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The prognostic value of *KRAS* mutation by cell-free DNA in cancer patients: A systematic review and meta-analysis

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Abstract

KRAS mutation has been found in various types of cancer. However, the prognostic value of *KRAS* mutation in cell-free DNA (cfDNA) in cancer patients was conflicting. In the present study, a meta-analysis was conducted to clarify its prognostic significance. Literature searches of Cochrane Library, EMBASE, PubMed and Web of Science were performed to identify studies related to *KRAS* mutation detected by cfDNA and survival in cancer patients. Two evaluators reviewed and extracted the information independently. Review Manager 5.3 software was used to perform the statistical analysis. Thirty studies were included in the present meta-analysis. Our analysis showed that *KRAS* mutation in cfDNA was associated with a poorer survival in cancer patients for overall survival (OS, HR 2.02, 95% CI 1.63–2.51, $P<0.01$) and progression-free survival (PFS, HR 1.64, 95% CI 1.27–2.13, $P<0.01$). In subgroup analyses, *KRAS* mutation in pancreatic cancer, colorectal cancer, non-small cell lung cancer and ovarian epithelial cancer had HRs of 2.81 (95% CI 1.83–4.30, $P<0.01$), 1.67 (95% CI 1.25–2.42, $P<0.01$), 1.64 (95% CI 1.13–2.39, $P = 0.01$) and 2.17 (95% 1.12–4.21, $p = 0.02$) for OS, respectively. In addition, the ethnicity didn't influence the prognostic value of *KRAS* mutation in cfDNA in cancer patients ($p = 0.39$). Prognostic value of KRAS mutation was slightly higher in plasma than in serum (HR 2.13 vs 1.65), but no difference was observed ($p = 0.37$). Briefly, *KRAS* mutation in cfDNA was a survival prognostic biomarker in cancer patients. Its prognostic value was different in various types of cancer.

Introduction

In recent years, the molecular biomarkers are increasingly being regarded as both predictive and prognostic tools for cancer patients. Currently, the alterations detection in biomarkers is considered the standard of care in many types of cancer, including lung, pancreatic, and colorectal cancer. For example, the National Comprehensive Cancer Network (NCCN) guidelines

recommend testing for the *KRAS* alterations as a part of the initial diagnostic check for metastatic colorectal cancer (CRC) [1].

KRAS, which is known as an important member of *RAS* family and encoded by the *KRAS* gene, is a small GTPase which cycles between active guanosine triphosphate (GTP)-bound (*KRAS*-GTP) and inactive guanosine diphosphate (GDP)-bound (*KRAS*-GDP) conformations. It plays a critical important role in normal tissue signaling. *KRAS* mutation can impair the intrinsic GTPase activity and lead to the permanent activation of its downstream signaling pathways, such as PI3K/AKT/mTOR and RAF/MEK/ERK [2,3]. Several studies have reported that *KRAS* mutation could enhance the cellular proliferation, induce the malignant transformation [4–6]. As a result, the continuous activation would contribute to the development and maintenance in cancer.

A growing number of studies indicated that *KRAS* mutation was a prognostic biomarker to predict the survival outcomes in cancer patients. A previous meta-analysis had suggested that *KRAS* mutation was associated with a poorer overall survival in patients with pancreatic cancer, especially when the mutation detection was performed by the circulating tumor DNA [7]. However, the prognostic value of *KRAS* mutation detected by cfDNA on survival in other cancer patients is still not completely clear. Thus, in the present study, we conducted a meta-analysis to investigate the effect of *KRAS* mutation detected by cfDNA on survival in patients with cancer.

Methods

Data sources and search strategy

The literature searches of EMBASE databases, Cochrane Library databases, and Web of Science were performed on June, 2016 and PubMed performed on March 2017. The main keywords used for the search were *K-ras* or *KRAS* or kirsten-ras or Kirsten ras or ki-ras, neoplasm or cancer or tumor or tumour or other subtypes/synonyms for cancer, liquid biopsy or serum or plasma or cell-free DNA or cell-free plasma DNA or cfDNA, and prognosis or survival. The detailed search terms and strategies were shown in S1 Table. Additionally, the full articles published were limited to English-language. The citation lists of retrieved articles were manually screened independently by two authors (ZYR and LS). All selected studies were checked according to a Newcastle-Ottawa Quality assessment Scale which was developed previously [8].

Selection criteria

The inclusion criteria of our meta-analysis was as follows: (1) independently published observational study (case–control or cohort study) investigating the association between *KRAS* mutation detected by liquid biopsy and survival in cancer patients; (2) a study had reported the HR and its 95% CI for the association between *KRAS* mutation detected by cfDNA and survival in cancer patients; (3) a study had reported other indexes which could be used to calculate the HR and its 95% CI according to previously published methods[9,10]. In addition, the following exclusion criteria were also used: (1) abstracts and reviews; (2) studies without enough information; and (3) repeated or overlapping publications.

Data extraction and quality assessment

The data extraction and quality assessment were performed by two investigators independently. The detailed information (first author, year of publication, period of study, the age of study population, country of study, ethnicity, cancer types and HR estimates) of each eligible study was collected. If several publications were overlapped, we selected the most recently

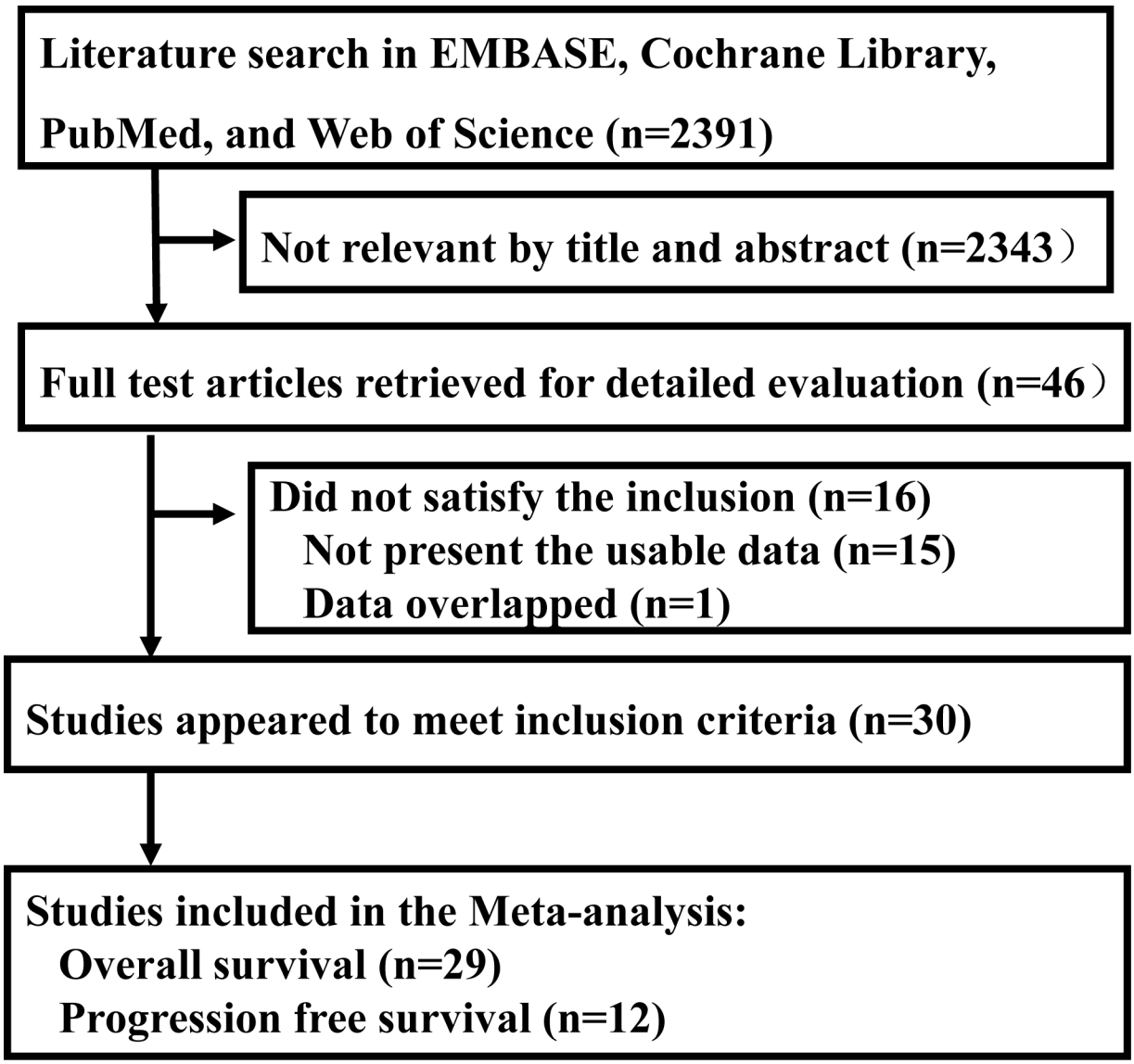

Fig 1. Flow chart of selection process for the eligible studies.

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published study or study with the largest numbers of subjects to be further analyzed. In addition, the discrepancies were reviewed and resolved in the present a third author (mainly SH).

The nine-star Newcastle–Ottawa Scale (NOS) was performed to assess the quality of each eligible study. With a NOS score equal or greater than seven, a study would be considered to be with high quality. An investigator would examine and adjudicate the information independently after data extraction and assessment.

Statistical analysis

The HR and its related 95% CI reported or obtained by calculating in each study were performed to estimate the association between *KRAS* mutation in cfDNA and survival in cancer patients. If there was no heterogeneity existed, the fixed effects model was choose to assess the pooled HRs and its related 95%CIs; otherwise, the random effects model would be selected.

Table 1. The main characteristic of the studies included in the meta-analysis.

Study	Country	Study Period	Age (years)	Tumor Types	Stage	KRAS mutation/ Total	Detection methods	Outcomes	HR estimates
Camps,2005[14]	Spain	1999–2002	Median 64	Non-small cell lung cancer	IIIB-IV	20/67	Serum PCR-RFLP	OS, PFS	OS-KM
Camps,2011[13]	Spain	NA	Median 60	Non-small cell lung cancer	IIIB-IV	27/251	Plasma Allelic Discrimination with RT-PCR	OS, PFS	KM
Castells,1999[15]	Spain	1996–1997	Mean 62.6	Pancreatic cancer	I–IV	12/44	Plasma RELP-PCR	OS	KM
Chen,2010[16]	China	2007–2008	Median 60	Pancreatic cancer	III–IV	30/91	Plasma Sequence	OS	HR+CI (m)
Dobrzycka,2011[17]	Poland	2002–2005	Median 58.3	Ovarian epithelial cancer	I–IV	27/126	Plasma PCR-RFLP	OS	KM
Earl,2015[18]	Spain	2009–2014	Median 68	Pancreatic cancer	LA, IV	8/31	Plasma ddPCR	OS	HR+P
El Messaoudi, 2016 [19]	France	2010–2012	Median 66.6	Colorectal Cancer	IV	38/91	Plasma AS-PCR	OS	HR+CI
Gautschi,2007[20]	Switzerland	2001–2003	Median 61	Lung cancer	I–IV	16/175	Plasma PCR-RFLP	OS	HR+CI
Hadano,2016[21]	Japan	2007–2013	Median 69	Pancreatic cancer	I–IV	86/105	Plasma ddPCR	OS	KM
Han,2016[22]	Korea	NA	Median 58	non-small cell lung cancer	IIIB- IV	19/135 (OS) 7/59 (PFS)	Plasma PNA-PCR	OS, PFS	KM
Hara,2017[23]	Japan	2010–2013	Median 67	colorectal cancer	I-III	26/71	Plasma NA	OS, PFS	OS-KM PFS-HR+CI (m)
Janowski,2017[24]	United States	2011–2015	Median 56	colorectal cancer	IV	27/49	Plasma qPCR	OS	HR+CI(m)
Kim,2015[25]	Korea	2008–2011	Median 62	Colorectal Cancer	Advanced	26/65	Serum RFLP-PCR	OS	KM
Kimura,2004[26]	United States	2000–2002	Median 63	Non–Small-Cell Lung Cancer	IIIB-IV	5/25	Plasma RFLP-PCR	OS	KM
Kingham,2016[30]	United States	1990 to 2014	Age 59	Colorectal Cancer	I–IV	15/43	Serum qRT-PCR	OS	Survival rate
Kinugasa,2015[27]	Japan	2008–2010, 2011–2013	Median 66	Pancreatic cancer	I–IV	101/141	Serum ddPCR-PHFA	OS	HR+CI
Laethem,2017[40]	German	NA	Median 63	Pancreatic cancer	II-IV	39/60	Plasma BEAMing	OS	HR+CI
Nygaard,2013[28]	Denmark	2007–2010	Median 66	Non-small cell lung cancer	II-IV	43/246	Plasma ARMS-qPCR	OS, PFS	HR+CI(m)
Nygaard,2014[29]	Denmark	NA	Median 64	Non-small cell lung cancer	III-IV	7/58	Plasma ARMS-qPCR	OS, PFS	HR+CI
Ramirez,2003[31]	Spain	1998–1999	Median 62	Non-small cell lung cancer	I–IV	9/50	Serum RFLP-PCR	OS	KM
Semrad,2015[32]	United States	2009–2012	Median 67	Pancreatic cancer	Advanced or IV	10/27	Plasma ARMS	OS, PFS	KM
Singh,2015[33]	India	2007–2011	Mean 55	Pancreatic cancer	42% of IV	34/110	Plasma RFLP-PCR	OS	HR+CI
Spindler,2014[36]	Denmark	2010–2012	Median 62	Colorectal Cancer	IV	29/86	Plasma ARMS-qPCR	OS, PFS	HR+CI(m)
Spindler,2015[35]	Denmark	2010–2013	Median 63	Colorectal Cancer	IV	30/140	Plasma AS-PCR	OS, PFS	HR+CI (m)
Tabernero,2015[37]	Spain	2010–2011	Median 61	Colorectal Cancer	IV	349/503	Plasma BEAMing	OS, PFS	HR+P
Takai,2015[38]	Japan	2011–2014	Median 66	Pancreatic cancer	I–IV	83/259	Plasma ddPCR	OS	HR+CI (m)
Tjensvoll,2016[39]	Norway	2012–2014	Median 64	Pancreatic cancer	Advanced	10/14	Plasma ddPCR	OS, PFS	HR+P
Wang,2010[41]	China	2005–2008	>60 (53.8%)	non-small cell lung cancer	IIIB or stage IV	35/273	Plasma RFLP-PCR	PFS	KM
Xu,2014[42]	China	2007–2011	Median 56	Colorectal cancer	IV	76/242	Plasma PNA-PCR	OS	HR+CI (m)
Yamada,1998[43]	Japan	1994–1997	Mean 63.9	Pancreatic cancer	I–IV	11/15	Plasma MASA-PCR	OS	OS value

HR, hazard ratio; CI, confidential interval; KM, Kaplan–Meier curve; AS-PCR, Allele-specific real-time quantitative PCR; m, multivariate analysis; p, p value

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The Chi^2 and I^2 statistic was used to assess and present the heterogeneity between the eligible studies. The funnel plot and Egger's test were performed to assess the potential publication

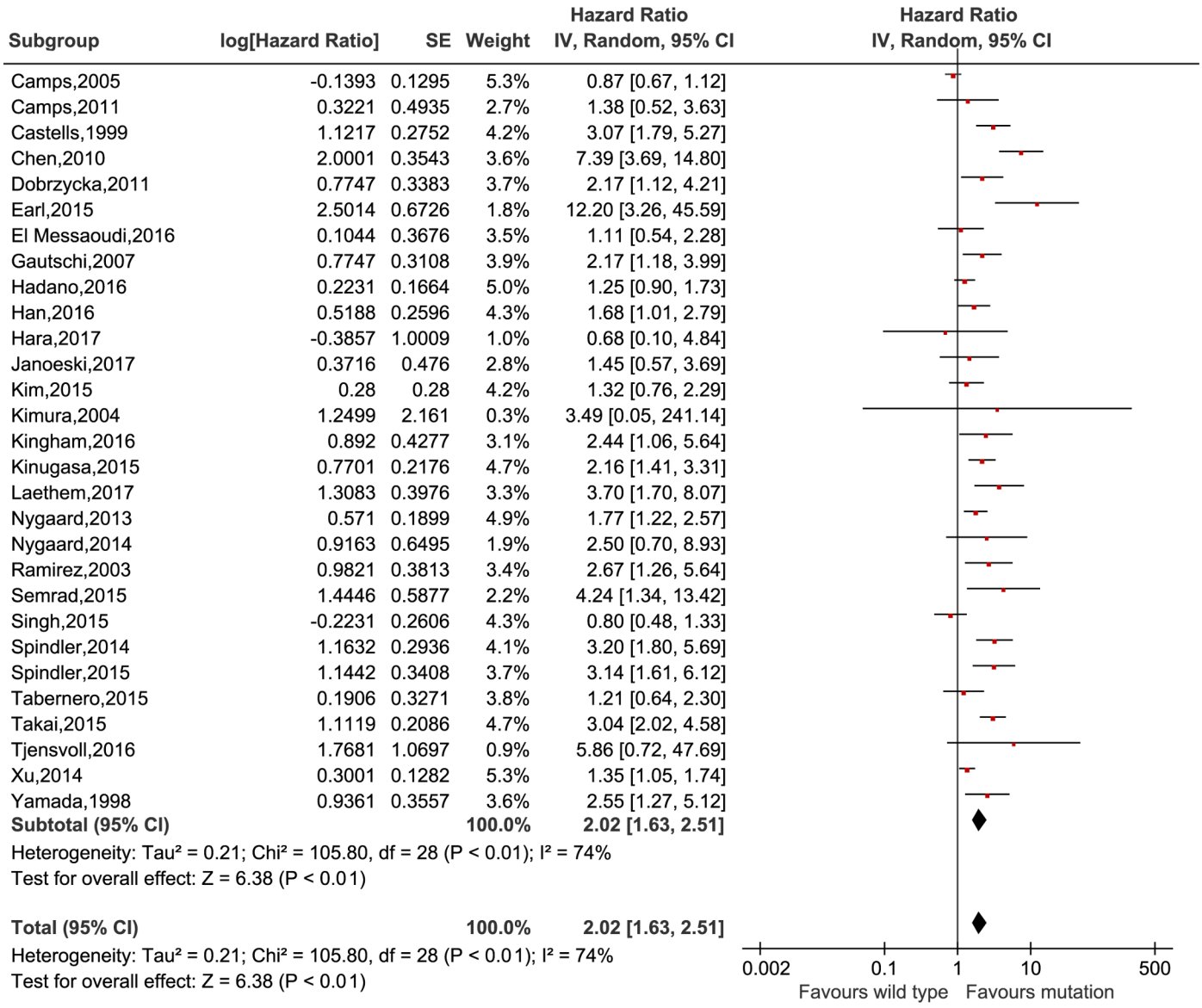

Fig 2. Forest plot for the association between *KRAS* mutation detected by cell-free DNA and overall survival in cancer patients.

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bias [11,12]. We considered that no publication bias existed, if the shape of the funnel plot was symmetrical and the *P* value of the Egger's test was more than 0.05. In addition, a HR<1 indicated *KRAS* mutation was associated with a better outcome while HR>1 indicated *KRAS* mutation was associated with a worse outcome. *P* values were two sided and less than 0.05 were considered statistically different. The meta-analysis was performed through the Review Manager 5.3 software (Cochrane Collaboration).

Results

Literature search and study selection

The literature searches resulted in 2391 studies at first. Then, 2343 records were excluded because of the duplications or no information on *KRAS* mutation detected by cfDNA and

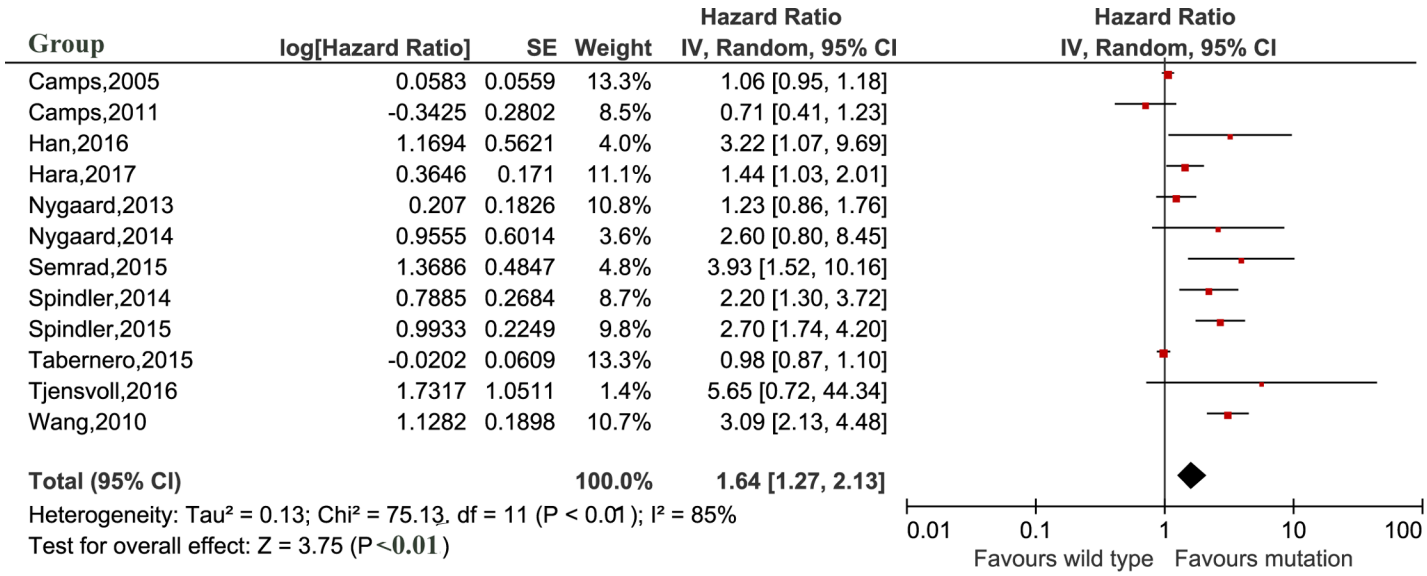

Fig 3. Forest plot for the association between *KRAS* mutation detected by cell-free DNA and progression free survival in cancer patients.

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survival in cancer patients through the screening of the titles and abstracts of all studies. The rest of 46 records were screened by full texts. At last, there were 30 studies included in our meta-analysis [13–43]. The selection process for the eligible studies was shown in Fig 1.The main characteristics of the eligible studies were summarized in Table 1. In addition, quality assessment of the eligible studies was shown in S1 Table.

Among thirty included studies, 12 studies and 29 studies which reported the association of *KRAS* mutation detected by cfDNA with OS and PFS in cancer patients respectively. The cancer types of the eligible studies included pancreatic cancer, colorectal cancer, non-small cell lung cancer and ovarian epithelial cancer. Among 29 studies reporting OS, there were 10 studies focusing on Asian population and 19 studies on non-Asian population. Serum samples and plasma samples were used to detect *KRAS* mutation in 4 studies and 25 studies respectively.

Qualitative assessment

The quality assessment of studies was shown in S2 Table. The scores of the eligible studies ranged from 6 to 8. The average NOS score of the eligible studies was 7.2 which indicating that most of the studies were with a high quality.

Survival prognosis of *KRAS* mutation in cfDNA in cancer patients

The meta-analysis was performed to investigate the prognostic value of *KRAS* mutation detected by cfDNA on survival in cancer patients. Our analysis showed that *KRAS* mutation detected by cfDNA was associated with a poorer survival in cancer patients for OS and PFS (HR = 2.02, 95% CI 1.63–2.51, $P<0.01$ and HR = 1.64, 95% CI 1.27–2.13, $P<0.01$, respectively) (Figs 2 and 3). In subgroup analyses, *KRAS* mutation detected by cfDNA in pancreatic cancer, colorectal cancer, non-small cell lung cancer and ovarian epithelial cancer had HRs of 2.81 (95% CI 1.83–4.30, $P<0.01$), 1.67 (95% CI 1.25–2.42, $P<0.01$), 1.64 (95% CI 1.13–2.39, $P = 0.01$) and 2.17 (95% 1.12–4.21, $p = 0.02$) (shown in Fig 4), respectively. Additionally, the ethnicity didn't influence the prognostic value of *KRAS* mutation detected by cfDNA in cancer patients. *KRAS* mutation detected by cfDNA was a significant prognostic biomarker in cancer

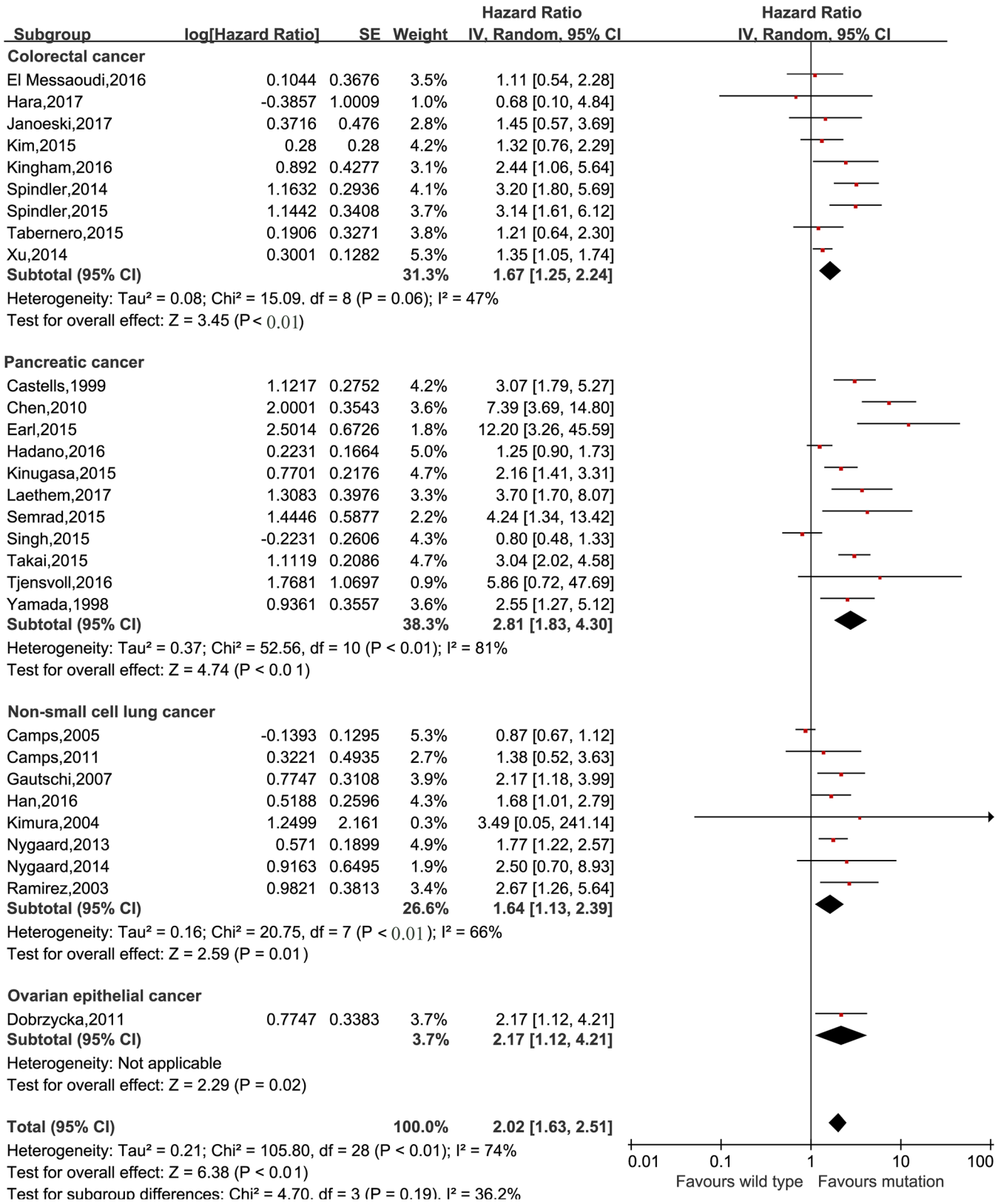

Fig 4. Forest plot for the subgroup analysis of cancer types.

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patients either in Asian (HR 1.81, 95% CI 1.29–2.53, $P<0.01$) or others population (HR 2.21, 95% CI 1.63–2.51, $P<0.01$) (Fig 5).

Sensitivity analysis

Sensitivity analyses were presented in Table 2. Firstly, the sensitivity analysis was performed through removing one single study one by one from the overall pooled analysis. The results

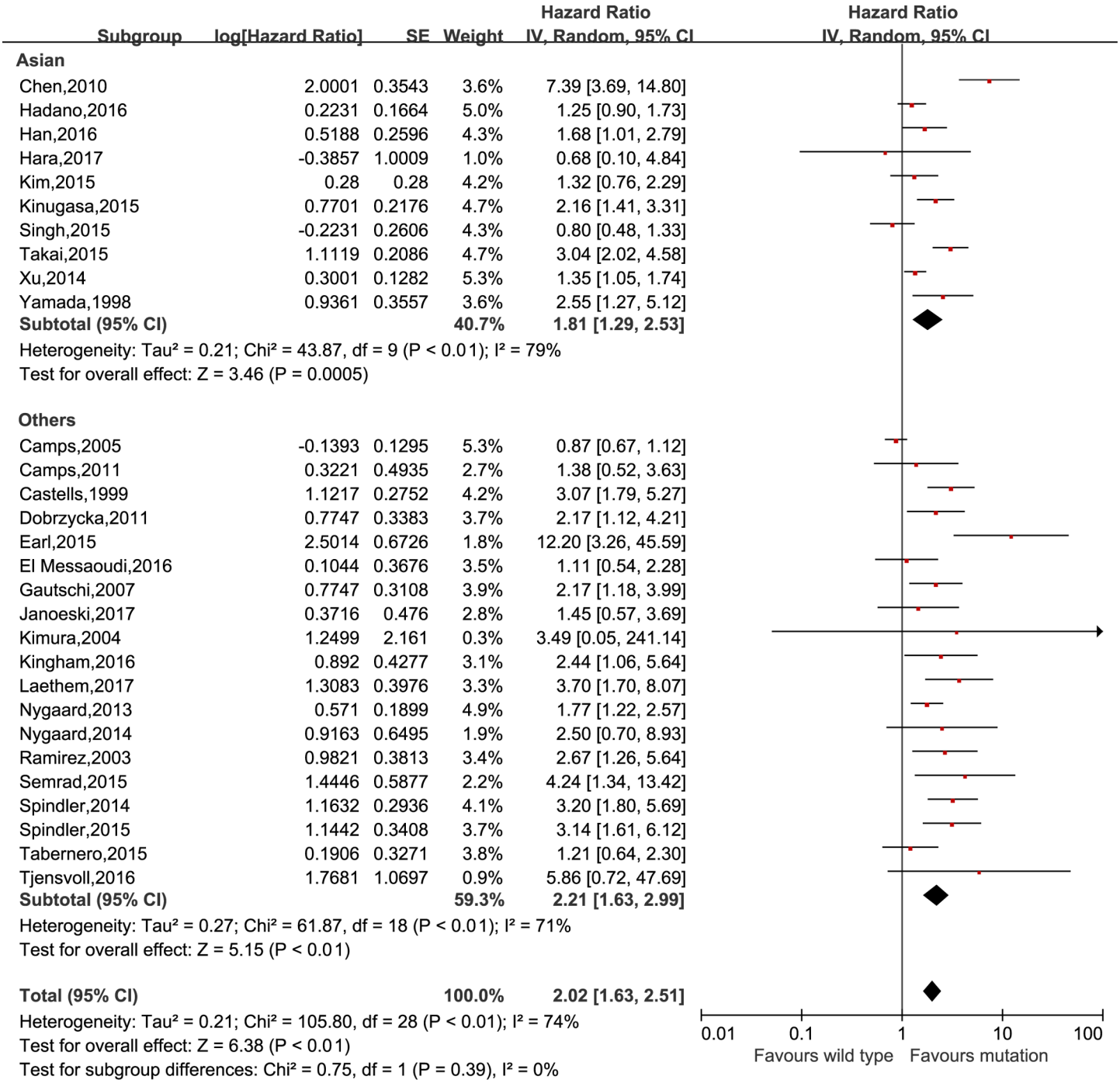

Fig 5. Forest plot for the subgroup analysis of ethnicity.

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Table 2. The sensitivity analysis for the meta-analysis.

Subgroup	HR (95%CI)	*p* value
Type of publication		
Reported	2.24 (1.66–3.01)	0.27
Recalculated (by Parmar's method)	1.76 (1.30–2.40)	
Analysis of hazard ratio		
Multivariate	2.53 (1.66–3.86)	0.74
Univariate	2.29 (1.50–3.50)	
Sample collection		
Serum	1.65 (0.99–2.74)	0.37
Plasma	2.13 (1.69–2.70)	

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showed that there was no significant alteration of the pooled HRs after removing one single study in turn which indicating the results of our meta-analysis was relative stable (data not showed). Additionally, studies with reported HRs of OS tended to have higher HRs compared with studies with recomputed HRs using Parmar's method (2.24 vs 1.76, $p = 0.24$) for all studies. There was no significant difference compared multivariate HRs with univariate HRs (2.53 vs 2.29, $p = 0.74$). For samples collection, no significant difference between serum samples and plasma samples was observed (HRs, 1.65 vs 2.13, $p = 0.37$).

Publication bias

The funnel plot and Egger's test were used to assess the publication bias of the eligible studies. It seemed that the shape of the funnel plot was not symmetrical (shown in Fig 6A and 6B). In addition, the Egger's test suggested that publication bias was existed ($P<0.05$).

Discussion

Circulating cell-free DNA (cfDNA), which exists as small DNA fragments in blood, could be isolated from serum or plasma by less-invasive approach to diagnosis cancers, detect drug resistance and overcome the problem of tumor heterogeneity[44–46]. *KRAS* mutation is one of the most frequent molecular abnormalities found in several types of cancer such as pancreatic cancer, colorectal cancer, non-small cell lung cancer [47]. Spindler et al reported that there was strong relationship between the plasma levels of total cfDNA and the plasma *KRAS* mutated alleles in metastatic colorectal cancer [35]. Several studies found that cfDNA and the presence of mutant *KRAS* in plasma or serum cfDNA was significantly associated with the metastasis in patients with cancer [28,33,38,48]. In recent years, many studies found that *KRAS* mutation was associated with the recurrence [49–51] and with survival prognosis in various types of cancer [32,35][38], but several studies suggested that *KRAS* mutation in cfDNA was not associated with survival outcome of patients with pancreatic, lung or colon cancer [14,25,33]. The prognostic values of *KRAS* mutations in cfDNA as a biomarker remain to confirm. A meta-analysis had clarified that *KRAS* mutations in cfDNA had a more significant impact on overall survival of patients with pancreatic cancer compared with *KRAS* mutation detected in tumor tissue [7]. Our results indicated that *KRAS* detected in cfDNA was a prognostic marker for OS and PFS of pancreatic cancer, colorectal cancer and NSCLC. But another meta-analysis could not support *KRAS* mutation as survival marker in NSCLC [52]. One reason might be that more studies was included in our study (8 publications) compared with

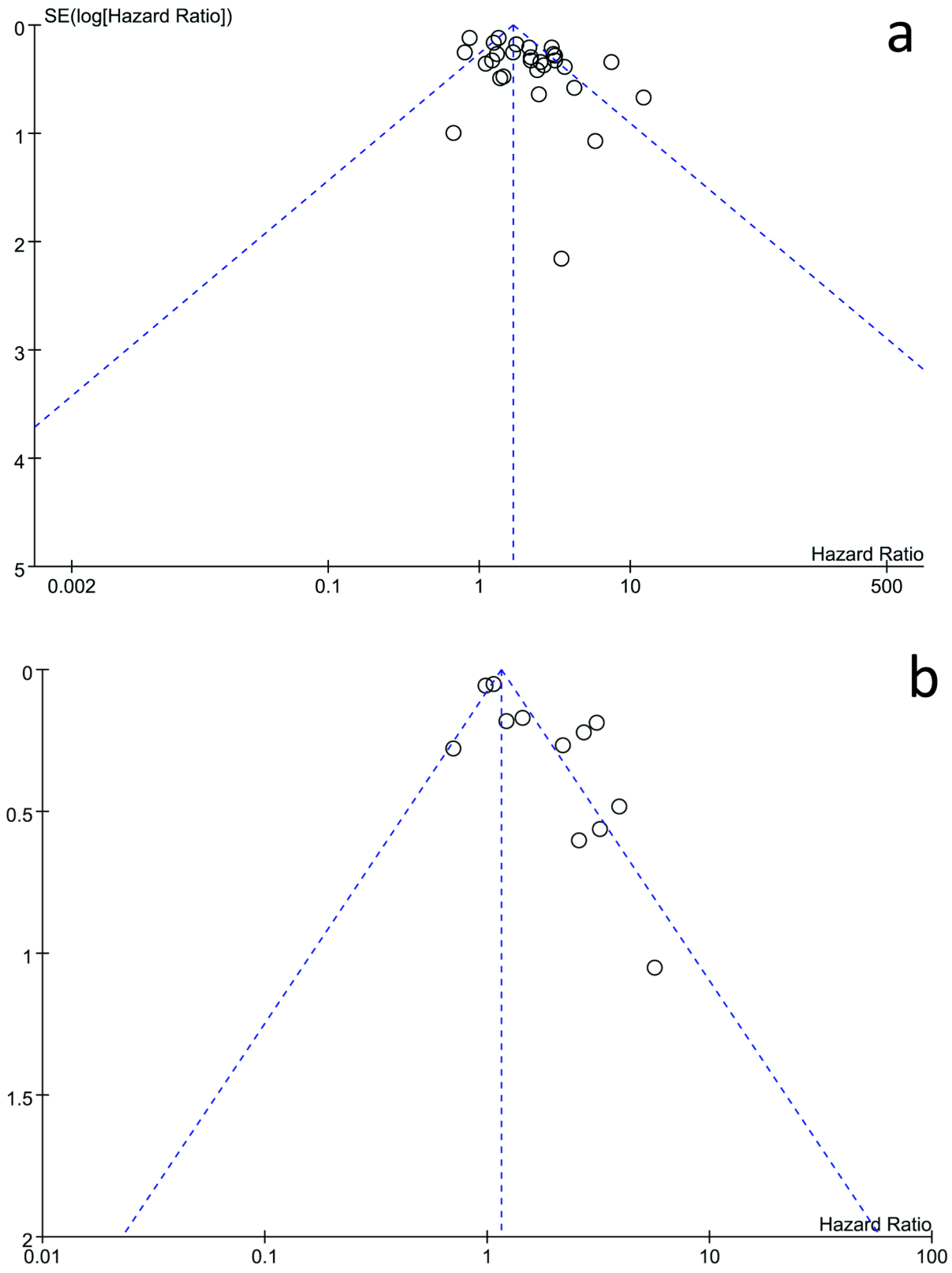

Fig 6. Funnel plot of the association between *KRAS* mutation detected by cell-free DNA and survival in cancer patients for publication bias. a, overall survival; b, progression-free survival.

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previous studies (4 publications). Maybe, large-scaled clinicltrials are necessary to confirm our results.

Currently, gene type analysis of tumor tissue is becoming a common practice in the clinical oncology, but there are some disadvantages such as tumor heterogeneity and samples being difficult to obtain. On the contrary, cfDNA is a non-invasive procedure and its samples would be easy to be collected [44,53]. Thus, considering the tumor heterogeneity of tumor tissue and the advantages of cfDNA, the cfDNA was selected according to the sample source in the present study. Furthermore, KRAS mutations in cfDNA is high correlated with mutations detected in the matched tumors [33,41]. Our meta-analysis showed that *KRAS* mutation detected in cfDNA was a significant prognostic biomarker of cancer patients, especially in pancreatic cancer.

Studies have suggested that the level of cfDNA is increased in both cancer patient and in various non-malignant pathological conditions compared to healthy individuals [48]. Even minority of healthy subjects demonstrated mutant KRAS in cfDNA [54], so the *KRAS* mutation used for disease diagnosis should be cautious. Generally, the prevalence of *KRAS* mutations in tumor tissues was high than that of cfDNA in pancreatic cancer and colorectal cancer [7,42]. Previous studies have suggested that the detection of tumor derived cfDNA is more trend in the setting of large tumor burden and tumor high turnover which are both independent predictors of a poor prognosis [38,48,55]. Result from Spindler et al [35] indicated there was strong relationship between the plasma levels of total cfDNA and the plasma *KRAS* mutated alleles in metastatic colorectal cancer. Several studies found that cfDNA and the presence of mutant *KRAS* in plasma or serum cfDNA was significantly associated with the metastasis in patients with cancer [28,33,38,48]. However, others reported there were no association observed between *KRAS* mutation and age, sex, tumor stage, histopathologic type and so on in advanced cancers [25,41]. In order to clarify this issue, we conducted a sensitivity analysis to compare univariate and multivariate analysis about prognostic value of *KRAS* in cfDNA in sensitivity analysis, and results proved *KRAS* mutation in cfDNA was an independent marker of poor prognosis of overall survival (HR = 2.53, 95%CI: 1.66–3.86, $p<0.01$).

There were some limitations in the present meta-analysis. At first, most of the studies included in our meta-analysis were retrospective, which may bring about some potential bias. Second, some studies of other databases might be lost and some relevant studies were excluded in our meta-analysis because of the publication limitations or incompletely raw data. Third, several studies didn't report HR and its related 95% CI and needed to be calculated according to Parmar's method [9] which might cause imprecise values and potential bias. In addition, there was heterogeneity existed in the eligible studies which might lead to an inaccurate conclusion.

In conclusion, our meta-analysis demonstrated that *KRAS* mutation detected in cfDNA was a prognostic biomarker in cancer patients. Its prognostic value was different in different types of cancer. However, because of the limitations existed in our meta-analysis, more studies are still needed to support our conclusions.

Supporting information

S1 Table. The search strategy of the prognostic value of *KRAS* mutation detected by cell-free DNA in cancer patients.
(PDF)

S2 Table. Application of the quality assessment tool NOS to the studies included in the meta-analysis.
(PDF)

S3 Table. PRISMA 2009 checklist.
(DOC)

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Author Contributions

Conceptualization: Tianshu Liu.

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Formal analysis: Rongyuan Zhuang.

Funding acquisition: Rongyuan Zhuang.

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Project administration: Rongyuan Zhuang, Tianshu Liu.

Resources: Rongyuan Zhuang, Song Li, Xi Guo.

Software: Rongyuan Zhuang, Hong Sun.

Supervision: Tianshu Liu.

Validation: Song Li, Qian Li, Xi Guo, Hong Sun.

Writing – original draft: Rongyuan Zhuang, Song Li.

Writing – review & editing: Rongyuan Zhuang, Song Li, Qian Li, Xi Guo, Feng Shen, Hong Sun, Tianshu Liu.

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