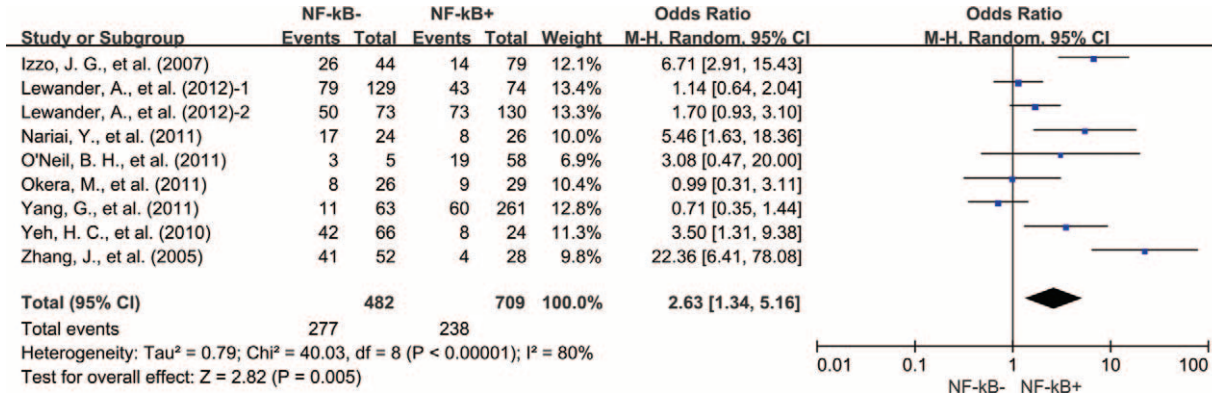

FIGURE 7. Ten-year OS by NF-κB expression. NF-κB = nuclear factor-kappaB, OS = overall survival, 1 = nuclear expression, 2 = cytoplasmic expression.

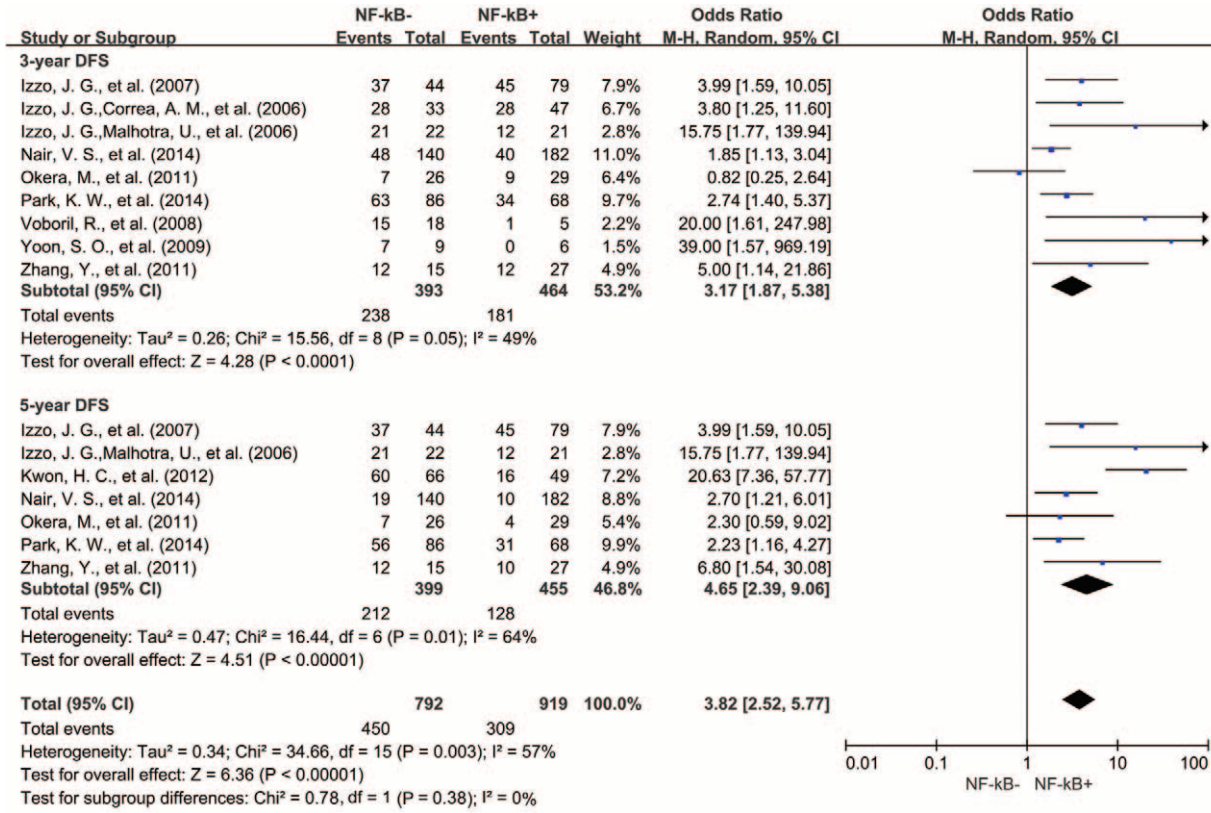

FIGURE 8. Forest plots showing odds ratios of human NF-κB overexpression versus normal NF-κB expression for DFS at 3 and 5 years. DFS = disease free survival, NF-κB = nuclear factor-kappaB.

This study has several important implications. First, it shows that NF-κB expression is associated with worse outcome of solid tumors, which suggests that NF-κB may be a potential therapeutic target. Second, it identifies a subgroup of tumors with unfavorable outcome potentially in colorectal cancer and esophageal carcinoma. Third, it highlights the relevance of NF-κB expression with adverse outcome of solid tumors is independent of cellular localization. Finally, it emphasizes the importance of the development of a valuable biomarker for prognostic assessment.

Some limitations also exist in this meta-analysis. First, the method and cut-off values for assessing NF-κB expression are inconsistent. Second, significant heterogeneity observed across studies cannot be completely accounted despite the use of appropriate meta-analytic techniques with random-effect models. Finally, small studies with negative results may not be published, which can cause publication bias.

In conclusion, our analyses show that overexpression of NF-κB in solid tumor tissues, as measured by IHC, is associated with a worse prognosis in different types of tumor, which suggests that targeting NF-κB could be a promising therapeutic approach for solid tumors.

ACKNOWLEDGMENTS

The authors thank the members of the laboratory for helpful discussions. This work was supported by grants from the National Natural Science Foundation of China (81572800, PW), the Science and Technology Department of Zhejiang Province (2011c13034~1, JH), and Natural Science Foundation of Zhejiang Province (Z2100366, JH and LY15H160041, PW).

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