REVEIW

Editorial Process: Submission:02/09/2018 Acceptance:10/20/2018

Prognostic Value of NRAS Gene for Survival of Colorectal Cancer Patients: A Systematic Review and Meta-Analysis

Yue Hu[1], Shuang-You Tao[2], Jie-Min Deng[1], Zheng-Kun Hou[3], Jia-Qi Liang[1], Qiu-Gu Huang[4], Liang-Hui Li[1], Hui-Biao Li[3], Yi-Ming Chen[1], Hua Yi[1], Xin-Lin Chen[1]*, Hui Liu[4]*

Abstract

Introduction: NRAS gene is associated with malignant proliferation and metastasis of colorectal cancer (CRC). But its prognostic value on CRC is still unknown. The objective of this study is to perform a meta-analysis to obtain its prognostic value on survival of CRC patients. **Methods:** The systematic review and meta-analysis was designed, undertaken and reported using items from the PRISMA statement. Relevant articles were identified through PubMed (containing Medline), Embase, Web of Science databases and Google scholar search engines from their inception up to October 3, 2016. The articles about NRAS on prognosis of CRC patients were enrolled. The association between NRAS and CRC survival time (including overall survival [OS], progression-free survival [PFS], and disease-free survival [DFS]) was evaluated using hazard ratio (HR) with its corresponding 95% confidence interval (CI). **Results:** A total of fifteen articles were included. High-expression of NRAS was significantly associated with poor OS (HR: 1.36, 95% CI: 1.15–1.61), and poor PFS (HR: 1.75, 95% CI: 1.04–2.94). The combined HR of NRAS on DFS was 0.87 (95% CI: 0.37–2.03). Subgroup analysis showed that NRAS was significantly associated with poor OS for patients from Western countries (HR: 1.38, 95% CI: 1.09–1.73), but not for those from Asian countries. **Conclusions:** This meta-analysis demonstrate that NRAS gene could predict the poor prognosis for the CRC patients. More large-sample cohort studies are needed to further confirm this conclusion.

Keywords: NRAS gene- colorectal cancer- prognosis- meta-analysis

Asian Pac J Cancer Prev, **19 (11)**, 3001-3008

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in males worldwide, with an estimated 1.4 million cases and 693,900 deaths occurring in 2012 (Torre et al., 2015). Incidence rates are highest in Australia/New Zealand, Europe and Northern America, and low in Africa and South-Central Asia (Torre et al., 2015). Decreasing colorectal cancer mortality rates have been observed in large numbers of countries worldwide, which ascribed reduced prevalence of risk factors and/or improved treatments to CRC screening (Edwards et al., 2010; Bosetti et al., 2011). However, the global burden of CRC is expected to increase under the diverse global CRC patterns and the number of patients with CRC will continue to increase in future decades (Arnold et al., 2017).

Activating RAS (including HRAS, NRAS and KRAS) mutations occurs in about 30% of human cancers (Schubbert et al., 2007). NRAS is one member of RAS gene family of oncoproteins, which is commonly mutated in melanoma and hematopoietic cancers via mapped on chromosome 1 (Wang et al., 2013; Funck-Brentano et al., 2016). NRAS mediates activation of both mitogen-activated protein kinase (MAPK) and PI3K/AKT/MYC signaling (Whitwam et al., 2007). NRAS induced classical MAPK signaling leads to cyclin D1 expression and cell cycle dysregulation and promotion of prosurvival pathways (Filmus et al., 1994; Boisvert-Adamo and Aplin, 2008). In addition, NRAS effectively prevents Glycogen Synthase Kinase3 (GSK3)-mediated phosphorylation of MYC via PI3K/AKT, which results in enhanced activity of endogenous MYC protein (Whitwam et al., 2007). Mutational NRAS causes Ras-GTP to be in a state of continuous activation, which results in malignant proliferation and metastasis (Mandala et al., 2014).

Many studies have been performed to assess the prognostic value of NRAS in patients with CRC, but the conclusions of these studies were still a matter of intense debate. For example, Schirripa et al., (2015) demonstrated that the NRAS mutations had a relevant incidence in

*[1]School of Basic Medical Science, [2]Spleen and Stomach Institute, [3]The First Affiliated Hospital, [4]The Third Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou, China. *For Correspondence: chenxlsums@126.com, ahl200@163.com. Yue Hu and Shuang-You Tao have equal contribution in this study.*

patients with metastatic colorectal cancer (mCRC) and it was an independent prognostic factor of the survival time for the CRC patients. However, some studies reported that there was no association between NRAS and survival time for the CRC patients (Gavin et al., 2012; Chang et al., 2016). Therefore, we systematically evaluated the correlation between NRAS and survival time of CRC patients and provided clinical guidance for the treatment of the CRC patients.

Materials and Methods

Search strategy

The systematic review and meta-analysis was designed, undertaken and reported using items from the PRISMA statement. A comprehensive literature search was performed in PubMed (containing Medline), Embase, Web of Science databases and Google scholar search engines. These databases were searched from their inception up to October 3, 2016. The following key words were used: colorectal cancer (including colon cancer, and rectal cancer), NRAS (including N-RAS, ALPS4, CMNS, NCMS1 and NS6), and prognosis. The detailed search strategy is presented in Appendix 1 (Supplementary material). References from any other relevant studies were also scanned to identify the eligible studies. Only English publications were included.

Selection criteria

Articles were included if they met the following criteria: (1) colorectal cancer, colon cancer, or rectal cancer; (2) NRAS gene; (3) the outcomes: such as overall survival (OS), disease-free survival (DFS), progression-free survival (PFS); Hazard ratio (HR) with corresponding 95% confidence interval (CI) were reported. The studies which reported sufficient data to calculate HR with corresponding 95% CI were also included. The articles were excluded if they contained insufficient information for data extraction, repeated or overlapped publications, review articles or comments.

Data collection

The following data were extracted from the eligible study: the name of the first author, year of publication, countries where the study was carried out, study period, age and gender of the patients, treatment time, treatment method, sample size, and follow-up time. HRs with their corresponding 95% CI for OS, PFS and DFS were also collected. Information from the studies was extracted independently by two of three authors (J.Q.L, Q.G.H and L.H.L). If there were discrepancies between reviewers, they discussed and resolved with fourth author (Y.H).

Statistical Analysis

Statistical analyses were carried out with STATA version 12.0. All statistical tests were two-sided. P value ≤ 0.05 was considered to be statistically significant. The primary outcomes of interest were OS, PFS and DFS. The HR and its 95% CI were used to measure the prognostic effect of NRAS on survival time. If the HR and its 95% CI were given explicitly in the studies, the crude values were used. If these indexes were indeterminate, they were calculated from the available numerical data or survival curve (Kaplan-Meier curves) using the methods reported by Tierney (Tierney et al., 2007). Statistical heterogeneity among studies was assessed by Cochran's Q test and inconsistency index (I^2) statistic. When the studies were homogenous, fixed-effects model was applied for HR estimation. When the studies were heterogeneous, random-effects model was chosen. An observed HR > 1 implied a worse prognosis for high-expression of NRAS in comparison to low expression.

If the eligible articles were adequate (for example 5 studies in any of the subgroups), subgroup analysis according to study countries (Asian, Western countries) was carried out. Publication bias was investigated using Begg's test and Egger's test. Sensitivity analysis was performed by removing each study in the meta-analysis at a time to determine its influence on pooled HR.

Results

Study characteristics

The literature review using the search criteria produced 756 articles from PubMed, Embase, Web of Science databases and Google scholar search engines. After screening the titles, abstracts and removal of duplicates, 46 full text articles were considered. Eventually, a total of 15 articles met our inclusion criteria and were used to perform this meta-analysis (Figure 1).

The study characteristics were shown in detail in Table 1. A total of 12,135 patients were included in our study. The age of the patient ranged from 25 to 108 years old. The median follow-up time ranged from 8.5 to 100.7 months. Among the fifteen studies, three studies reported both OS and PFS (De Roock et al., 2010; Takahashi et al., 2014; Modest et al., 2016), and one article reported OS and DFS (Chang et al., 2016). At last, ten studies

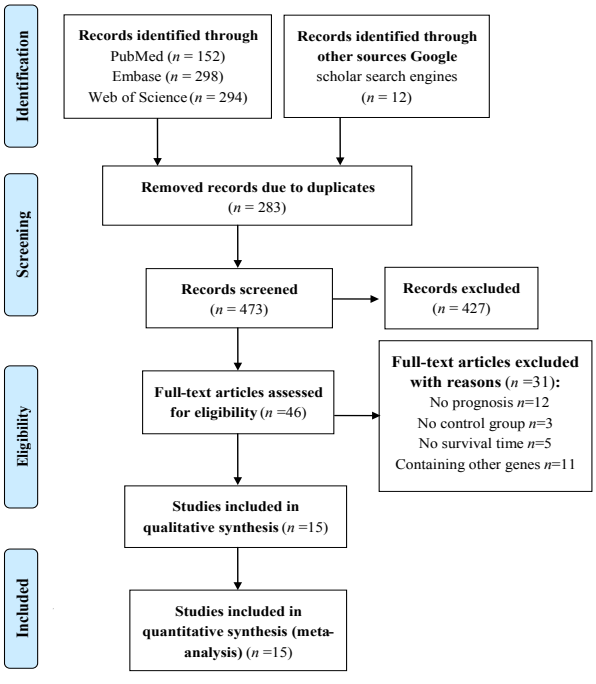

Figure 1. Flow Chart of the Search Strategy

Table 1. Characteristics and HR Results of the Included Studies

First Author, year of publication	Country	Study period	Sample size	Male (%)	Mean age (range)	Patients	Stage I+II (%)	Surgical therapy* (%)	Chemotherapy* (%)	Radiotherapy* (%)	Follow-up (median, months)
Schirripa 2015	Italy	2009-2012	786	59.2	NR (25–88)	mCRC	NR	NR	NR	0	8.5
Takahashi 2014	Japan	2008-2011	129	NR	NR (NR)	mCRC	NR	100	100	0	NR
Gavin 2012	USA	NR	2,299	NR	NR (NR)	Colon cancer	NR	100	NR	0	NR
Maroudov 2013	Australia	2002-2004	822	64.7	64.2 (NR)	CRC	48.9	100	63.9	63.9	58.5
Igarashi 2015	Japan	1997-2013	102	70.6	60.4 (NR)	mCRC	NR	100	100	0	48
Gleeson 2015	USA	2002-2011	102	64.7	69.9 (NR)	Rectal cancer	NR	100	NR	NR	69.6
Lee 2016	USA	2010-2013	179	NR	NR (NR)	mCRC	41.3	NR	NR	NR	NR
Hsu 2016	Taiwan	2010-2014	53	64.2	63.5 (28–93)	mCRC	NR	NR	100	100	17.1
Chang 2016	Taiwan	2000-2010	1,249	65.8	72 (27–108)	CRC	62.1	100	NR	NR	62
Osumi 2016	Japan	2012-2013	132	54.6	63 (NR)	mCRC	22	100	80.3	NR	84.1
Chang 2016	Taiwan	2000-2009	1,519	34.3	72 (50–93)	CRC	51.4	100	0	0	100.7
De Roock 2010	Europe&	2001-2008	1,022	36.9	61 (22–86)	mCRC	NR	NR	63.5	NR	NR
Ogura 2014	Japan	1999-2008	1,304	59.8	63.8 (NR)	CRC	50.2	100	NR	NR	67.2
Modest 2016	Germany	2000-2013	1,239	65.4	64 (25–83)	mCRC	NR	NR	4.3	NR	NR
Seymour 2013	UK	2006-2010	1,198	26.5	63 (56–70)	CRC	NR	NR	100	10.6	25.4

First Author, year of publication	Test sample	Test content	Test method	Analytic method	Outcome	OS		PFS		DFS	
						HR	95% CI	HR	95% CI	HR	95% CI
Schirripa 2015	Tissue	Protein	PCR	Multivariate	OS	1.75	1.13–2.72	NR	NR	NR	NR
Takahashi 2014	Tissue	DNA	IHC	Multivariate	OS, PFS	2.69	0.89–8.10	4.51	1.80–11.32	NR	NR
Gavin 2012	Tissue	DNA	IHC	Univariate	OS	1.25	0.80–1.95	NR	NR	NR	NR
Maroudov 2013	Tissue	DNA	Sanger sequencing	Multivariate	DFS	NR	NR	NR	NR	0.82	0.36–1.85
Igarashi 2015	Tissue	DNA/RNA	PCR	Univariate	PFS	NR	NR	2.61	0.89–6.16	NR	NR
Gleeson 2015	Tissue	DNA	PCR	Multivariate	DFS	NR	NR	NR	NR	0.42	0.19–0.94
Lee 2016	Tissue	RNA	PCR	Multivariate	PFS	NR	NR	2.27	1.25–4.13	NR	NR
Hsu 2016	Tissue	DNA	PCR	Univariate	PFS	NR	NR	0.66	0.19–2.23	NR	NR
Chang 2016	Tissue	DNA	PCR	Multivariate	OS	1.59	1.06–2.38	NR	NR	NR	NR
Osumi 2016	Tissue	DNA	Luminex xMAP	Univariate	OS	3.99	0.50–31.2	NR	NR	NR	NR
Chang 2016	Tissue, Plasma	DNA	PCR	Multivariate	OS, DFS	1.39	0.97–1.99	NR	NR	1.71	0.98–2.97
De Roock 2010	tissue	DNA	PCR	Multivariate	OS, PFS	1.82	1.01–3.30	1.79	1.00–3.20	NR	NR
Ogura 2014	tissue	DNA	HRM	Multivariate	OS	0.53	0.27–1.03	NR	NR	NR	NR
Modest 2016	tissue	DNA	RT-qPCR	Multivariate	OS, PFS	1.01	0.60–1.72	0.9	0.58–1.39	NR	NR
Seymour 2013	tissue	DNA	Pyrosequence	Multivariate	OS	1.15	0.60–2.21	NR	NR	NR	NR

CRC, colorectal cancer; DFS, disease-free survival; IHC, immunohistochemistry; mCRC, metastatic colorectal cancer; Multivariate, multivariate survival analyses; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; Univariate, univariate survival analyses; NR, not report;*, The proportion of the patients receiving the treatment; &, contained many countries in Europe (Belgium, Switzerland, Greece, Cyprus, France, Italy, Spain and Denmark)

Table 2. Meta-Analysis Results of NRAS Gene and Colorectal Cancer Risk

	Number of studies	Patients	HR (95% CI)	Heterogeneity		
				I^2	χ^2	P
Overall survival						
All	10	10,877	1.36 (1.15–1.61)	38.30%	14.6	0.103
Asian countries	5	4,333	1.34 (0.83–2.16)*	63.00%	10.82	0.029
Western countries	5	6,544	1.38 (1.09–1.73)	0.00%	3.76	0.44
Progression-free survival						
All	6	2,724	1.75 (1.04–2.94) *	69.30%	16.31	0.006
Disease-free survival						
All	3	2,443	0.87 (0.37–2.03) *	75.90%	8.28	0.016

*Results were based on a random-effects model

Table 3. The Results of Begg's and Egger's Tests

	Number of studies	Begg's test		Egger's test	
		Z value	P	*t* value	P
Overall survival	10	0.09	0.929	0.72	0.494
Progression-free survival	6	0.94	0.348	-0.33	0.756
Disease-free survival	3	-0.52	0.602	2.08	0.286

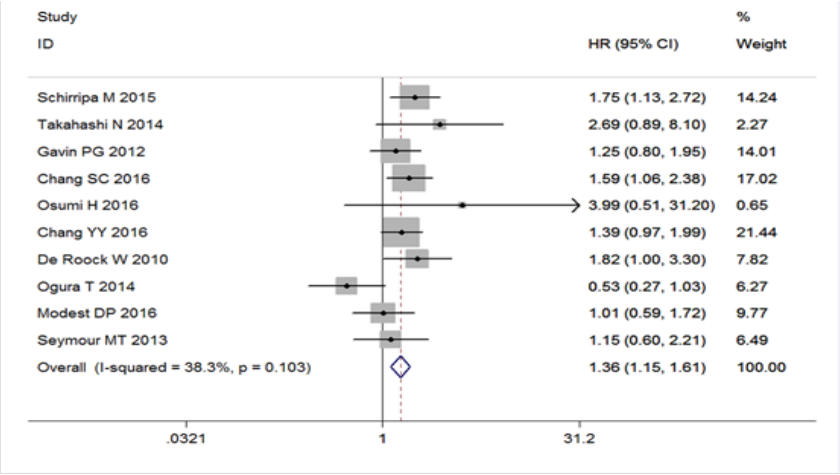

Figure 2. Forest Plot Evaluating the Combined HRs between NRAS and OS

reported OS, six articles presented PFS, and three articles presented DFS.

Meta-analysis of OS

Ten studies investigated the association between NRAS gene and OS for CRC patients (De Roock et al., 2010; Gavin et al., 2012; Seymour et al., 2013; Ogura et al., 2014; Takahashi et al., 2014; Schirripa et al., 2015; Chang et al., 2016; Chang et al., 2016; Modest et al., 2016; Osumi et al., 2016). The pooled HR of OS in ten studies was 1.36 (95% CI: 1.15–1.61) according to fixed-effects model (I^2 = 38.3%, P = 0.103) (Table 2, Figure 2).

Ten studies were included for OS, therefore subgroup analysis according to study countries (Asian, Western countries) was performed. There was a significant association between OS and NRAS gene in Western studies (HR = 1.38; 95% CI: 1.09–1.73, Table 2). There was not a significant association between OS and NRAS gene in Asian studies (HR = 1.34; 95% CI: 0.83–2.16, Table 2).

Meta-analysis of PFS and DFS

Six studies reported the association between NRAS gene and PFS for CRC patients (De Roock et al., 2010; Takahashi et al., 2014; Igarashi et al., 2015; Hsu et al., 2016; Lee et al., 2016; Modest et al., 2016). The summary HR was 1.75 (95% CI: 1.04–2.94, Figure 3), which were from random-effects model.

Three studies reported the association between NRAS gene and DFS for CRC patients (Mouradov et al., 2013; Gleeson et al., 2015; Chang et al., 2016). The pooled HR of DFS in three studies was 0.87 (95% CI: 0.37–2.03) basing on the result of random-effects model due to heterogeneity (I^2 = 75.9 %, P = 0.016, Figure 4).

Risk of bias

Begg's funnel plot and Egger's tests were used to assess the publication bias. No obvious publication bias was found in included studies, suggesting there is low

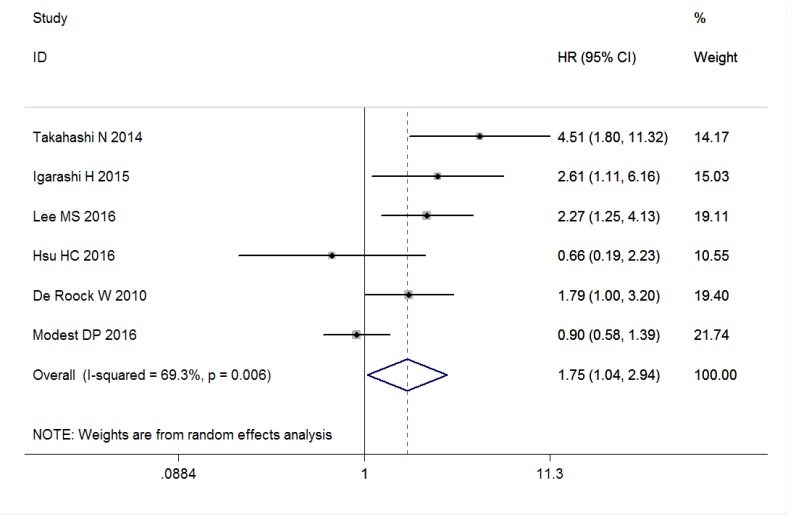

Figure 3. Forest Plot Evaluating the Combined HRs between NRAS and PFS

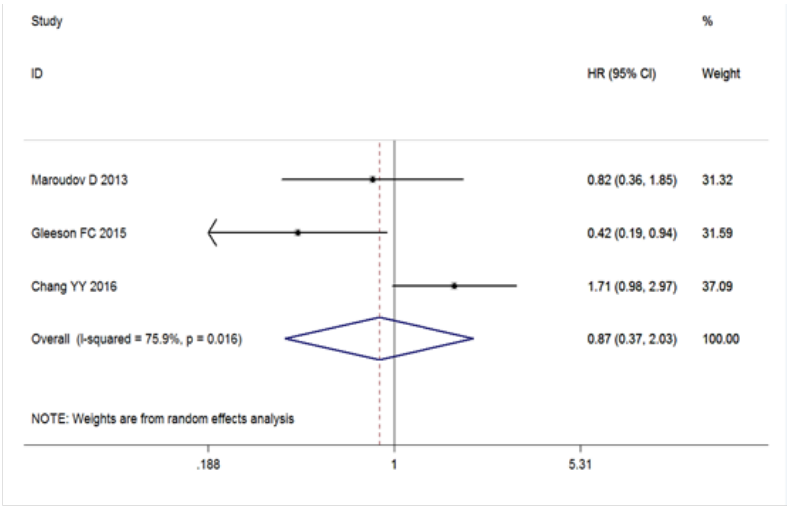

Figure 4. Forest Plot Evaluating the Combined HRs between NRAS and DFS

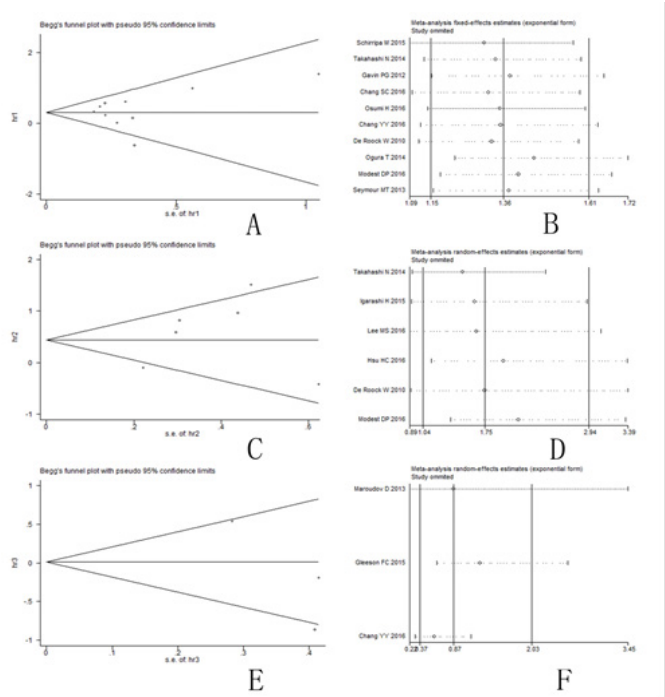

Figure 5. Begg's Funnel and Sensitivity Analysis Plot (A, Begg's funnel for OS; B, sensitivity analysis for OS; C, Begg's funnel for PFS; D, sensitivity analysis for PFS; E, Begg's funnel for DFS; F, sensitivity analysis for DFS)

publication bias (Table 3, Figure 5). The stability of the results was assessed by sensitivity analysis (Figure 5).

Discussion

The results of our meta-analysis provided supportive evidence that NRAS gene could be a prognostic indicator for CRC. With regard to OS (PFS), the mortality risk of patients with high-expression of NRAS was 1.36 (1.75) times higher than those with low-expression of NRAS. Similar results were also found in patients with lung cancer (Ohashi et al., 2013), gastric cancer (Takahashi et al., 2014), melanoma (Jakob et al., 2012; Birkeland et al., 2013), and autoimmune lymphoproliferative syndrome (Oliveira et al., 2007). For example, Birkeland et al., (2013) reported that NRAS expression levels influenced the prognosis in patients with advanced melanoma.

Normanno et al., (2015) reported that NRAS mutations were usually present in the majority of neoplastic cells.

NRAS was a prognostic indicator for the CRC patients, the following signaling pathway might explain the reasons. (1) The over-expression of NRAS contributed to survival time in CRC patients via the targeting of MAPK. The MAPK pathway was involved in apoptosis related to growth factors and cyclo-oxygenase 2 in CRC (Fang and Richardson, 2005). The MAPK pathway was associated with a poor prognosis in cancer (Hendrickx et al., 2003). (2) The second signaling pathway was related to MYC. MYC was an oncogenic transcription factor and could either activate or repress transcription (Walz et al., 2014). Further-more, MYC was deregulated in most types of cancer, and it controlled many cellular processes, including cell growth, metabolism, proliferation, differentiation and apoptosis (Amati et al., 2001; Dang, 2013; McMahon, 2014; Bretones et al., 2015). Recent evidences showed that MYC promoted proliferation and invasion of colon and gastric cancer cells (Yang et al., 2013; He et al., 2014; He et al., 2014).

NRAS-targeted therapy should be considered since the over-expression of NRAS was associated with poor prognosis in CRC. NRAS mutations in colorectal cancer play a critical role in clinical studies for treatment of metastatic CRC with anti-EGFR antibodies. In recent past years, epidermal growth factor receptor (EGFR) of depending pathway has been largely exploited for personalized therapies, and EGFR has become a key target of specific inhibitors to treat metastatic CRC (Therkildsen et al., 2014; Bronte et al., 2015; Ciardiello et al., 2016; Liu et al., 2016). One study demonstrated that EGFR expression has prognostic value for patients with metachronous mCRC (Huang et al., 2013). NRAS have been recently hypothesized to have involvement in resistance to anti-EGFR agents in CRC (Troiani et al., 2013; Ciardiello et al., 2014). Two studies reported that wild-type KRAS patients carrying NRAS mutations, had lower response rates for anti-EGFR therapy compared with those with dual wild-type genes (Andre et al., 2013; Di Bartolomeo et al., 2014). Peeters et al., (2013) reported that treatment with panitumumab resulted in improved PFS in patients with wild-type KRAS/NRAS rather than those with wild-type KRAS/mutational NRAS in randomized Phase III study. A poor prognostic effect was observed in patients with NRAS mutations in a randomized phase 3 metastatic CRC COIN trial (Maughan et al., 2011).

However, for DFS, the results indicated the prognostic value of NRAS gene was not associated with colorectal cancer (HR = 0.87, 95% CI: 0.37–2.03). The main reasons maybe contain: (1) Only three studies about DFS were included in our meta-analysis. The less the included studies, the more difficult it was to get statistically significant results. (2) The heterogeneity between the three studies was observed, which had an effect on the results. The reasons for the heterogeneity were as following: different follow-up time (One study follow-up time was twice longer than other two studies), and different characteristics of the patients.

Our study had several limitations. (1) The detection methods of NRAS were different from each other, such as PCR and IHC. However, the homogeneity among these studies was obtained. Thus, the confounding effects of different detection methods would not be substantial. (2) The methods of therapy also affected the survival time of CRC patients. Some studies chose surgery and chemotherapy (or/and radiotherapy), and some only surgery. Due to the lack of relevant information, we did not analyze their effects on survival time. (3) There was significant heterogeneity among DFS studies. Although we investigated the reasons of the heterogeneity and conducted subgroup analyses according to geographical regions, the heterogeneity remained significant.

In summary, the results from this meta-analysis showed that NRAS gene could be a prognostic indicator (including poor OS and PFS) for the patients with CRC. In this case, NRAS may be a promising, new therapeutic target for CRC and may enable clinical practitioners to better predict patient prognosis through the detection of NRAS levels in patients. However, well-designed randomized controlled trials will be needed to determine whether NRAS is a useful biomarker for predicting CRC into clinical decision-making in the future.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the support by the National Natural Science Foundation of China (81774451, 81403296), the Outstanding Youth Foundation of Guangdong Province Colleges and Universities (YQ2015041), the Young Talents Foundation of Guangzhou University of Chinese Medicine (QNYC20140101), Natural Science Foundation of Guangdong Province (2017A030313827, 2015A030313036), and Guangdong high level universities program of Guangzhou University of Chinese Medicine.

References

Amati B, Frank SR, Donjerkovic D, et al (2001). Function of the c-Myc oncoprotein in chromatin remodeling and transcription. *Biochim Biophys Acta*, **1471**, 135-45.

Andre T, Blons H, Mabro M, et al (2013). Panitumumab combined with irinotecan for patients with KRAS wild-type metastatic colorectal cancer refractory to standard chemotherapy: a GERCOR efficacy, tolerance, and translational molecular study. *Ann Oncol*, **24**, 412-9.

Arnold M, Sierra MS, Laversanne M, et al (2017). Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, **66**, 683-91.

Birkeland E, Busch C, Berge EO, et al (2013). Low BRAF and NRAS expression levels are associated with clinical benefit from DTIC therapy and prognosis in metastatic melanoma. *Clin Exp Metastasis*, **30**, 867-76.

Boisvert-Adamo K, Aplin AE (2008). Mutant B-RAF mediates resistance to anoikis via Bad and Bim. *Oncogene*, **27**, 3301-12.

Bosetti C, Levi F, Rosato V, et al (2011). Recent trends in colorectal cancer mortality in Europe. *Int J Cancer*, **129**, 180-91.

Bretones G, Delgado MD, Leon J (2015). Myc and cell cycle control. *Biochim Biophys Acta*, **1849**, 506-16.

Bronte G, Silvestris N, Castiglia M, et al (2015). New findings on primary and acquired resistance to anti-EGFR therapy in metastatic colorectal cancer: do all roads lead to RAS?. *Oncotarget*, **6**, 24780-96.

Chang SC, Lin PC, Lin JK, et al (2016). Mutation spectra of common cancer-associated genes in different phenotypes of colorectal carcinoma without distant metastasis. *Ann Surg Oncol*, **23**, 849-55.

Chang YY, Lin PC, Lin HH, et al (2016). Mutation spectra of RAS gene family in colorectal cancer. *Am J Surg*, **212**, 537-44.

Ciardiello F, Normanno N, Maiello E, et al (2014). Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial. *Ann Oncol*, **25**, 1756-61.

Ciardiello F, Normanno N, Martinelli E, et al (2016). Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): a randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX. *Ann Oncol*, **27**, 1055-61.

Dang CV (2013). MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med*, **3**, 1-15.

De Roock W, Claes B, Bernasconi D, et al (2010). Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*, **11**, 753-62.

Di Bartolomeo M, Pietrantonio F, Perrone F, et al (2014). Lack of KRAS, NRAS, BRAF and TP53 mutations improves outcome of elderly metastatic colorectal cancer patients treated with cetuximab, oxaliplatin and UFT. *Target Oncol*, **9**, 155-62.

Edwards BK, Ward E, Kohler BA, et al (2010). Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer*, **116**, 544-73.

Fang JY, Richardson BC (2005). The MAPK signalling pathways and colorectal cancer. *Lancet Oncol*, **6**, 322-7.

Filmus J, Robles AI, Shi W, et al (1994). Induction of cyclin D1 overexpression by activated ras. *Oncogene*, **9**, 3627-33.

Funck-Brentano E, Helias-Rodzewicz Z, Longvert C, et al (2016). Increase in NRAS mutant allele percentage during metastatic melanoma progression. *Exp Dermatol*, **25**, 472-4.

Gavin PG, Colangelo LH, Fumagalli D, et al (2012). Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res*, **18**, 6531-41.

Gleeson FC, Kipp BR, Voss JS, et al (2015). Endoscopic ultrasound fine-needle aspiration cytology mutation profiling using targeted next-generation sequencing: personalized care for rectal cancer. *Am J Clin Pathol*, **143**, 879-88.

He W, Li Y, Chen X, et al (2014). miR-494 acts as an anti-oncogene in gastric carcinoma by targeting c-myc. *J Gastroenterol Hepatol*, **29**, 1427-34.

He X, Tan X, Wang X, et al (2014). C-Myc-activated long noncoding RNA CCAT1 promotes colon cancer cell proliferation and invasion. *Tumour Biol*, **35**, 12181-8.

Hendrickx N, Volanti C, Moens U, et al (2003). Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. *J Biol Chem*, **278**, 52231-9.

Hsu HC, Thiam TK, Lu YJ, et al (2016). Mutations of KRAS/NRAS/BRAF predict cetuximab resistance in metastatic colorectal cancer patients. *Oncotarget*, **7**, 22257-70.

Huang CW, Tsai HL, Chen YT, et al (2013). The prognostic values of EGFR expression and KRAS mutation in patients with synchronous or metachronous metastatic colorectal cancer. *BMC Cancer*, **13**, 1-12.

Igarashi H, Kurihara H, Mitsuhashi K, et al (2015). Association of microRNA-31-5p with clinical efficacy of anti-EGFR therapy in patients with metastatic colorectal cancer. *Ann Surg Oncol*, **22**, 2640-8.

Jakob JA, Bassett RL Jr, Ng CS, et al (2012). NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer*, **118**, 4014-23.

Lee MS, McGuffey EJ, Morris JS, et al (2016). Association of CpG island methylator phenotype and EREG/AREG methylation and expression in colorectal cancer. *Br J Cancer*, **114**, 1352-61.

Liu J, Hu J, Cheng L, et al (2016). Biomarkers predicting resistance to epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer with wild-type KRAS. *Onco Targets Ther*, **9**, 557-65.

Mandala M, Merelli B, Massi D (2014). Nras in melanoma: targeting the undruggable target. *Crit Rev Oncol Hematol*, **92**, 107-22.

Maughan TS, Adams RA, Smith CG, et al (2011). Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet*, **377**, 2103-14.

McMahon SB (2014). MYC and the control of apoptosis. *Cold Spring Harb Perspect Med*, **4**, 1-9.

Modest DP, Ricard I, Heinemann V, et al (2016). Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. *Ann Oncol*, **27**, 1746-53.

Mouradov D, Domingo E, Gibbs P, et al (2013). Survival in stage II/III colorectal cancer is independently predicted by chromosomal and microsatellite instability, but not by specific driver mutations. *Am J Gastroenterol*, **108**, 1785-93.

Normanno N, Rachiglio AM, Lambiase M, et al (2015). Heterogeneity of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer and potential effects on therapy in the CAPRI GOIM trial. *Ann Oncol*, **26**, 1710-4.

Ogura T, Kakuta M, Yatsuoka T, et al (2014). Clinicopathological characteristics and prognostic impact of colorectal cancers with NRAS mutations. *Oncol Rep*, **32**, 50-6.

Ohashi K, Sequist LV, Arcila ME, et al (2013). Characteristics of lung cancers harboring NRAS mutations. *Clin Cancer Res*, **19**, 2584-91.

Oliveira JB, Bidere N, Niemela JE, et al (2007). NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc Natl Acad Sci U S A*, **104**, 8953-8.

Osumi H, Shinozaki E, Suenaga M, et al (2016). RAS mutation is a prognostic biomarker in colorectal cancer patients with metastasectomy. *Int J Cancer*, **139**, 803-11.

Peeters M, Oliner KS, Parker A, et al (2013). Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase III study of metastatic colorectal cancer. *Clin Cancer Res*, **19**, 1902-12.

Schirripa M, Cremolini C, Loupakis F, et al (2015). Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J Cancer*, **136**, 83-90.

Schubbert S, Shannon K, Bollag G (2007). Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer*, **7**, 295-308.

Seymour MT, Brown SR, Middleton G, et al (2013). Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol*, **14**, 749-59.

Takahashi N, Yamada Y, Taniguchi H, et al (2014). Clinicopathological features and prognostic roles of KRAS, BRAF, PIK3CA and NRAS mutations in advanced gastric cancer. *BMC Res Notes*, **7**, 271.

Takahashi N, Yamada Y, Taniguchi H, et al (2014). Combined assessment of epidermal [corrected] growth factor receptor dual color in situ hybridization and immunohistochemistry with downstream gene mutations in prediction of response to the anti-EGFR therapy for patients with metastatic colorectal cancer. *Arch Med Res*, **45**, 366-74.

Therkildsen C, Bergmann TK, Henrichsen-Schnack T, et al (2014). The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. *Acta Oncol*, **53**, 852-64.

Tierney JF, Stewart LA, Ghersi D, et al (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*, 8, 16.

Torre LA, Bray F, Siegel RL, et al (2015). Global cancer statistics, 2012. *CA Cancer J Clin*, **65**, 87-108.

Troiani T, Zappavigna S, Martinelli E, et al (2013). Optimizing treatment of metastatic colorectal cancer patients with anti-EGFR antibodies: overcoming the mechanisms of cancer cell resistance. *Expert Opin Biol Ther*, **13**, 241-55.

Walz S, Lorenzin F, Morton J, et al (2014). Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. *Nature*, **511**, 483-7.

Wang Y, Velho S, Vakiani E, et al (2013). Mutant N-RAS protects colorectal cancer cells from stress-induced apoptosis and contributes to cancer development and progression. *Cancer Discov*, **3**, 294-307.

Whitwam T, Vanbrocklin MW, Russo ME, et al (2007). Differential oncogenic potential of activated RAS isoforms in melanocytes. *Oncogene*, **26**, 4563-70.

Yang F, Xue X, Bi J, et al (2013). Long noncoding RNA CCAT1, which could be activated by c-Myc, promotes the progression of gastric carcinoma. *J Cancer Res Clin Oncol*, **139**, 437-45.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.