Cancer and Metastasis Reviews (2018) 37:173–187
https://doi.org/10.1007/s10555-017-9726-5

NON-THEMATIC REVIEW

Mutations of key driver genes in colorectal cancer progression and metastasis

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Published online: 10 January 2018

Abstract

The association between mutations of key driver genes and colorectal cancer (CRC) metastasis has been investigated by many studies. However, the results of these studies have been contradictory. Here, we perform a comprehensive analysis to screen key driver genes from the TCGA database and validate the roles of these mutations in CRC metastasis. Using bioinformatics analysis, we identified six key driver genes, namely APC, KRAS, BRAF, PIK3CA, SMAD4 and p53. Through a systematic search, 120 articles published by November 30, 2017, were included, which all showed roles for these gene mutations in CRC metastasis. A meta-analysis showed that KRAS mutations (combined OR 1.18, 95% CI 1.05–1.33) and p53 mutations (combined OR 1.49, 95% CI 1.23–1.80) were associated with CRC metastasis, including lymphatic and distant metastases. Moreover, CRC patients with a KRAS mutation (combined OR 1.29, 95% CI 1.13–1.47), p53 mutation (combined OR 1.35, 95% CI 1.06–1.72) or SMAD4 mutation (combined OR 2.04, 95% CI 1.41–2.95) were at a higher risk of distant metastasis. Subgroup analysis stratified by ethnic populations indicated that the BRAF mutation was related to CRC metastasis (combined OR 1.42, 95% CI 1.18–1.71) and distant metastasis (combined OR 1.51, 95% CI 1.20–1.91) in an Asian population. No significant association was found between mutations of APC or PIK3CA and CRC metastasis. In conclusion, mutations of KRAS, p53, SMAD4 and BRAF play significant roles in CRC metastasis and may be both potential biomarkers of CRC metastasis as well as therapeutic targets.

Keywords Key driver gene · Mutation · Colorectal cancer · Metastasis · Comprehensive analysis

1 Introduction

Colorectal cancer (CRC) remains one of the leading types of malignancy and causes of deaths worldwide [1]. Despite early detection and therapeutic advances, metastasis, including lymphatic and distant metastases, remains the major cause of death in newly diagnosed CRC patients, and the overall survival of advanced CRC remains unsatisfactory.

Multiple alternative genetic pathways exist in CRC development. APC, as a tumour suppressor gene, plays a principal role in CRC development. Contributing to tumour progression, deletion of the APC protein triggers accumulation of beta-catenin and transcriptional activation of TCF-responsive genes [2]. Additionally, KRAS and BRAF encode a protein that belongs to the Ras-Raf-MEK-ERK signalling pathway. Activation of this pathway is considered to be a molecular switch that leads to cellular growth and proliferation [3]. Occurring late in the pathogenesis of CRC, loss of

Dongdong Huang, Wenjie Sun and Yuwei Zhou contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10555-017-9726-5) contains supplementary material, which is available to authorized users.

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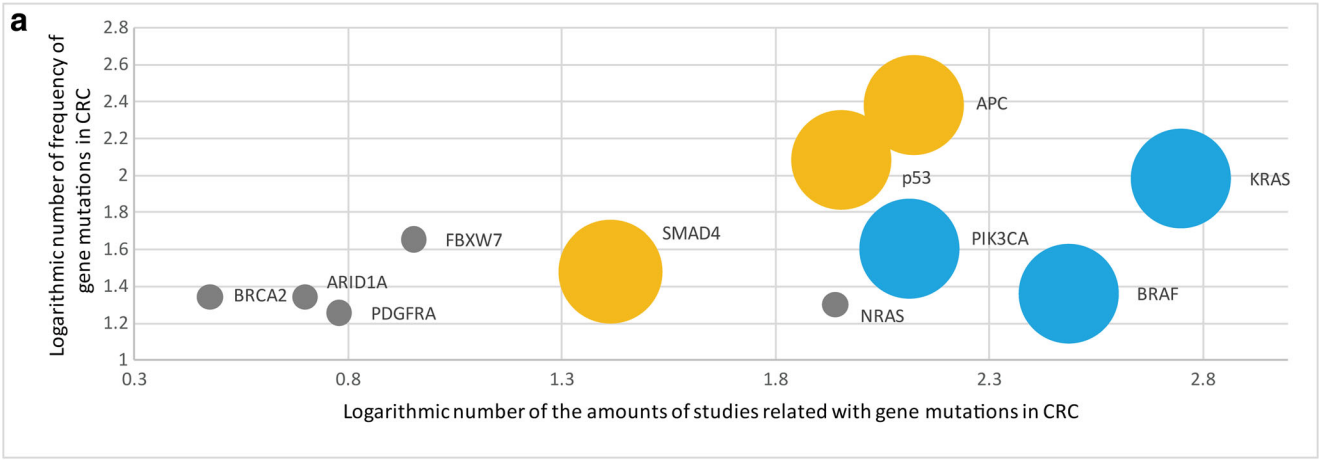


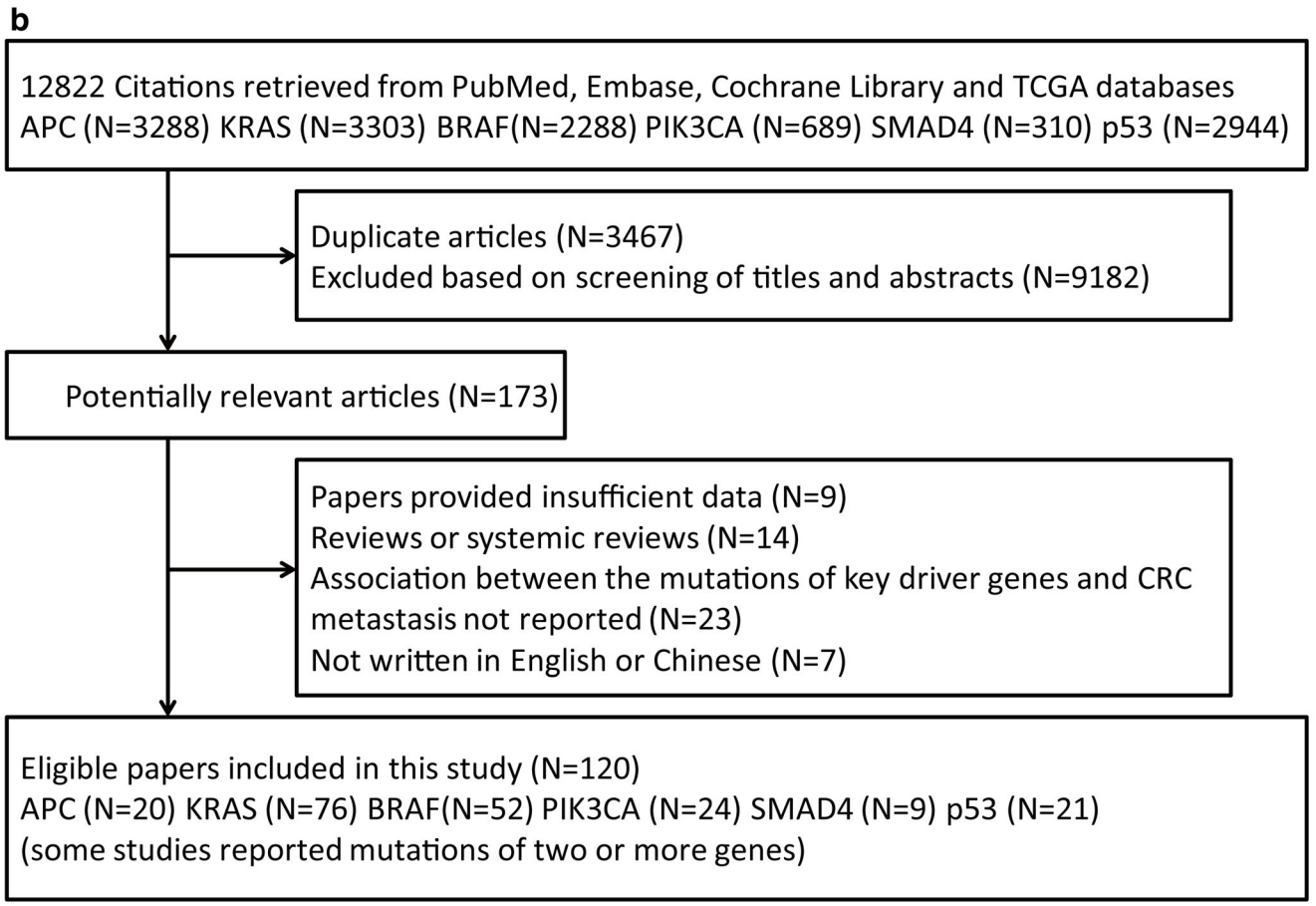
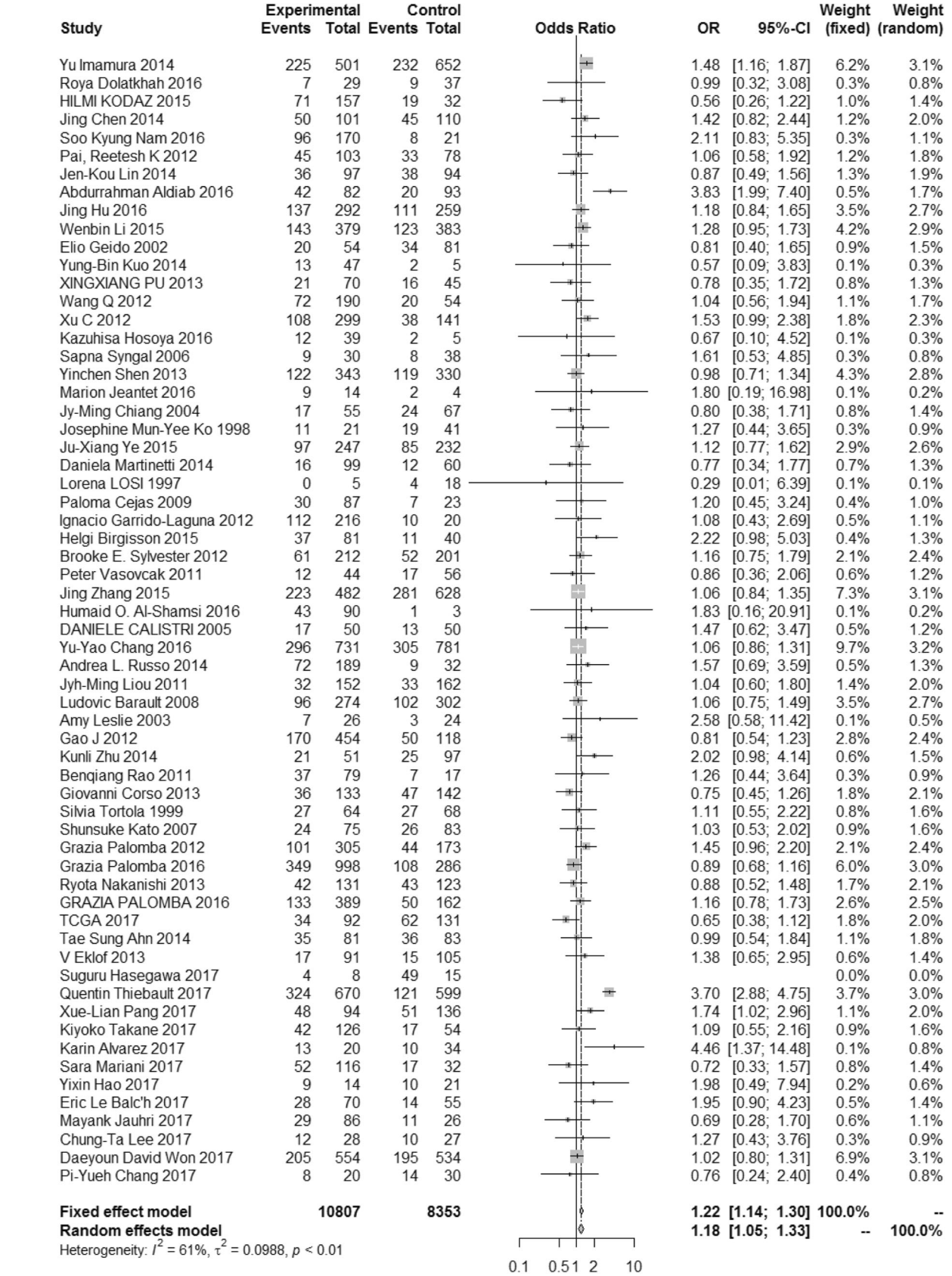


Fig. 1 The process of gene screening, literature search and study selection. **a** Bubble diagram of gene screening. Blue represents an oncogene, orange represents a tumour suppressor gene and grey represents an unselected gene. **b** The flow chart for the study selection. This figure provides detailed information for study inclusion and exclusion

p53 function is proposed to be one of the major events in the development of CRC [4].

Additionally, as CRC evolves from benign to malignant lesions, some key driver genes acquire a series of mutations over time [5]. Among these key driver genes is APC, and its mutation regulates growth advantages in epithelial cells and results in the formation of a small adenoma. Subsequently, KRAS and BRAF mutations provide a second round of expansion for cells, involving transformation to a large adenoma. Eventually, mutations of PIK3CA, SMAD4 and p53 generate a malignant tumour that has potential for invasion

Fig. 2 Forest plots of the association between KRAS mutations and CRC metastasis, including lymphatic and distant metastases. There was a significant association between KRAS mutations and CRC metastasis, including lymphatic and distant metastases ▶



Experimental : mutations of KRAS in CRC with metastasis*;
Control: mutations of KRAS in CRC wtihout metastasis*;
*including lymphatic and distant metastases.

and metastasis. In this genetic model of CRC, mutations of APC, KRAS and BRAF are significant early events in the transition from normal colonic epithelium to adenoma. In addition, mutations of PIK3CA, SMAD4 and p53 are related to the late stage, the transition from adenoma to carcinoma. However, whether early or late genetic events contribute to CRC metastasis remains unknown. It also remains unclear which mutations of these key driver genes are involved in the metastasis of CRC.

Thus far, many studies have estimated the association between mutations of the above-mentioned key driver genes and CRC metastasis, but the results of these studies have been contradictory. For example, the study performed by Josephine Mun-Yee Ko et al. showed that no essential correlations were found between KRAS mutations and Dukes staging, metastasis or recurrence [6]. By contrast, Bazan et al. reported that KRAS mutations were significantly associated with advanced Dukes staging and lymphatic metastasis [7]. Consequently, in the present study, we screened several promising key driver genes by analysing the CRC mutation rank combined with a literature search and investigated the association between mutations of these key driver genes and CRC metastasis. This study may provide further insight into more effective preventive strategies and adjuvant therapies against CRC.

2 Methods

2.1 Gene screening, literature search and study selection

We defined key driver genes in CRC progression and metastasis using the mutation frequency and number of publications. To determine the most well-studied gene mutations in CRC, literature search was applied. Specifically, the search query "('colon cancer' or 'colon carcinoma' or 'colon tumour' or 'colon neoplasm' or 'rectal cancer' or 'rectal carcinoma' or 'rectal tumour' or 'rectal neoplasm' or 'colorectal cancer' or 'colorectal carcinoma' or 'colorectal tumour' or 'colorectal neoplasm' or 'CRC') AND ('mutation' or 'mutations') AND ('metastasis')" was used to retrieve available studies from PubMed that were published by November 30, 2017. The R package, pubmed.mineR, was used to fetch the genes (HGNC proved Symbol) from the abstracts of the articles and tally the number of articles in which a gene was discussed. Furthermore, the most frequently mutated genes in the TCGA Colorectal Adenocarcinoma cohort were retrieved from cBioPortal (TCGA Colorectal Adenocarcinoma datasets, http://www.cbioportal.org/study? id=coadread_tcga# mutations).

To validate the role of mutations of the screened genes in CRC metastasis, we performed systematic literature searches of the PubMed, Embase, Cochrane Library and TCGA databases of articles published by November 30, 2017. The corresponding search term combinations were ('colon cancer' or 'colon carcinoma' or 'colon tumour' or 'colon neoplasm' or 'rectal cancer' or 'rectal carcinoma' or 'rectal tumour' or 'rectal neoplasm' or 'colorectal cancer' or 'colorectal carcinoma' or 'colorectal tumour' or 'colorectal neoplasm' or 'CRC') AND ('mutation' or 'mutations') AND ('APC' or 'p53' or 'SMAD4' or 'KRAS' or 'BRAF' or 'PIK3CA'). After excluding potential duplicates, we subsequently manually reviewed the titles, abstracts and full papers for potential eligible studies.

The studies were selected if they met the predefined selection criteria: (1) the outcome of interest was metastasis of CRC, (2) study of interest was mutations of key driver genes, (3) OR estimates with 95% CIs were available and (4) studies published in English or Chinese were included. Of note, only the most recent or detailed information was extracted from overlapping publications.

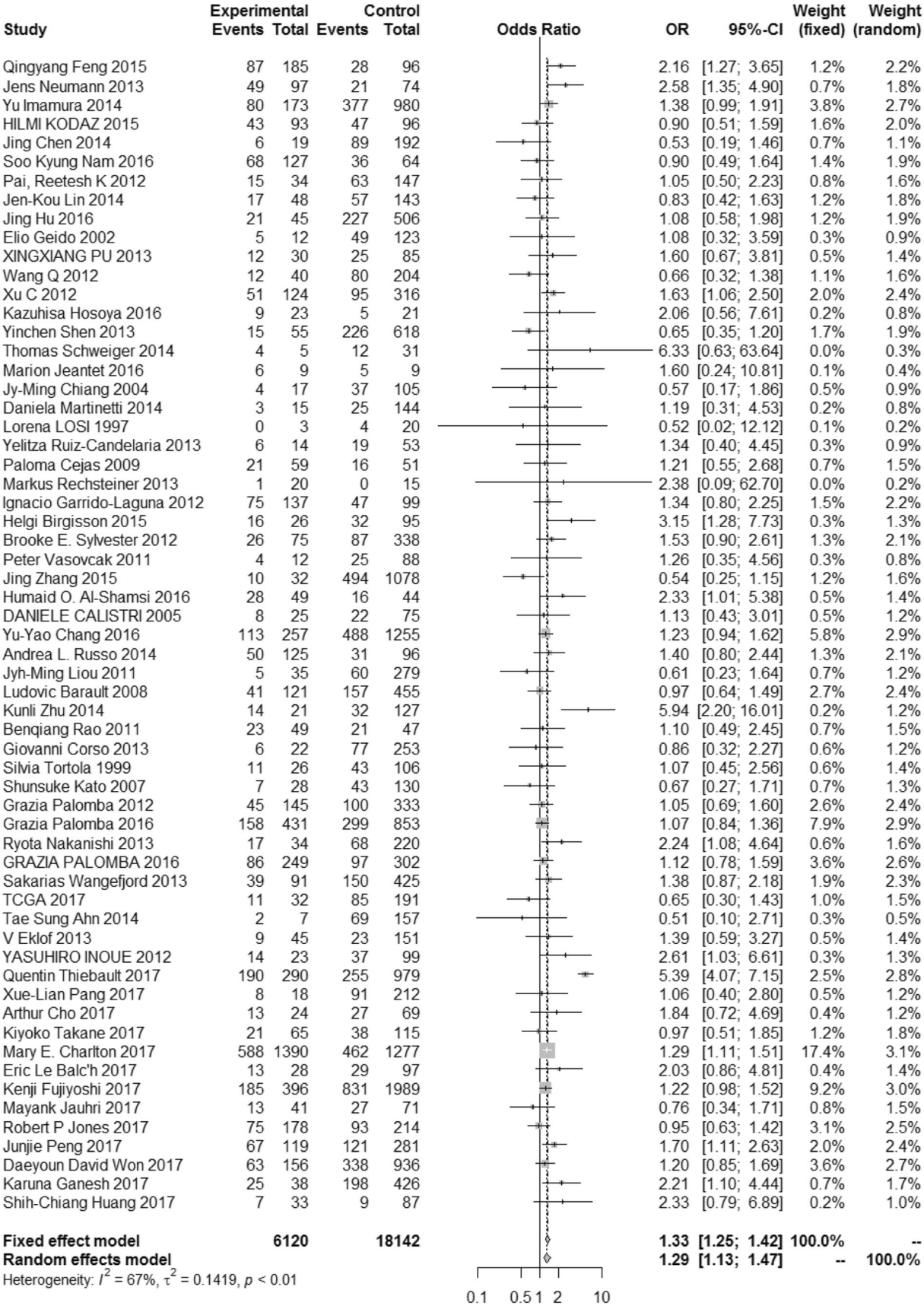
Fig. 3 Forest plots of the association between KRAS mutations and CRC distant metastasis. KRAS mutation was significantly related to CRC distant metastasis ▶

2.2 Data extraction

Two authors independently extracted all of the datasets from the selected studies, and any disagreements were resolved by consensus or by consulting with a third reviewer. The following information was collected from each study: first author, publication, year of publication and title, study design and participants' characteristics, including number, age, gender, race and metastasis status as well as tumour stage. The principal author or corresponding author was contacted to acquire additional or unavailable information. The Newcastle-Ottawa Scale was used to estimate the methodological and reporting quality of the included studies ranked in order of scores (8 to 9 points represented high quality; 5 to 7 points represented medium quality; fewer than 5 points represented low quality) [8].

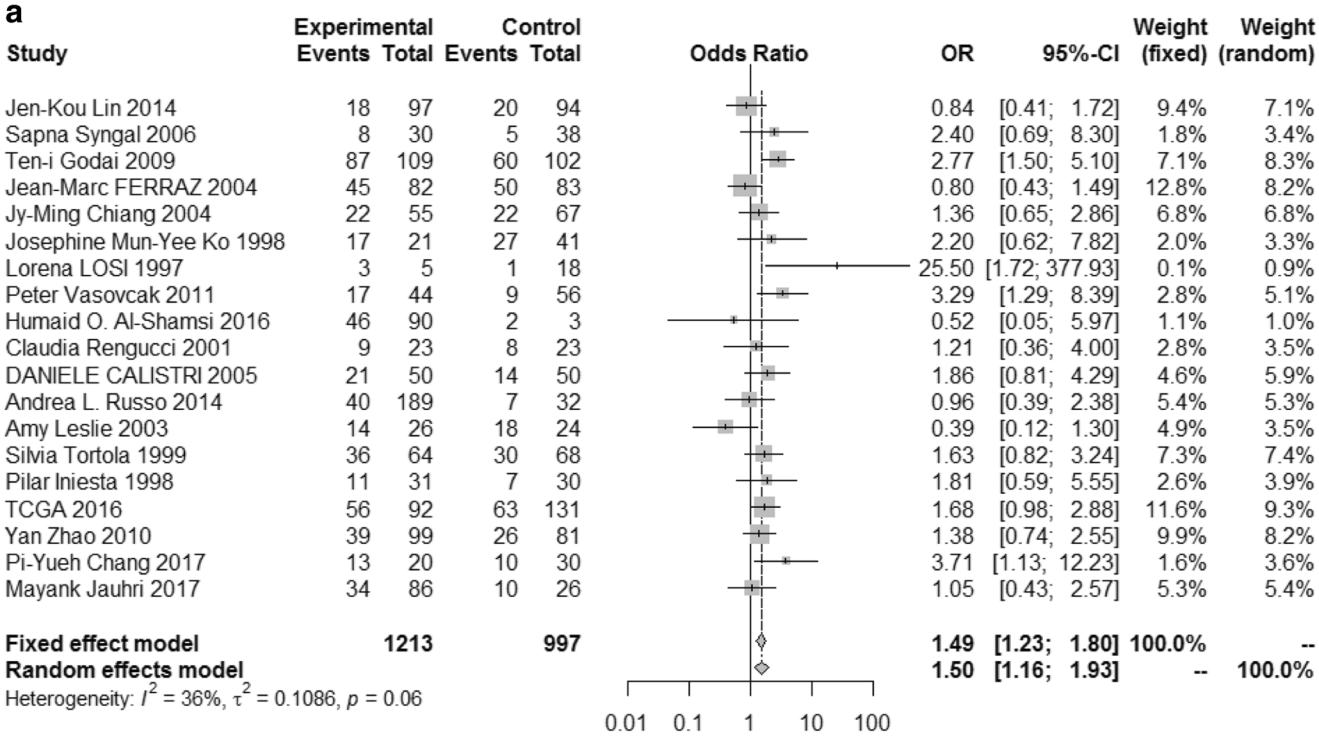
2.3 Statistical analysis

All analyses were conducted using the R software (version 3.4.1). The I^2 test was applied to assess the heterogeneity of each study [9]. Displayed in the forest plot, pooled ORs and 95% CIs were calculated by applying a random-effects model for the study with substantial heterogeneity ($I^2 > 50\%$); otherwise, a fixed-effects model was applied ($I^2 < 50\%$). We conducted primary meta-analyses to investigate the association between mutations of key driver genes and CRC metastasis and further performed subgroup analyses according to ethnic populations. Funnel plots as well as Begg's and Egger's tests were performed to identify the risk of publication bias.



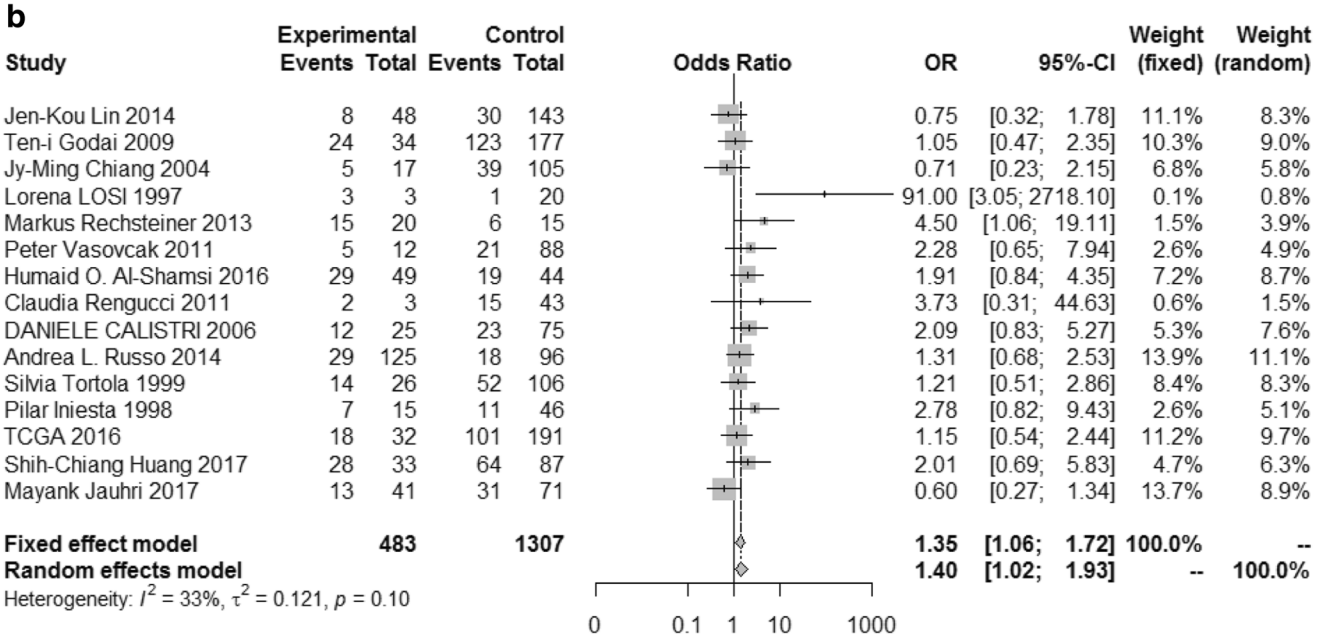
Experimental : mutations of KRAS in CRC with distant metastasis;
Control: mutations of KRAS in CRC wtihout distant metastasis.

a



Experimental : mutations of p53 in CRC with metastasis*;
Control: mutations of p53 in CRC wtihout metastasis*;
*including lymphatic and distant metastases.

b



Experimental : mutations of p53 in CRC with distant metastasis;
Control: mutations of p53 in CRC wtihout distant metastasis.

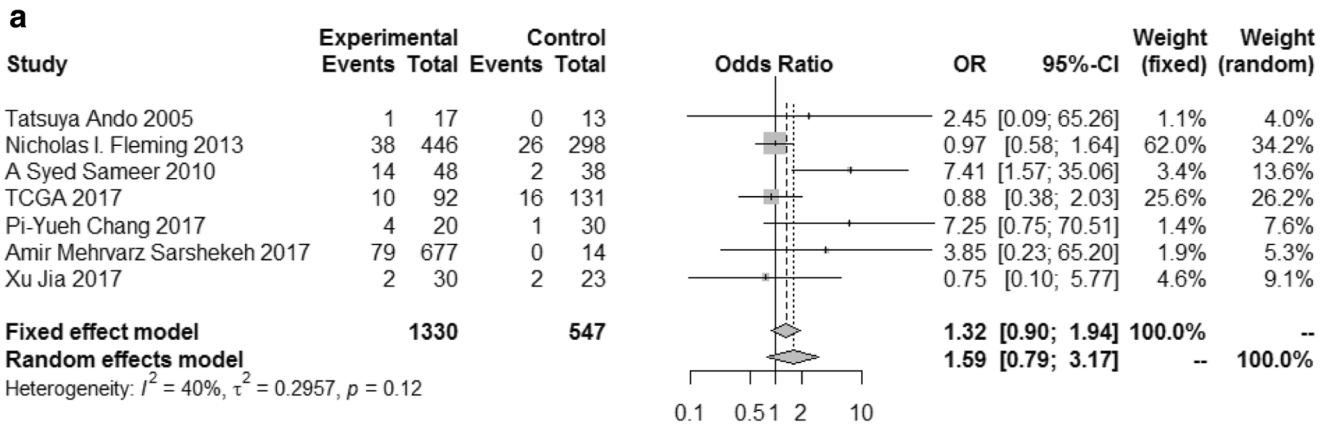
Fig. 4 Forest plots of p53. **a** Forest plots of the association between p53 mutations and CRC metastasis, including lymphatic and distant metastases. p53 mutations were associated with metastasis in CRC patients. **b** Forest plots of the association between p53 mutations and CRC distant metastasis. p53 mutations yielded a higher risk of distant metastasis

3 Results

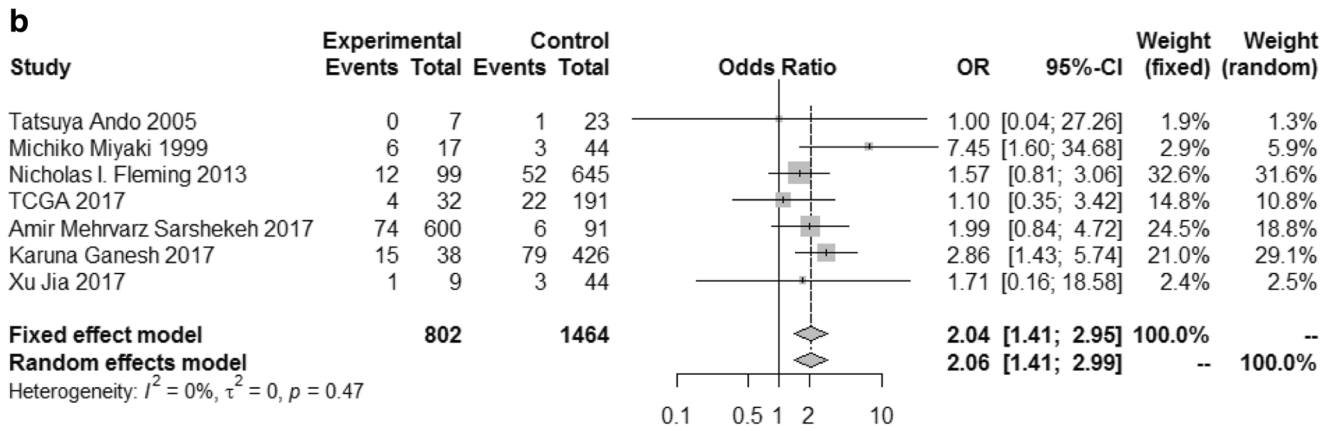
3.1 Screening analysis and description of the included studies

The key driver genes were identified by combining the literature search results with the mutation rank of the TCGA Colorectal Adenocarcinoma cohort as well as biological relevance. The results of the literature search and mutation rank (top 100 genes) are given in Table S1. As shown in Fig. 1a, the intersection of the two lists resulted in a list of 11 genes, including APC, KRAS, BRAF, PIK3CA, SMAD4, p53, NRAS, FBXW7, PDGFRA, ARID1A and BRCA2. Among these genes, APC, KRAS, PIK3CA and p53 were well studied and the most frequently mutated in CRC. Given that BRAF regulates the Ras-Raf-MEK-ERK pathway and is associated with a poor prognosis in many CRC studies, it was selected as one of the key driver genes, although its mutation rate is slightly lower than those of the other genes. SMAD4 was also selected because of its significant role as a critical regulator of the TGF-beta pathway in the metastasis process. Consequently, APC, KRAS, BRAF, PIK3CA, SMAD4 and p53 were selected for further investigation and comprehensive analysis.

Additionally, a total of 12,822 records were retrieved from the PubMed, Embase, Cochrane Library and TCGA databases. After exclusion of duplicated and irrelevant articles by data analysis and title and abstract scanning, 173 citations remained for the full-text review. Finally, a total of 120 relevant articles were included in the analysis, with 20 studies for APC, 76 studies for KRAS, 52 studies for BRAF, 24 studies for PIK3CA, 9 studies for SMAD4 and 21 studies for p53, and some studies reported mutations of two or more genes in the same article [3, 4, 6, 10–125]. The details for selection are



Experimental : mutations of SMAD4 in CRC with metastasis*;
Control: mutations of SMAD4 in CRC wtihout metastasis*;
*including lymphatic and distant metastases.



Experimental : mutations of SMAD4 in CRC with distant metastasis;
Control: mutations of SMAD4 in CRC wtihout distant metastasis.

Fig. 5 Forest plots of SMAD4. **a** Forest plots of the association between SMAD4 mutations and CRC metastasis, including lymphatic and distant metastases. **b** Forest plots of the association between SMAD4 mutations and CRC distant metastasis. SMAD4 mutations correlated with distant metastasis

Table 1 Subgroup analysis for the association between KRAS, BRAF and p53 mutations and CRC metastasis (including lymphatic and distant metastases)

Gene	Population	No. of studies	Combined OR	95% CL	I^2 (%)	*p* value
KRAS	Asian population	33	1.12	1.03 to 1.22	10	0.3
	European and American populations	29	1.21	0.98 to 1.51	74	< 0.01
BRAF	Asian population	24	1.42	1.18 to 1.71	29	0.09
	European and American populations	21	0.89	0.65 to 1.22	59	< 0.01
p53	Asian population	8	1.55	1.16 to 2.06	33	0.17
	European and American populations	11	1.44	1.11 to 1.85	44	0.06

presented in Fig. 1b. All of the included studies were from Asia, Europe and America. Among them, 16 studies enrolled only colon cancer patients and three studies included only rectal cancer patients, whereas the other studies included patients with both colon and rectal malignancy. Most of the studies investigated CRC patients at all TNM stages (I–IV), whereas three studies enrolled only stage I–III patients and another four studies reported II–IV stage patients. The quality of the analysed studies was generally moderate to good.

3.2 Association between mutations of driver genes and CRC metastasis

A total of 39,313 patients were evaluated for the association between these key driver gene mutations and CRC metastasis. There was a significant statistical association between KRAS mutations and CRC metastasis, including lymphatic and distant metastases (combined OR 1.18, 95% CI 1.05–1.33; Fig. 2). Additionally, p53 mutations were also associated with metastasis in CRC patients (combined OR 1.49, 95% CI 1.24–1.80; Fig. 4a).

Moreover, KRAS mutations were significantly related to CRC distant metastasis in 61 studies that included 24,262 patients (combined OR 1.29, 95% CI 1.13–1.47; Fig. 3). Similarly, p53 mutations resulted in a higher risk of distant metastasis (combined OR 1.35, 95% CI 1.06–1.72; Fig. 4b). Furthermore, there was a trend that SMAD4 mutations correlated with distant metastasis (combined OR 2.04, 95% CI 1.41–2.95; Fig. 5b). Of note, no significant association was found between mutations of APC, BRAF, and PIK3CA and CRC metastasis (Figs. S1, S2, S3 and S4).

3.3 Subgroup analyses according to population

Because there was a significant heterogeneity in some gene mutations, subgroup analyses stratified by ethnic populations were further performed for KRAS, BRAF and p53 (Tables 1 and 2). KRAS mutations (combined OR 1.12, 95% CI 1.03–1.22), BRAF mutations (combined OR 1.42, 95% CI 1.18–1.71) and p53 mutations (combined OR 1.55, 95% CI 1.16–2.06) were all significantly related to CRC metastasis, including lymphatic and distant metastases in an Asian population; however, p53 mutations (combined OR 1.44, 95% CI 1.11–1.85) were also associated with CRC metastasis in European and American populations.

In addition, significant associations between gene mutations and CRC distant metastasis were observed in an Asian population for BRAF (combined OR 1.51, 95% CI 1.20–1.91). Furthermore, p53 mutations (combined OR 1.72, 95% CI 1.23–2.40) were correlated with CRC distant metastasis in European and American populations. Interestingly, the CRC distant metastasis risk was enhanced by KRAS mutations both in an Asian population (combined OR 1.23, 95% CI 1.11–1.37) or European and American populations (combined OR 1.35, 95% CI 1.10–1.65). However, CRC metastasis was not influenced by other gene mutations in different ethnic populations according to the present results.

Table 2 Subgroup analysis for the association between KRAS, BRAF and p53 mutations and CRC distant metastasis

Gene	Factor	No. of studies	Combined OR	95% CL	I^2 (%)	*p* value
KRAS	Asian population	28	1.23	1.11 to 1.37	47	< 0.01
	European and American populations	33	1.35	1.10 to 1.65	75	< 0.01
BRAF	Asian population	18	1.51	1.20 to 1.91	32	0.09
	European and American populations	21	0.86	0.70 to 1.06	41	0.03
p53	Asian population	6	1.03	0.72 to 1.47	21	0.27
	European and American populations	9	1.72	1.23 to 2.40	25	0.22

3.4 Publication bias

Funnel plots are displayed in Fig. S4, and the results of Begg's test are shown in Table S2. No publication bias was found for the included studies for the key driver gene mutations.

4 Discussion

It is well established that many mutations of oncogenes and tumour suppressor genes are involved in the process of tissue transformation from normal epithelial cells to carcinomas in

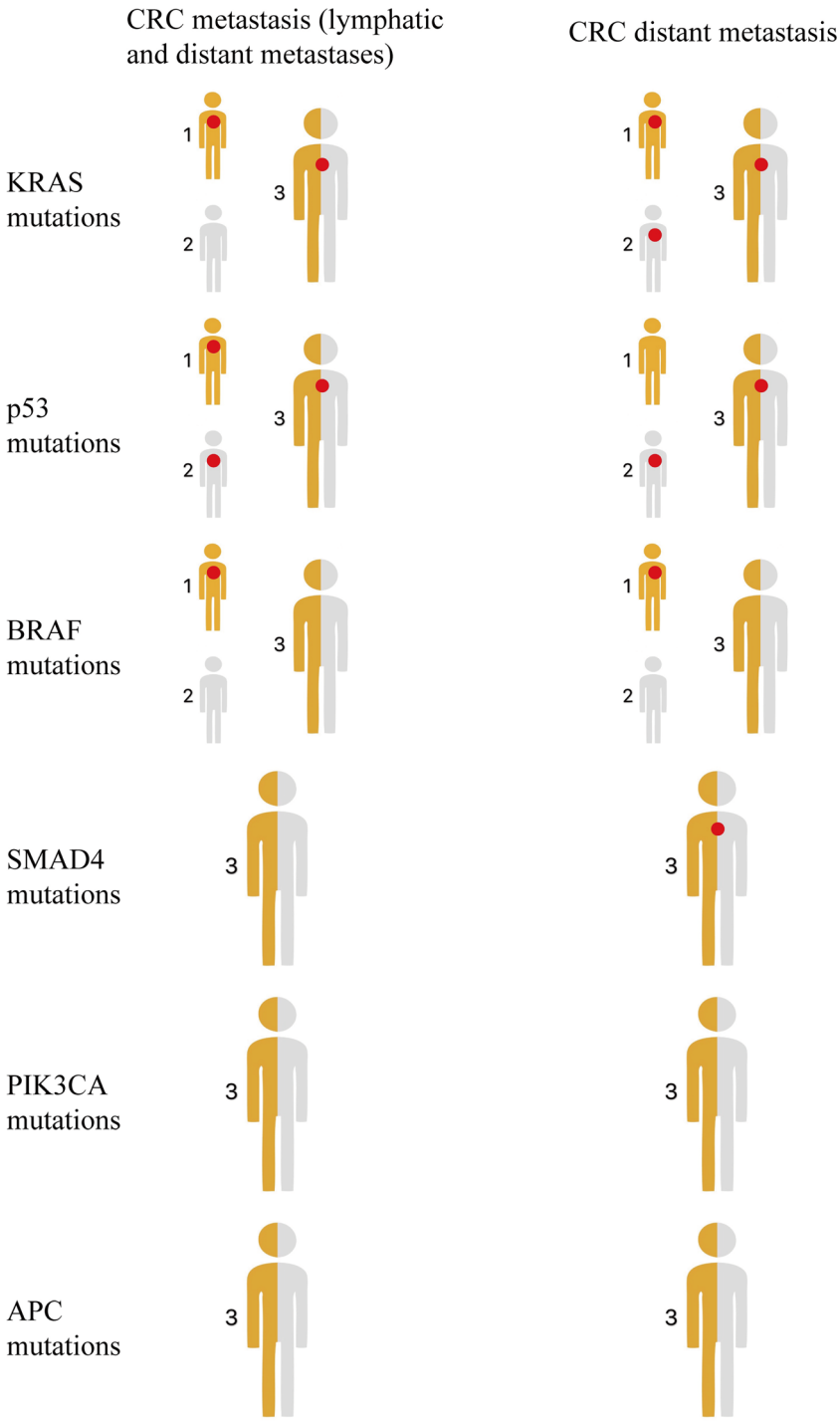


Fig. 6 Summary of our study for the association between key driver gene mutations and CRC metastasis. KRAS mutations and p53 mutations were associated with CRC metastasis, including lymphatic and distant metastases in Asian, European and American populations. Moreover, CRC patients with KRAS mutations, p53 mutations or SMAD4 mutations were at a higher risk of distant metastasis in Asian, European and American populations. No significant associations were found between mutations of APC, BRAF, PIK3CA and CRC metastasis in Asian, European and American populations. However, KRAS, BRAF and p53 mutations were associated with CRC metastasis, including lymphatic and distant metastases, in an Asian population, whereas only p53 mutations promoted CRC metastasis, including lymphatic and distant metastases, in European and American populations. In addition, the CRC distant metastasis risk was facilitated by KRAS and BRAF mutations in an Asian population and by KRAS and p53 mutations in European and American populations

CRC [29]. Indeed, the accumulation of molecular alterations of these key driver genes plays a crucial role in the tumourigenesis and progression of CRC. However, it remains controversial whether mutations of these key driver genes can influence CRC metastasis.

In the present study, we performed a comprehensive analysis to screen several promising key driver genes, APC, KRAS, BRAF, PIK3CA, SMAD4 and p53, and to evaluate the association between mutations of these key driver genes and CRC metastasis (Fig. 6). The results indicated that the frequencies of KRAS as well as p53 mutations were significantly enhanced in patients with CRC metastasis. In addition, SMAD4 mutations were associated with CRC distant metastasis. Of note, the results of the subgroup analysis stratified by ethnic populations indicated that KRAS, BRAF and p53 mutations were associated with CRC metastasis, including lymphatic and distant metastases in an Asian population, whereas only p53 mutations promoted CRC metastasis, including lymphatic and distant metastases, in European and American populations. In addition, the CRC distant metastasis risk was facilitated by KRAS and BRAF mutations in an Asian population and by KRAS and p53 mutations in European and American populations.

KRAS mutations occur early during the progression from colorectal adenoma to malignant carcinoma, accumulating a sequential tumour growth advantage [97]. Large cohort studies, such as the collaborative RASCAL study, indicated that KRAS mutations increased the risk of recurrence and death [64]. Of note, the Ras-Raf-MEK-ERK and PI3K/AKT pathways are strongly interconnected, forming a signalling network. Activation of the different partners in this network by mutation deregulates survival, mobility and proliferation of cells [91]. In the present study, we found that KRAS mutations had a significant impact on both lymphatic metastasis and distant metastasis.

It is well known that p53 mutations are relatively late events of CRC progression. p53, as a tumour suppressor gene, causes apoptosis, releasing oncogenic stress and terminating DNA damage [86]. By repressing epithelial marker expression and increasing mesenchymal marker expression, the absence of p53 led to EMT transition and provided potential for the invasion and metastasis of CRC cells [126]. In the present study, p53 mutations were also observed to have significant associations with both lymphatic metastasis and distant metastasis in CRC.

TGF-beta signalling regulates tumourigenesis and tumour progression and is also involved in metastasis, particularly as an important EMT inducer [123]. As the pivotal factor of the TGF-beta pathway, in the present study, SMAD4 mutations tend to promote distant metastasis rather than lymphatic metastasis of CRC, because of different molecular mechanism between lymphatic and distant metastases or lymphatic and distant metastases originated from independent subclones in colorectal cancer [127]. In addition, as a downstream molecule and mutual exclusion mutation of KRAS, BRAF mutations were only shown to promote CRC metastasis including lymphatic or distant metastases in an Asian population, but not in European and American populations, in the current study. The reason for this difference is that the mutation rate of BRAF in the Asian population included in our study was 5.7%, which was consistent with a previous report that found a mutation rate of 5.0–10.0% for BRAF mutations in CRC patients [128]. Nevertheless, the BRAF mutation rate in the European and American populations included in the current study reached 14.8%, which was higher than a previous study [20].

Our study comprehensively analysed the association between mutations of several key driver genes and CRC metastasis, and the results suggested that some CRC metastasis-related driver genes, such as KRAS and p53, may be more important for clinical practice and diagnosis of CRC than other metastasis-related driver genes. However, the current analysis has several limitations. First, subgroup analysis for certain genes, such as SMAD4, could not be performed due to a lack of sufficient studies. Second, the heterogeneity of the studies for some gene analyses could not be fully explained. In the current study, ethnic populations were focused on to explain the heterogeneity. However, the effects of genetic factors on metastasis risk may be confounded by gender, age and mutational site. Therefore, further individual participant data analysis is warranted to avoid these confounding factors.

In conclusion, the results from the current study demonstrated that mutations of key driver genes, particularly KRAS and p53, promote CRC metastasis. This study highlights that KRAS, p53, SMAD4 and BRAF are potential markers to estimate prognosis, including the status of metastasis for patients with CRC. Future studies are needed to confirm the mechanisms and effects and to provide deeper insight into the role of mutations of these key driver genes in CRC metastasis.

Acknowledgments The authors thank Prof. Pengyuan Liu (Zhejiang University), an expert bio-statistician, for reviewing the analysis portion of the manuscript.

Author contributions H.Z. and Y.W. contributed to the conception and design of the study. D.H., W.S. and Y.Z. contributed to the conception, design of the study and editing of the manuscript. D.H., Y.Z., P.L., F.C., H.C. and D.X. contributed to the statistical analysis. E.X. and M.L. contributed to the analysis and interpretation of data. All of the authors commented on drafts of the paper and approved the final draft of the manuscript.

Funding information This work is supported by the National Natural Science Foundation of China (81672730 to H.Z. and 81572716 to M.L.), the Fundamental Research Funds for the Central Universities (2017FZA7005 to H.Z.), the Natural Science Foundation of Zhejiang Province (LY17H160025 to E.X.) and the Department of Science and Technology of Zhejiang Province (2016C33150 to E.X.)

Compliance with ethical standards

Competing interests The authors declare that they have no conflicts of interest.

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