BRAF Mutations in Patients with Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis

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Abstract

Background: BRAF mutations have been well described in non-small cell lung cancer (NSCLC) for several years, but the clinical features of patients harboring BRAF mutations are still not well described. We performed a meta-analysis to identify common clinical features in NSCLC patients carrying BRAF mutations.

Methods: We identified clinical studies that examined the association between BRAF mutations and features of NSCLC within PubMed, Embase and ISI Science Citation Index database up to October 2013. The effect size of clinical features was estimated by odds ratios (ORs) with 95% confidence interval (CI) for each study, using a fixed-effects or random-effects model.

Results: Ten studies with a total of 5599 NSCLC patients were included. There was a 3% (170/5599) BRAF mutation rate. BRAF mutations in NSCLC were significantly associated with adenocarcinomas (ADCs) (compared with non-ADCs, OR = 4.96, 95%CI = 2.29–10.75). There were no significant differences in gender, smoking and stage in patients with and without BRAF mutations. The BRAFV600E mutation was more frequent in women than non-BRAFV600E mutations (OR = 0.27, 95%CI = 0.12–0.59), and was closely related to never smokers (OR = 0.14, 95%CI = 0.05–0.42).

Conclusions: These findings have important implications for the prediction of the NSCLC sub-types more accurately combined with other genetic changes.

Introduction

Lung cancer is the leading cause of cancer death, annually resulting in more than one million deaths worldwide [1]. Approximately 85% of lung cancer patients have a histologic diagnosis of non-small cell lung cancer (NSCLC), and the overall 5-year survival rate is about 17% [2,3]. Treatment decisions for patients with lung cancer have historically been based on tumour histology. One promising treatment strategy has focused on the further subdivision of NSCLC into clinically relevant molecular subsets. The classification schema was based on specific so-called driver mutations including activating mutations in the epidermal growth factor receptor (EGFR), KRAS, BRAF, HER2, PIK3CA, and others in frequencies exceeding 1% [4,5]. Besides, Seidel et al. have successfully predicted each of the NSCLC sub-types by using a combination of immunohistochemistry and genomic markers [6].

BRAF, one of three members of the RAF kinase family: A-RAF, BRAF, C-RAF, belongs to the group of serine-threonine kinases and plays vital role in mitogen-activated protein kinase (MAPK) pathways [7,8]. Mutations in BRAF have been found in different kinds of cancers, predominantly melanoma, metastatic colorectal cancer and papillary thyroid cancer. The frequency of mutation has been about 50%, 9%, and 45%, respectively [9–11]. BRAF mutations were found in 1–3% of NSCLCs [4].

The mutations in the above genes are closely related to specific demographic or clinicopathologic characteristics, including smoking habits, gender, clinical stage, and tumor histology [12,13]. This information may be useful for the selection of patients for treatment with specific gene inhibitors. While BRAF mutations in NSCLC have been described for several years, the actual prevalence and clinical features of patients with NSCLC who harbor BRAF mutations are not well defined due to the relatively low number of patient cases investigated [14–16].

We performed a meta-analysis of a large number of lung tumors with BRAF mutations from published studies in order to quantitatively review the association between BRAF mutation and the demographic or clinicopathologic characteristics.
Materials and Methods

Publication search

We performed a systematic literature search in PubMed, EMBASE, and the Science Citation Index databases. The following search terms were used to identify relevant publications: “BRAF”, “B-RAF”, “non-small cell lung cancer”, “non-small cell lung carcinoma”, “non-small cell carcinoma”, “NSCLC”, “squamous-cell lung cancer”, “squamous-cell lung carcinoma”, “large-cell lung cancer”, “large-cell lung carcinoma”, “lung adenocarcinoma”. The literature search was limited to human studies. No limitations were placed on the language of publication or type of study. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were hand searched to find additional eligible studies.

Inclusion criteria

Studies eligible for inclusion in this meta-analysis (1) were published as a full text in English. (2) the number of patients with BRAF mutations was more than 1; (3) the articles were involved with the association between BRAF and demographic or clinicopathologic features of NSCLC. When the same author or group reported results from the same patient population in more than one article, the most recent report or the most informative one was included.

Data extraction

Information was carefully extracted from all eligible studies. The following data were collected from each study: first author’s name, year of publication, number of patients included, number of patients with BRAF mutations, number of patients with BRAF mutants, mutations, screening methods, demographic and clinicopathologic characteristics of patients. Data extraction was done independently by two of the authors and discrepancies were resolved by consensus including a third author. All of the procedures conformed to the guidelines for the meta-analysis of observational studies in epidemiology [17].

Statistical methods

We used RevMan (version 5) to calculate the summary odds ratios (ORs) with 95% confidence intervals (CIs), using a random or fixed effects model for all of the analyses. We assessed the heterogeneity of the studies using the chi-square test of heterogeneity and the I² measure of inconsistency. Significant heterogeneity was defined as a chi-square test P value <0.10 or as an I² measure >50% [18]. If ORs were found to have fine homogeneity (I²<50%), a fixed effects model was used for secondary analysis. If not (I²>50%), a random-effects model was used. Sensitivity analysis was performed to examine the influence of each study on the pooled OR by serially omitting an individual study and pooling the remaining studies. Possible publication bias was evaluated by visual assessment of a funnel plot. Subgroup analyses were performed by ethnicity and number of BRAF mutations.

Results

Study Selection

A total of 1480 abstracts and titles were obtained through electronic searches. 349 records were excluded because of duplicates. The remaining 1131 records were screened by the titles and abstracts and 1088 studies were excluded. 43 full-text papers were deemed relevant and were examined in detail. 35 of these full-text articles were excluded (Figure 1). Ten studies met the inclusion criteria and were included in the meta-analysis.

Study Characteristics

There were 5599 patients in the identified 10 studies [3,14–16,19–24]. Only 170 (3.0%) of these patients had BRAF mutations in the NSCLC tumors (Table 1). The earliest study was in November 2008 by Pratilas et al. [19], while the latest study was in August 2013 by Cardarella et al. [5]. The sample size ranged from 96 to 1046, with only one study over 1000 patients [16]. The incidence of BRAF mutations in individual studies ranged from 0.9% to 8.9%. Patients in four studies were Asian, five studies were Non-Asian, and one study consisted of a mixed population from 4 countries. Five studies screened for BRAF mutations using polymerase chain reaction (PCR) and direct sequencing. Three studies detected the BRAF mutations using the above methods plus matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), single strand conformation polymorphism (SSCP) analysis, or high-resolution melting analysis (HRMA). The remaining two studies used the single methods MALDI-TOF MS or HRMA.

BRAF mutations and demographic and clinicopathologic characteristics of NSCLC

The pooled results of the association between BRAF mutations and demographic and clinicopathologic characteristics of NSCLC are reported in Table 2 and Figure 2. Nine studies presented data on the association between BRAF mutations and gender. BRAF mutations were detected in 83 of 2224 male patients (3.73%) and 79 of 1972 female patients (4.01%). There was no significant difference in the frequency of mutation by gender (OR = 0.79, 95%CI = 0.57–1.10). Data regarding the association between BRAF mutations and smoking was presented in eight studies. BRAF mutations were detected in 120 of 2557 former or current smokers (4.69%) and 38 of 1248 never smokers (3.04%). There was no significant difference in BRAF mutation rate in former or current smokers and never smokers (OR = 0.95, 95%CI = 0.45–2.02). Six studies reported the association between BRAF mutation
and tumor histology. \textit{BRAF} mutations were detected in 98 (4.04\%) of 2224 ADC and 6 (0.58\%) of 1037 non-ADCs. There were significant differences between ADCs and non-ADCs in \textit{BRAF} mutation rate (OR = 4.96, 95\%CI = 2.29–10.75). Only four studies contained information about clinical stage of NSCLC with \textit{BRAF} mutations. \textit{BRAF} mutations were detected in 46 of 1132 patients with stage I or II (4.06\%) and 71 of 931 in patients with stage III or IV (7.63\%). There were no significant difference in the \textit{BRAF} mutation rate in stage I and II, and stage III and IV (OR = 1.05, 95\%CI = 0.55–2.01).

\textit{BRAF}V600E mutation and demographic and clinicopathologic characteristics

The association of \textit{BRAF}V600E mutation and demographic and clinicopathologic characteristics was evaluated (Figure 3). Three studies evaluating the \textit{BRAF}V600E mutation in NSCLC were systematically analyzed using a fixed effects model. \textit{BRAF}V600E mutations accounted for 53.6\% (60/112) of all the \textit{BRAF} mutations. \textit{BRAF}V600E mutations were detected in 23 of 60 male patients (38.3\%) and 37 of 52 female patients (71.2\%). There was a significant differences in male and female expression of this mutation (OR = 0.27, 95\%CI = 0.12–0.59). Of 84 former or current smokers, 36(42.9\%) had \textit{BRAF}V600E mutations. 24 of 28 never smokers (85.7\%) had this mutation. The difference was significant between former or current smokers and never smokers (OR = 0.14, 95\%CI = 0.05–0.42).

\textit{BRAF} mutations were detected in 20 of 42 patients with stage I or II (47.6\%) disease and 40 of 70 in patients with stage III or IV (57.1\%) disease. There was no significant difference in expression of this mutation in the two stage groups (OR = 0.54, 95\%CI = 0.23–1.28).

Subgroup analyses of \textit{BRAF} mutations and demographic and clinicopathologic characteristics

When the combined studies included were stratified according to ethnicity, there were no statistically significant associations between \textit{BRAF} mutation and gender, smoking history or stage, in agreement with the overall effect (Table S1). However, the OR of histology was 3.49 (95\%CI: 0.79–15.45) for Asians, 5.75 (95\%CI: 0.94–35.25) for non-Asians, and 3.59 (95\%CI: 0.82–15.80) for mixed ethnicity. There was no statistically significant association between \textit{BRAF} mutation and ADC, which was different from the overall effect (OR = 4.96, 95\%CI = 2.29–10.75). Similarly, when the studies were grouped by the number of \textit{BRAF} mutations, the combined OR for histology was 3.49 (95\%CI: 0.79–15.45) for fewer mutations (<10). In this subgroup \textit{BRAF} mutations were more frequently associated with ADCs. (Table S1).

Sensitivity analysis and publication bias

Sensitivity analysis was conducted to ascertain whether modification of the inclusion criteria of this meta-analysis affected the final results. The sensitivity analysis revealed that none of the studies significantly affected the pooled ORs and CIs (data not shown). To investigate the presence of publication bias, a funnel plot of effects calculated from individual studies examining the association between \textit{BRAF} mutations and demographic or clinicopathologic features was performed. There was no strong indication of publication bias among the studies included in this meta-analysis.

Discussion

Studies of mutations of the \textit{BRAF} gene have generated considerable interest because these mutations may be associated with increased sensitivity to agents directly targeting \textit{BRAF} or
BRAF-mediated downstream signaling pathways [25]. BRAF mutations have been analyzed using meta-analysis studies in melanoma, colorectal cancer and papillary thyroid cancer [26–29]. Mutations in the BRAF gene were closely related to particular demographic or clinicopathologic characteristics, including smoking habits, gender, clinical stage, differential and tumor histology. The effect of BRAF mutations on the clinical features of NSCLC have been reported for several years. A consensus has not been reached due to the small number of patients evaluated. We performed this meta-analysis to investigate the prevalence and characteristics of NSCLC patients with BRAF mutations in a large pooled sample of patients.

We systematically reviewed the literature describing the relationship between BRAF mutations and demographic or clinicopathologic features from 10 studies involving over 5500 patients with NSCLC. The rate of BRAF mutations was on average around 3% (170/5599), in agreement with previously published data [4,7,21]. The mutation rate from published studies varied from 0.9% to 8.9% [20,24]. Ilie et al. explained the variation may be due to the exclusive evaluation of BRAF mutation in Caucasian patients with EGFR, KRAS, PI3KCA, HER2 or ALK alterations [24]. Mutation rates of 2% and 3.5% were reported by Pratilas et al and Marchetti et al. in series comprised of 916 and 1046 patients, respectively [16,19]. These were close to the frequency found in our study.

One of the aims of our study was to identify features that would help enrich patients for tumor mutation analysis. The meta-analysis was carried out to find an association between BRAF mutation and four clinicopathologic features. There were no significant association with gender and the incidence of BRAF mutation (OR = 0.79 95%CI = 0.57–1.10). An association between BRAF mutations and female has been reported in patients with colorectal cancer [29–31]. However, such an association has not been made for NSCLCs [5,24], which corroborates with our results. Subgroup analyses by ethnicity finds a weak association with Asians having a slightly higher propensity for having BRAF mutation, but this was not statistically significant. Most of the studies also showed no association of BRAF mutation and smoking status. However, Paik et al reported that all patients with a BRAF mutation were current or former smokers [21]. However we see no such association in this analysis.

In recent years, scientists have made great progress toward understanding specific mutations of the cancer and targeting them with appropriate drugs [6]. Seidel et al. performed a great work with two cohorts studies including more than 6000 lung cancer patients, and demonstrated the association between lung tumor subtype and its predominant mutations, and the benefit of genetic testing and targeted therapy in these patients [6]. They found that most mutations showed histological subtype specificity and provided a blueprint for genomic diagnosis of lung tumors [6]. Therefore, we also performed the meta-analysis to found the association between BRAF mutation and histological subtype and clinical stage of NSCLC.

NSCLC is comprised of three different histologic types, squamous-cell carcinoma, large-cell carcinoma, and ADC. ADC accounts for more than 50% of all cases. We found that BRAF mutations as a whole were more common in ADCs than in other histologies (OR = 4.96 95%CI = 2.29–10.75), similar to a previous study [16]. There was no significant heterogeneity between studies ($I^2 = 0.0\%$, $P = 0.70$). An interesting finding of our study was that the pooled results in the subgroup stratified by ethnicity and number of mutations were significantly different from the overall effect of histology (Table S1). There was no significant difference in the rate of BRAF mutations and ADCs in the subgroup, while

### Table 2. Association between BRAF mutation and gender, smoking, histology and stage in NSCLC.

| Outcome | Mutant BRAF (%) | Test of association | OR (95%CI) | P | Chi^2 | I^2 | P |
|---------|----------------|---------------------|------------|---|-------|-----|---|
| Gender | Male 83/2224(3.73) | M-H, Fixed, 95%CI | 0.79 [0.57, 1.10] | 0.16 | 9.23 | 13% | 0.32 |
| Female | 79/1972(4.01) | M-H, Fixed, 95%CI | 0.95 [0.65, 2.02] | 0.00 | 19.25 | 64% | 0.01 |
| Smoking | Former/current 120/2557(4.69) | M-H, Random, 95%CI | 4.96 [2.20, 10.75] | 0.00 | 3.00 | 0% | 0.70 |
| Non-smoker | 38/1248(3.04) | M-H, Fixed, 95%CI | 0.89 [0.45, 2.02] | 0.00 | 19.25 | 64% | 0.01 |
| Histology | ADC 98/2424(4.04) | M-H, Fixed, 95%CI | 4.96 [2.20, 10.75] | 0.00 | 3.00 | 0% | 0.70 |
| Non-ADC | 6/1037(0.58) | M-H, Fixed, 95%CI | 0.89 [0.45, 2.02] | 0.00 | 19.25 | 64% | 0.01 |
| Stage | I, II 46/1132(4.06) | M-H, Random, 95%CI | 1.05 [0.55, 2.01] | 0.89 | 5.73 | 48% | 0.13 |
| III, IV | 71/931(7.63) | M-H, Fixed, 95%CI | 4.96 [2.20, 10.75] | 0.00 | 3.00 | 0% | 0.70 |

OR, Odds Ratio; CI, confidence interval. doi:10.1371/journal.pone.0101354.t002
Figure 2. The association of BRAF mutations with gender (A), smoking (B), histology (C) and stage (D).
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the differences were significant in the overall patient population. This may be explained by the number of cases included. When the number of patients was small in the subgroup, the association was not significant. When we investigated this association in a large number of patients, there was greater power to detect the association between \textit{BRAF} mutation and ADC. This is the most valuable finding of this study. Clinical stage is an important factor in determining the prognosis of NSCLC. We found no significant association between low or high stage and \textit{BRAF} mutation. Larger studies are needed to better examine this relationship.

Among the different mutations occurring in the \textit{BRAF} gene, \textit{BRAF}V600E is the most common [32]. So far three studies have investigated the association between \textit{BRAF}V600E mutation and demographic or clinicopathologic features [5,16,24]. The number of V600E and non-V600E mutations detected was low, not allowing us to perform a separate analyse in other studies. The three studies found significant differences in the clinical features of patients with NSCLC with and without \textit{BRAF}V600E mutation [5,16,24]. Two reports found \textit{BRAF}V600E mutations more frequent in females and never-smokers, and not with any other clinicopathologic features [16,24]. We also found the \textit{BRAF}V600E mutation was significantly more frequent in women. The \textit{BRAF}V600E mutation was also significantly more frequent in never-smokers compared to current or former smokers (OR = 0.14; 95\%CI = 0.05–0.42). Ilie et al. reported that non-\textit{BRAF}V600E mutations were significantly associated with early-stage tumours [24]. We did see a trend for earlier-stage disease but that was not statistically significant.

Heterogeneity is a potential problem that may affect the interpretation of all meta-analyses. To investigate the potential sources of heterogeneity that might modify effects of the \textit{BRAF} mutations on smoking and stage, we performed subgroup analyses according to the ethnicity and number of \textit{BRAF} mutations. The results in smoking and stage were not substantially changed, as indicated by subgroup and sensitivity analysis. Subgroup analysis by ethnicity demonstrated no clinical heterogeneity regarding the association between \textit{BRAF} mutations and gender, smoking or stage. This indicates that overall estimation of the association between \textit{BRAF} mutations and the clinical features of NSCLC is legitimate.

Our study had several limitations that need to be taken into consideration when interpreting the findings. Firstly, the number of included studies was small. More studies are needed to extend and confirm our results. Secondly, we did not collect data on the treatment and clinical outcomes of patients with \textit{BRAF} mutations which will be done in a future study. Finally, we did not describe the association between \textit{BRAF} mutation and smoking habit which grouped by former and current smokers separately due to lack of the data.
Conclusions

Despite the limitations, our meta-analysis had some significant findings. We found that BRAF mutations were more frequent in ADCs, and were not associated with other histologic types. The BRAFV600E mutation was significantly correlated with female and non-smoker NSCLC patients. The conclusions obtained here confirmed the reported association of BRAF mutations with specific demographic or clinicopathologic characteristics, which may be useful for the prediction of the NSCLC sub-types more accurately combined with other genetic changes.

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Supporting Information

Table S1 Subgroup analysis of the relationship between BRAF mutation and tumor characteristics according to ethnicity and number of mutations.

Checklist S1 PRISMA 2009 Checklist.

Author Contributions

Conceived and designed the experiments: WLF QH DC. Analyzed the data: JFH KL. Wrote the paper: DC. Performed literature search: ZRC. DCS QL. Data extraction: LQZ YXW ZY. Performed the meta-analysis: LQZ YXW ZY. Discovered the reported association: WLF QH DC. Designed the study: LQZ YXW ZY. Drafted the manuscript: LQZ YXW ZY.

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