Research Paper

Clinicopathological Significance of \(BRAF^{V600E}\) Mutation in Colorectal Cancer: An Updated Meta-Analysis

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Abstract

**Background and Aims:** Numerous studies have identified \(BRAF^{V600E}\) mutation as a predictive factor of anti-EGFR antibodies in colorectal cancer (CRC). However, the association between \(BRAF^{V600E}\) mutation and clinicopathological features remains unclear. Therefore, we aimed to conduct an updated and comprehensive meta-analysis to evaluate the above issues.

**Methods:** We performed a systematic literature search from PubMed, Web of Science, Embase, and PMC database examining the association between \(BRAF^{V600E}\) mutation and clinicopathological features in CRC patients. Odds ratio with 95% confidence interval were used to estimate the effects of \(BRAF^{V600E}\) mutation on each clinicopathological parameter with fixed-effect model or random-effect model.

**Results:** Sixty-one studies published, including 32407 CRC patients from multiple countries, were included in the meta-analysis. The overall \(BRAF^{V600E}\) mutation rate was 11.38%, and \(BRAF^{V600E}\) mutation was positively related to high disease stage (OR=0.81; 95% CI=0.72–0.92; \(P<0.001\)), high T stage (OR=0.51; 95% CI=0.40–0.65; \(P<0.0001\)), proximal colon (OR=4.76; 95% CI=3.81–5.96; \(P<0.00001\)) or right colon (OR=5.15; 95% CI=4.35–6.10; \(P<0.00001\)) tumor location, poor tumor differentiation (OR=0.27; 95% CI=0.21–0.34; \(P<0.00001\)), mucinous histology (OR=2.97; 95% CI=2.37–3.72; \(P<0.00001\)), K-ras-wild type (OR=0.04; 95% CI=0.02–0.07; \(P<0.00001\)), TP53-wild type (OR=0.50; 95% CI=0.31–0.78; \(P=0.003\)), deficient DNA mismatch repair (OR=2.93; 95% CI=1.78–4.82; \(P<0.00001\)), high microsatellite instability (OR=11.15; 95% CI=8.51–14.61; \(P<0.00001\)) and high CpG island methylator phenotype (OR=0.04; 95% CI=0.03–0.08; \(P<0.00001\)).

**Conclusions:** Our updated meta-analysis demonstrated that \(BRAF^{V600E}\) mutation was related to poor prognosis of CRC and associated with the distinct molecular phenotypes.

Key words: colorectal cancer, BRAF mutation, prognosis, meta-analysis

Introduction

Colorectal cancer (CRC), the third most common cancer, causes the fourth most frequent cancer-related deaths worldwide [1]. It has been widely recognized that constitutive activation of the RAS-RAF-MEK-ERK (MAPK) pathway plays a critical roles in CRC development and progression [2]. Gain-of-function mutations of the key protein BRAF in this pathway will constitutively activate this pathway, suggesting the crucial role of BRAF mutation in CRC [3]. The \(BRAF^{V600E}\) mutation, inducing the substitution of valine for glutamate at position 600 of the b-raf protein, accounts for approximately 90% of BRAF mutations and has more important significance compared to other BRAF mutation types in CRC, and...
about 10% of CRC patients harbor the BRAFV600E mutation [3]. Increasing studies have discussed the relationship between BRAFV600E mutation and the effect of anti-EGFR inhibitors in CRC, but the effects of BRAFV600E mutation on the clinicopathological characteristics of CRC remains limited. Therefore, in this article we comprehensively estimate the association between BRAFV600E mutation and clinicopathological characteristics of CRC patients.

Methods

Literature search strategy

We searched PubMed, Web of Science, Embase, and PMC database for relevant publications with the following search terms: (“colorectal cancer” or “rectal cancer” or “colon cancer”) and (“BRAF mutation” or BRAFV600E). Original articles about human studies written in English published before June 18, 2018 were included.

Inclusion criteria

The studies were gone through in accordance to the predetermined selection. The inclusion criteria were: (1) the association between BRAFV600E mutation and clinicopathological characteristics was studied; (2) sufficient published data for calculating an odds ratio (OR) and 95% confidence interval (CI) were reported; (3) the most appropriate article was selected when multiple articles associated with the same patient population were published. The exclusion criteria were: (1) review articles; (2) articles without enough data to analyzed; and (3) single case reports. The quality of each study was assessed using the Newcastle-Ottawa Scale (NOS).

Data extraction

For every appropriate study, the relevant data were extracted, including name of the first author, publication year, country where the study was conducted, follow-up time, number of patients with BRAFV600E mutation, total number of patients, patient demographics (age and gender); clinicopathological characteristics including tumor site, disease stage, T stage, N stage, metastasis status, tumor size, tumor differentiation and mucinous histology; molecular characteristics including KRAS mutation status, CpG island methylator phenotype (CIMP), TP53 mutation status, DNA mismatch repair (MMR) status and microsatellite instability (MSI) status).

Statistical analysis

Meta-analysis was performed using RevMan (Cochrane Collaboration, Oxford, UK). The strength of the association between the BRAFV600E mutation and clinicopathological parameters was assessed by odds ratio (OR) with the corresponding 95% confidence interval (CI). In the course of data pooling, statistical heterogeneity was defined by using chi-square-based Q-test. The P value indicates the degree of heterogeneity. A P-value<0.10 and/or I2>50% are considered significant heterogeneity, and then a random-effect model is used. Otherwise, a fixed-effect model is used.

Results

Characteristics of eligible literatures

According to the search terms, a total of 1332 eligible citations were obtained. After screening the abstract, 1228 citations were excluded. Among the remaining 104 citations, 43 citations were excluded because of the reasons shown in Figure 1. Finally, 61 studies published from 2006 to 2018 were included in the meta-analysis (Figure 1). A total of 32407 CRC patients from China, Japan, South Korea, India, French, Sweden, Greece, American, Netherlands, Italy, Germany, Australia, and so on were included, and among these patients, 3688 patients were with BRAFV600E mutation (11.38%). The study sample sizes ranged from 69 to 1980 cases. BRAFV600E mutation rate among all studies ranged from 3.14% to 23.14%, which was consistent with the results in the previous study [4]. All specimens were derived from CRC tissues by either biopsy or surgical resection, and were detected for BRAF mutation status mainly by direct sequencing, pyrosequencing, allele-specific PCR and immunohistochemistry (IHC) method.

The basic characters of the 61 eligible studies were summarized in
Supplementary Table 1. Thirty-five studies are with sample size below 500 [5-37, 64], whereas twenty-six studies are with sample size over 500 [38-63]. The earliest study was published in July 2005 [51], and the latest study was published in August 2017 [49]. Most of these studies involved patients with stage I-IV CRC [5, 6, 8, 10, 12, 14, 15, 19, 21, 22, 24, 26, 27, 31, 32, 34, 38, 39, 42, 44-47, 51, 52, 54, 55, 57-60, 64], and six studies only involved patients with stage IV CRC [23, 29, 33, 35, 43, 56]. All the studies have a NOS score of ≥5, and 18 studies have a NOS score of ≥6 (Supplementary Table 1).

Correlation of BRAFV600E mutation with clinicopathological characteristics of CRC patients

Demographic characteristics (Age and Gender)

A total of 14 studies investigated the association between BRAFV600E mutation and age. Of 2434 patients younger than 60 years, 182 (7.47%) patients were BRAFV600E mutation positive, and 551 (22.46%) of 3864 patients 60 year or older were BRAFV600E mutation positive. The association between BRAFV600E mutation and age did not reach statistical significance (OR=0.66; 95% CI=0.43–0.90; P=0.05) (Figure 2A, Table 1). Fifty-six studies analyzed the association between BRAFV600E mutation and gender. Of 14453 male patients, 1214 (8.40%) CRC patients were with BRAFV600E mutation, and 1822 (15.04%) of 12048 female patients were with BRAFV600E mutation. There was a significantly negative association between BRAFV600E mutation and male gender (OR=0.53; 95% CI=0.40–0.65; P<0.00001) (Figure 2B, Table 1).

Clinical Facts (Disease stage, T stage, N stage, tumor size, metastasis status, tumor site, tumor differentiation, and mucinous histology)

Twenty-six studies analyzed the association between disease stage and BRAFV600E mutation. Of 5457 patients with stage I or II, 528 (9.68%) patients were BRAFV600E mutation positive, and 733 (11.65%) patients was BRAFV600E mutation positive from 6290 patients diagnosed with stage III or IV disease. Stage I or II were negatively related to BRAFV600E mutation (OR=0.81; 95% CI=0.72–0.92; P=0.001), indicating that CRC patients with BRAFV600E mutation trend to have more advanced disease stage (Figure 3A, Table 1). Furthermore, patients with BRAFV600E mutation were also negatively associated with low T stage (OR=0.51; 95% CI=0.40–0.65; P<0.00001) (Figure 3C, Table 1). However, the overall analysis showed BRAFV600E mutation did not statistically significant correlated with tumor size (OR=0.83; 95% CI=0.45–1.55; P=0.56) (Figure 3B, Table 1), N stage (OR=0.85; 95% CI=0.73–1.00; P=0.05) (Figure 3D, Table 1) and metastasis status (OR=1.30; 95% CI=0.90–1.88; P=0.16) (Figure 3E, Table 1).

In total, forty-five studies investigated the relationship between BRAFV600E mutation and tumor site. And among these studies, twenty-four studies categorized tumors as proximal colon, distal colon or rectal tumor, and another twenty-one studies classified the tumor as right colon, left colon or rectal tumor. The final results showed that BRAFV600E mutation was significantly associated with proximal colon tumor location (OR=4.76; 95% CI=3.81–5.96; P<0.00001) or right colon tumor location (OR=5.15; 95% CI=4.35–6.10; P<0.00001) (Figure 4A-B, Table 1).

Twelve studies assessed the association between BRAFV600E mutation and tumor differentiation. 592 (7.81%) patients were with BRAFV600E mutation of 7579 patients with well or moderate differentiation, and 310 (26.34%) patients were with BRAFV600E mutation of 1177 patients with poor differentiation. It was obvious that BRAFV600E mutation was negatively associated with well or moderate differentiation, indicating that CRC patients with BRAFV600E mutation trend to have aggressive tumor phenotype (OR=0.27; 95% CI=0.21–0.34; P<0.00001) (Figure 4C, Table 1). Besides, BRAFV600E mutation was also strikingly related to mucinous histology (OR=2.97; 95% CI=2.37–3.72; P<0.00001) (Figure 4D, Table 1).

Molecular Features (K-ras mutation status, TP53 mutation status, MMR capacity, MSI status, and CIMP)

Twelve studies analyzed the association between BRAFV600E mutation and K-ras mutation status. Notably, K-ras mutation and BRAFV600E mutation were negatively related (OR=0.04; 95% CI=0.02–0.07;
P<0.00001) (Figure 5A, Table 1). Of 1616 K-ras-mutated patients, only nine (0.56%) ones were BRAF^V600E mutated, while 468 (15.38%) patients of 3043 K-ras-wild patients were BRAF^V600E mutated. Interestingly, BRAF^V600E mutation was also negatively associated with TP53 mutation (OR=0.50; 95% CI=0.31–0.78; P=0.003) (Figure 5B and Table 1).

Figure 2. The association of BRAF^V600E mutation with demographics, including age (A) and gender (B).
Figure 3. Meta-analysis of association between BRAF\textsuperscript{V600E} mutation and clinical features, including disease stage (A), tumor size (B), T stage (C), N stage (D) and metastasis status (E).
Figure 4. The association of BRAFV600E mutation with tumor characteristics, including tumor site (A and B), tumor differentiation (C) and mucinous histology (B).
Only two studies investigated the relationship between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and mismatch repair (MMR) capacity. The results showed that 24 (18.75\%) patients were \textit{BRAF}^{\textit{V}600\textit{E}} mutation positive from 128 patients with deficient MMR (dMMR) capacity, and 90 (7.51\%) patients were \textit{BRAF}^{\textit{V}600\textit{E}} mutation positive from 1198 patients with proficient MMR (pMMR) capacity. \textit{BRAF}^{\textit{V}600\textit{E}} mutation was significantly related to dMMR (OR=2.93; 95\% CI=1.78–4.82; \(P<0.00001\)) (Figure 5C, Table 1). Twenty-seven studies investigated the \textit{BRAF}^{\textit{V}600\textit{E}} mutation and microsatellite instability (MSI). Of 1872 patients with high microsatellite instability (MSI-High), 864 (46.15\%) patients were \textit{BRAF}^{\textit{V}600\textit{E}} mutated, and of 11668 patients with low microsatellite instability (MSI-low) or microsatellite stable (MSS), 810 (6.94\%) patients were \textit{BRAF}^{\textit{V}600\textit{E}} mutated. There was a significant association between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and MSI-high (OR=11.15; 95\% CI=8.51–14.61; \(P<0.00001\)) (Figure 5D, Table 1). Ten studies were analyzed for CpG island methylator phenotype (CIMP) and \textit{BRAF}^{\textit{V}600\textit{E}} mutation. Of 4112 patients with low or negative CIMP, 179 (4.35\%) patients were with \textit{BRAF}^{\textit{V}600\textit{E}} mutation, and of 834 patients with high CIMP, 359 (43.05\%) patients were with \textit{BRAF}^{\textit{V}600\textit{E}} mutation. According to the result, \textit{BRAF}^{\textit{V}600\textit{E}} mutation was negatively associated with high CIMP (OR=0.04; 95\% CI=0.03–0.08; \(P<0.00001\)) (Figure 5E and Table 1).

Additional analyses

A funnel plot of effects calculated from individual studies examining the association between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and disease stage was conducted to estimate the presence of publication bias. Because there are small studies with negative results in the literature, no strong indication of publication bias exist among the series of studies included in this meta-analysis.

Discussion

\textit{BRAF}^{\textit{V}600\textit{E}} mutation was an important molecular alternation in CRC patients. In our study, the highest \textit{BRAF}^{\textit{V}600\textit{E}} mutation rate reached to 23.14\% and the average \textit{BRAF}^{\textit{V}600\textit{E}} mutation rate was 11.35\% among all the involved studies, similar to other reports [4]. Clinicopathological parameters have crucial roles in predicting the prognosis of cancer patients. It is necessary to clarify the relationship between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and clinicopathological parameters in CRC patients [65]. Our meta-analysis indicated that \textit{BRAF}^{\textit{V}600\textit{E}} mutation was significantly associated with female, advanced disease stage, high T stage, proximal or right tumor location, poor tissue differentiation and mucinous phenotype. As high disease stage, high T stage, poor tissue differentiation and mucinous histology were the multiple risk factors of the prognosis in CRC patients, it may be deemed that \textit{BRAF}^{\textit{V}600\textit{E}} mutation was a poor predictive indicator [20, 51, 65]. Our study also showed that \textit{BRAF}^{\textit{V}600\textit{E}} mutation had a crucial association with disease stage, T stage, N stage and tissue differentiation, which demonstrated the important role of \textit{BRAF}^{\textit{V}600\textit{E}} mutation in occurrence and development of CRC.

Intriguingly, tumors located in the proximal colon were 4.76-fold more likely to have \textit{BRAF}^{\textit{V}600\textit{E}} mutation than tumor located in the distal or rectal colon. Moreover, the \textit{BRAF}^{\textit{V}600\textit{E}} mutation was 5.15-fold more frequent in tumors located in the right colon than tumors located in the left or rectal colon. The association between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and tumor sites was very strong and the reason of the association has not been clarified clearly. Previous studies have indicated that colorectal tumors located in different sites have totally different outcomes and specific biomolecular characteristics [66]. Our study also demonstrated that the difference in regard to \textit{BRAF}^{\textit{V}600\textit{E}} mutation in different tumor location. Moreover, the different \textit{BRAF}^{\textit{V}600\textit{E}} mutation rates among different tumor sites might be useful for formulating treatment therapy for CRC located in different tumor sites [67, 68].

Nowadays, in addition to clinicopathologic stage and histological morphology, molecular markers play increasing roles in making therapeutical decision for cancer patients. Melanoma with \textit{BRAF}^{\textit{V}600\textit{E}} mutation is more sensitive to immunotherapy [69]. Deficient MMR status has been demonstrated to predict the response of PD-1 blockade in metastatic CRC [70], and MSI-High also has been recognized as a predictive factor of programmed death ligand-1 inhibitor pembrolizumab in metastatic/refractory CRC [71]. Our results revealed that \textit{BRAF}^{\textit{V}600\textit{E}} mutation was significantly related to K-ras-wild type, TP53-wild type, deficient MMR, high MSI and high CIMP. This association between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and other molecular features cloud be important to understand the molecular distinction between CRC patients with or without \textit{BRAF}^{\textit{V}600\textit{E}} mutation. However, the association between \textit{BRAF}^{\textit{V}600\textit{E}} mutation and the therapeutical response in CRC needs more prospective investigations.

In this article, although we conducted comprehensive and detailed meta-analysis, there are still some limitations. Firstly, with regard to some clinicopathologic characteristics, the number of the involved patients was limited. Small studies are prone to introduce unstable results and related to publication bias. Secondly, most of these studies were retrospective or observational studies (data not...
shown), which might induce heterogeneity. Thirdly, the mutation detection assays were different among these studies. The most two commonly used methods are direct sequencing and pyrosequencing. Different BRAF<sup>V600E</sup> mutation assays also affected the accuracy and precision of the pooled estimates.

Figure 5. The association of BRAF<sup>V600E</sup> mutation with molecular features, including Kras mutation status (A), TP53 mutation status (B), MMR capacity (C), MSI status (D) and CIMP phenotype (E).
In conclusion, our updated and comprehensive meta-analysis based on a large number of clinical data demonstrated that BRAFV600E mutation is a biological predictor for poor prognosis in CRC patients, which helps to elucidate the mechanisms of progression and metastasis of CRC and to develop novel therapeutic strategies for CRC.

Competing Interests
The authors have declared that no competing interest exists.

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