

Review Article

BRAF mutation in papillary thyroid carcinoma

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Abstract: BRAF mutation is the most common genetic alternation in thyroid cancer, particularly in papillary thyroid cancer (PTC). Excessive activation of BRAF/MAPK signaling pathway due to BRAF mutation plays a central role in the tumorigenesis and development of PTC. The association of BRAF mutation with poor clinicopathological characteristics of PTC further demonstrated the importance of the BRAF mutation alternation in PTC. Detection of BRAF mutation on FNA specimen before surgery is recommended as a useful diagnostic marker and prognostic indicator for PTC, and thus influences surgeon's decision on management of PTC. Recent studies have focused on inhibition of BRAF activation and several small molecules have been developed as targeting therapy.

Keywords: BRAF mutation, papillary thyroid carcinoma, BRAF/MAPK signaling pathway, targeting therapy

Introduction

In the United States, thyroid cancer shows the fastest rising incidence among all major human cancers, currently with 44,670 new cases per year and > 400,000 existing cases [1]. Papillary thyroid cancer (PTC) is the most common histological type of thyroid malignance and the rising incidence of thyroid cancer is mainly attributed to the increased diagnosis of PTC, particularly the small PTC [1, 2]. The majority of thyroid cancers generally have a good prognosis after appropriate treatment including surgical procedure and radioiodine therapy. However, the recurrence rate of differentiated thyroid cancer increased up to 30%, and the cancer death rate was 8% after initial treatment at 30 years of follow-up [3]. During the past decade, understanding of genetic alternations regarding thyroid cancer has rapidly expanded. These improvements underlying the development of thyroid cancer offered novel diagnostic tools and therapeutic strategies.

Similar to other cancer types, thyroid cancer progression and dedifferentiation involves a number of genetic alterations including two distinct molecular mechanisms: point mutation or

chromosomal rearrangement. Most mutations in thyroid cancer involve the MAPK and PI3K-AKT signaling pathways. BRAF is a serine-threonine kinase that is translocated to the cell membrane after being bound and activated by RAS, which results in the phosphorylation and activation of mitogen activated protein kinase (MAPK) and other downstream targets of MAPK signaling pathway. According to present studies, BRAF mutation exclusively exists in PTC and PTC-derived anaplastic thyroid cancer (ATC), yet has not been found in other histological types of thyroid cancer such as follicular thyroid cancer (FTC) and medullar thyroid cancer (MTC). The BRAF^{V600E} mutation [a valine to glutamic acid mutation at position 600] is found in more than 50% of overall thyroid malignances [4-7]. In this review, we focus on the association of BRAF mutation with clinicopathological characteristics and BRAF mutation as a diagnostic marker and therapeutic target of thyroid cancer.

BRAF V600E mutation related molecular events in PTC

The most common genetic alterations in PTCs include BRAF mutation, RAS mutation, and RET/PTC rearrangement which mainly involve in the

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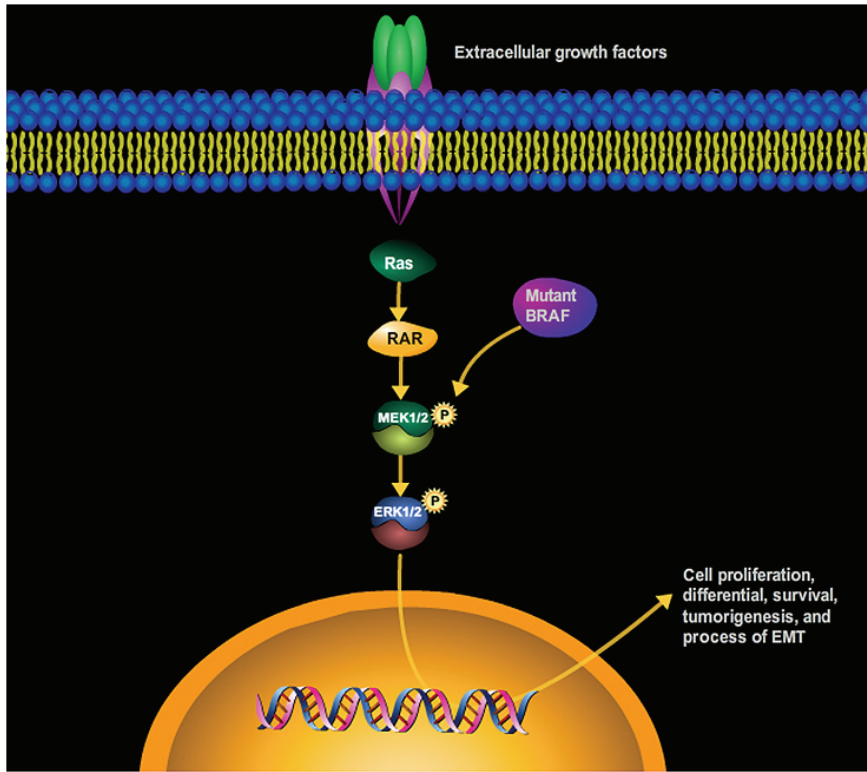


Figure 1. The activation of the mitogen-activated protein kinase (MAPK) pathway in thyroid cancer by mutant BRAF. The most common genetic alteration in papillary thyroid cancer, BRAF mutation, stimulates constitutive signaling which bypasses the need for extracellular mitogenic signals. Subsequently phosphorylation of downstream MER1/2 and ERK1/2 results in transcriptional regulation of various genes which are involved in cell proliferation, differential, survival, tumorigenesis, and the process of EMT.

RAS/BRAF/MAPK signal pathway. Interestingly, these molecular alterations are exclusive in PTC patients, suggesting that each of them alone is sufficient for malignant transformation of thyroid cells. BRAF V600E mutation strongly increases BRAF kinase activity by eliciting ERK1/2 phosphorylation which is 480-fold higher than wild type BRAF [8]. The markedly increased ERK1/2 phosphorylation in BRAF V600E mutation mainly attributed to a negatively charged residue adjacent to the phosphorylation site at T598 and mimicking phosphorylation at Thr598 and Ser601 residues [9].

Activation of MAPK pathway by BRAF V600E was believed to play a dominant role in the development and progression of thyroid cancer. Effectors of MAPK signaling pathway representing an early molecular event in PTC regulate a number of genes related to cell proliferation, differential and survival. Gene expression analyses using DNA microarrays showed that differential transcriptional profiles were associated with BRAF, RET/PTC and RAS mutation groups in humane PTCs [10]. The differential expression profiles between these genetic alternations may explain why BRAF mutation has more close cor-

relations with poor clinicopathological features compared with RET/PTC and RAS mutation. The mutant BRAF can stimulate constitutive signaling which bypasses the need for extracellular growth factors. Subsequently phosphorylation of downstream MER1/2 and ERK1/2 leads to the expression of a number of specific genes involved in cell proliferation, differential, survival, tumorigenesis, and even the microenvironment promoting the process of epithelial-mesenchymal transition (EMT) [11, 12]. The mutant BRAF/MAPK pathway is illustrated in **Figure 1**.

It has been demonstrated by Xing et al [13] that methylation of several tumor suppressor genes including tissue inhibitor of metalloproteinase-3 (TIMP3), SLC5A8, death-associated protein kinase(DAPK), and retinoic acid receptor b2 (RARb2) was closely associated with BRAF mutation in PTC. The silencing of these tumor suppressor genes represented an important molecular mechanism in BRAF mutation-induced progression and invasiveness of PTC. Another study showed that PTCs harboring BRAF mutation were significantly associated with the expression of both MMP-2 and MMP-9, which pre-

sented with more frequency of extrathyroidal invasion with respect to those MMP negative PTCs [14]. The process of absorption and accumulation of radioiodine relies on the sodium/iodide symporter (NIS) in the basal membrane that transports iodide into the cells from the blood stream. Impairment of NIS expression and other iodide-metabolizing genes including TPO, TG, and pendrin was found to be associated with BRAF mutation in PTCs. One explanation was the gene-silencing by methylation regulated by BRAF mutation, which resulted in not only the loss of radioiodine resistance but also increased aggressiveness of PTC.

Association of BRAF mutation with clinicopathological characteristics of PTC

BRAF^{V600E} mutation in thyroid cancer has been proved to be associated with high-risk clinicopathological characteristics, tumor recurrence and reduced sensitivity of radioiodine therapy. Conventional factors that demonstrate the high-risk clinicopathological characteristics included increased age, male gender, larger tumor size, extrathyroidal invasion, local lymph node metastasis, distant metastasis, and advanced disease stages. Recent studies have showed that significant correlations of BRAF mutation with the reliable prognostic predictors such as extrathyroidal invasion, lymph nodal metastasis, and advanced TNM stage [15-17]. A meta-analysis revealed that PTC patients harboring BRAF V600E mutation achieved a 1.5- to 2.1-fold increase in extrathyroidal extension, lymph node metastasis, and advanced TNM stages compared with those holding wild-type BRAF [16]. The above aggressive features of PTC were also confirmed to correlate independently with BRAF mutation when general data of patients such as age, gender, and residence and tumor size, multifocality, and histology subtype were adjusted for multivariate logistic regression analysis [14]. However, a number of studies showed conflicting results that demonstrated there was no association between BRAF mutation and poor clinicopathological factors [18, 19]. The number of cases, enrollment criteria, and tumor classification involved in these studies were believed to be the reasons why the results were not consistent and did not reach a statistical significance.

The association of BRAF mutation with PTC recurrence was displayed in majority of the stud-

ies including multivariate analysis with adjustment for all the known prognostic factors, even in patients with early tumor stages I and II [6, 15, 20]. With respect to micro-PTC (diameter no more than 10mm) which usually has a satisfactory prognosis, BRAF mutation may be a more important indicator for high risk of poor clinicopathological features and progression to advanced stage. The relatively low prevalence of BRAF mutation in micro-PTCs may also be explained by that more of them progressed to larger counterparts than wild-type BRAF. Recently, a conflicting outcome was reported in a Finnish cohort of TNM stage I or II PTC patients after 16 years follow-up, suggesting that the BRAF V600E mutation was not associated with the recurrence of PTC after initial treatment with total thyroidectomy and radioiodine remnant ablation [21]. The rather aggressive initial treatment in this study may contribute to the absence of significant recurrence in patients with BRAF mutation. It has also been indicated that BRAF V600E mutation correlated strongly with radioiodine resistance in PTC patients due to the reduced expression of sodiumiodide symporter and lost capacity for iodine uptake [22]. The impairment of iodide metabolism in PTC patients harboring BRAF mutation makes it reasonable for radioiodine ablation therapy with an aggressive dose.

Detection of BRAF^{V600E} mutation as a molecular marker in thyroid cancer

Fine-needle aspiration biopsy (FNAB) as the gold standard for preoperative evaluation of thyroid lesions has recently been used in conjunction with molecular testing to improve the accuracy of diagnosis from cytology. These molecular markers including BRAF, RAS, RET/PTC and PAX8/PPAR γ were proved to be feasible and helpful to increase the diagnostic accuracy for patients with indeterminate thyroid nodules [23, 24]. The indeterminate thyroid nodules accounted for 10-15% of overall outcomes on FNABs and were grouped to three subcategories that are follicular lesions of undetermined significance, follicular and oncocytic neoplasms, and suspicious nodules for malignancy [25].

A number of published papers reported that BRAF mutational analysis improved the sensitivity of biopsy for PTC and the evaluation of BRAF mutation on FNAB specimens was well documented [26]. These studies showed that the

sensitivity, specificity, negative predictive value, and positive predictive value were 15%~84%, 97.3%~100%, and 17.9% ~ 93.6%, and 95.7% ~ 100% respectively. The testing techniques for detection of BRAF mutation included direct DNA sequencing, colorimetric gene detection method, pyrosequencing, PCR-based single-strand conformation polymorphism, and restriction fragment length analysis, and dual-priming oligonucleotide (DPO)-based multiplex PCR analysis, which can detect BRAF mutation in 2% -20% of cells within a wild-type background. The diversity and liability of these testing techniques should contribute to the variability of the outcomes. It has been proposed that preoperative knowledge of BRAF mutation of the thyroid nodules may help surgeons make proper decision regarding the extent of operation, such as subtotal thyroidectomy vs. total thyroidectomy and neck dissection vs. no neck dissection. Appropriate surgical plan before surgery may seem to be possible especially for patients at early stages and reduce the postoperative complications accordingly. Compared with clinicopathological evaluation using traditional indicators, testing of BRAF mutation seems to be more useful to identify the risk of tumor recurrence [15, 27].

Targeting BRAF signaling pathway as therapeutic target to treat thyroid cancer

The BRAF mutation leads to the activation of BRAF kinase and stimulation of MAPK pathway, which is crucial for tumor initiation of PTCs. Many studies demonstrated the role of MAPK signaling pathway in thyroid tumorigenesis and therefore BRAF has become an attractive target [28]. Several molecule inhibitors of BRAF have been developed including sorafenib, PLX4032, RAF265, PLX4720, and XL281 with different selectivity [29]. Indeed, encouraging results with the BRAF inhibitors sorafenib and PLX4032 were recently reported in clinical trials with malignant melanoma which has a high prevalence of BRAF mutations [30, 31]. These drugs showed markedly inhibition of cell proliferation, survival, motility, and invasion in vivo and in vitro. PLX4032, a compound of selectively targeted BRAF V600E, can effectively inhibit the proliferation of PTC cell lines bearing BRAF mutation [32]. However, incomplete inhibition of ERK activation by PLX4032 was also revealed in comparison with the MEK inhibitor PD0325901 by which ERK activation was fully inhibited. The

disabled feedback mechanisms and wild-type BRAF proteins may contribute to the incomplete inhibition of ERK activation. Moreover, recent studies suggested that BRAF activates nuclear factor κ B (NF- κ B) and this pathway is MEK-independent [33, 34].

Although increasing clinical trials using these selective pathway inhibitors have shown promising results in patients with tumors harboring BRAF^{V600E} mutations, acquired resistance to these agents is an emerging problem. The clinical effectiveness and safety of the inhibitors tested was generally limited, raising the question on the effectiveness of inhibiting only the MAPK pathway to target resistant and aggressive tumors with BRAF mutations.

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