

BRAF mutations in papillary thyroid carcinoma and emerging targeted therapies (Review)

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Abstract. Papillary thyroid carcinoma (PTC) is the most common histotype among the thyroid cancer types. Although PTC is a curable malignancy, many patients relapse after treatment. Thus, there is a need to identify novel factors involved in the pathogenesis of PTC that may be used as targets for new therapies. The MAPK pathway has been implicated in the pathogenesis of PTC. Therefore, in this review, we summarize the role of the BRAF V600E mutation in the development and progression of thyroid cancer. The clinical implication of this molecular abnormality is also discussed. It is evident that the detection of the BRAF V600E mutation is crucial in order to identify novel avenues for thyroid cancer treatment.

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1. Introduction

Papillary thyroid carcinoma (PTC) is the most common histotype among thyroid tissue malignancies, accounting for approximately 80% of all thyroid cancers (1). The growing

incidence of thyroid cancer is almost entirely ascribed to PTC (2,3). The epidemiology of PTC has evoked much interest. Dietary iodine deficiency appears to influence the incidence of the disease, and in some cases, the morphology of the papillary carcinomas (4). However, as regards the etiology of PTC, a number of studies have revealed an association between its incidence and development with radiation exposure (5-7).

Differentiated thyroid cancer, including PTC, tends to be biologically indolent, highly curable and has an excellent prognosis. A number of clinicopathological features are considered as high-risk factors in PTC (8,9). These include: old patient age at the time of diagnosis, male gender, large tumor size, extrathyroidal invasion, lymph node metastasis, distant metastasis, and advanced disease stages (8,10-12). All of these clinical characteristics are associated with poor prognosis as they reflect an aggressive tumor behavior with an increased rate of progression and recurrence. Finally, the histological criteria are important to stratify the risk of disease aggressiveness for the patients (13-16). All the cited elements are useful for a more appropriate therapeutic approach of PTC and subsequent follow-up. It has been shown that the accuracy of this clinicopathological criteria-based method can be unreliable for patients with conventionally low clinicopathological stages (11,17). Although PTC is highly curable with standard surgical treatment and radioiodine ablation therapy, a significant recurrence rate (approximately 20% at 10 years and 30% at 30 years of follow-up) is observed after treatment and many patients still succumb to the disease. Additionally, there are no therapeutic options for those patients who develop radioiodine resistance and are not eligible for surgery. Risk stratification is the chief consideration in determining the appropriate management of PTC-affected patients. Therefore, it is important to improve the reliability of this system in order to reduce the recurrence rate as well as the mortality of PTC.

Thyroid cancer carries several highly prevalent genetic alterations, some of which are observed only in this type of cancer. These oncogenic alterations include: Ras mutations (19,20), RET/PTC rearrangements (21,22) and PAX8-peroxisome proliferator-activated receptor γ (PPAR γ) fusion oncogene (23,24). In PTC tumors, RET/PTC rearrangement and point mutations of the BRAF and RAS genes are found in over 70% of papillary carcinomas and they rarely overlap in the same tumor (25).

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The BRAF gene is highly mutated in tumor cells and over 40 different mutations have been identified. The BRAF V600E mutation is the most common and accounts for more than 90% of all the mutations found in the BRAF gene (26). Moreover, this mutation has been found to occur frequently in thyroid cancer (27-32). Intriguingly, the BRAF mutation in thyroid cancer occurs exclusively in PTC and PTC-derived anaplastic thyroid carcinoma (ATC) and it does not occur in follicular thyroid carcinoma (FTC) or other types of thyroid tumors (33). As highlighted in the study by Xing, the prevalence and specificity of the BRAF mutation in PTC cells may be associated with a pathogenic role of this mutation in this tumor. The fine-needle aspiration is a pre-operative approach useful for diagnosis that should be completed by screening for the BRAF V600E mutation. Targeted therapy may prove to be advantageous for PTC patients harboring this molecular aberration (33).

In the present review, the role of BRAF mutations in the development and progression of thyroid cancer and their implications for novel therapeutic strategies are summarized.

2. BRAF V600E mutations

The 'MAPK pathway' [the RET/PTC → Ras → Raf → mitogen extracellular kinase (MEK) → MAPK/ERK pathway] is a fundamental intracellular signaling pathway that plays a central role in cellular functions, such as proliferation, differentiation, apoptosis and survival (34-38). The aberrant activation of the MAPK pathway, through the activation of genetic alterations of its elements, has been observed in many human cancers (39-43) (Fig. 1). The B-type Raf kinase (BRAF) mutation was discovered to be a leading cause of aberrant activation of the MAPK pathway in human cancers (44). There are three Raf kinases, A-Raf, BRAF (BRAF) and C-Raf. Among the three, BRAF is the most potent activator of the MAPK pathway in many cells (37).

The wild-type BRAF gene is localized on chromosome 7 and is composed of 119 base pairs (bp). Most of BRAF mutations are clustered within exons 11 and 15 (44). The T1799A mutation results in a V600E amino acid substitution in the protein product and subsequent constitutive activation of the BRAF kinase and ERK phosphorylation (44-46). Accordingly, dysregulated signaling through the activation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways is often the result of genetic alterations in critical components in these pathways, as well as mutations in upstream growth factor receptors (47). Another BRAF mutation detected in thyroid tumors is the K601E mutation which has been observed in two benign thyroid adenomas (31,48) and three follicular-variant PTCs (49). The mutations in exon 11 of the BRAF gene found in other human cancers have not been found in thyroid cancer (27-30,50-52). The *in vivo* fusion of the BRAF gene with the AKAP9 gene through a paracentric inversion of the long arm of chromosome 7 may cause BRAF activation. This aberration has been observed in PTC patients with a history of radiation exposure (53,54).

3. Effects of BRAF mutation on PTC

Strong molecular bases have now been revealed for BRAF mutation-promoted invasiveness and the progression of PTC. It has been shown that transgenic mice develop PTC after

injection of the BRAF V600E protein in the thyroid gland (55). Previous studies have shown that BRAF V600E promotes the invasion of thyroid cells in a rat cell line model (56). Ouyang *et al* demonstrated that BRAF inhibitors can inhibit the growth and proliferation of cells harboring the BRAF V600E mutation (57). As previously shown, siRNA transfection of PTC cells harboring the BRAF mutation resulted in the persistent suppression of BRAF, sustained the inhibition of cell proliferation, prevented transformation even after long-term culture and inhibited xenograft tumor growth in nude mice (58,59).

As already mentioned above, the activating genetic alterations in the MAPK pathway, including RET/PTC rearrangement, Ras mutation and BRAF mutation, are mutually exclusive in PTC, suggesting that each of these genetic alterations in PTC may be sufficient on their own to drive PTC tumorigenesis (25). Conversely, as shown in a previous study, the expression of RET/PTC did not cause genetic instability that was observed in rat thyroid cells after induction of BRAF V600E (60). Mesa *et al* showed that in rat thyroid cell lines, an increased Matrigel cell invasion was observed with the induced expression of BRAF V600E, but not with RET/PTC rearrangement (56).

Microarray gene expression analyses have displayed different gene expression patterns in cells harboring the BRAF V600E mutation or RET/PTC rearrangement, suggesting a difference in the genes affected by the two genetic alterations and indicating that the BRAF mutant is possibly a stronger activator of the MAPK pathway than RET/PTC (56,61,62).

It has been also demonstrated in PTC that the BRAF mutation is linked with the aberrant methylation of different tumor suppressor genes, including the genes, tissue inhibitor of matrix metalloproteinase-3 (TIMP3), death-associated protein kinase (DAPK), SLC5A8 and retinoic acid receptor β 2 (RAR β 2) (63-66). It is known that TIMP3 suppresses tumor growth, angiogenesis, invasion and metastasis by preventing the interstitial matrix destruction promoted by matrix metalloproteinase (MMP)-3 (67) and by blocking the binding of vascular endothelial growth factor (VEGF) to the VEGF receptor (68). The BRAF mutation has been reported to be associated with alterations in the expression of various micro-RNAs that appear to have tumor-suppressor potential in PTC (69-71). The BRAF V600E mutation in thyroid cell lines is also involved in the upregulation of tumor-promoting molecules, such as MMPs (56,61,72). In the study by Palona *et al*, the authors also showed that BRAF V600E promoted the activation of the nuclear transcription factor κ B (NF- κ B)-coupled signaling, which in turn promoted the Matrigel invasion of thyroid cancer cells (72). Intriguingly, BRAF V600E promoted the activation of NF- κ B through signaling directly from BRAF, independently of the downstream MEK/MAPK/ERK signaling pathway (72-74). Moreover, it has been demonstrated that the overexpression of VEGF is associated with BRAF mutation in PTC (75). Recently, Grabellus *et al* showed that glucose transporter 1 (GLUT1) is a target of the constitutive activation of the RAF/MEK/ERK pathway, hypothesizing that the BRAF mutation in PTC may contribute to the initiation of the glycolytic phenotype and may confer growth advantages in cancer cells (76).

Taken together, all these elements suggest the critical role played by the BRAF mutation in promoting extrathyroidal invasion and metastasis (which involves vigorous angiogenesis

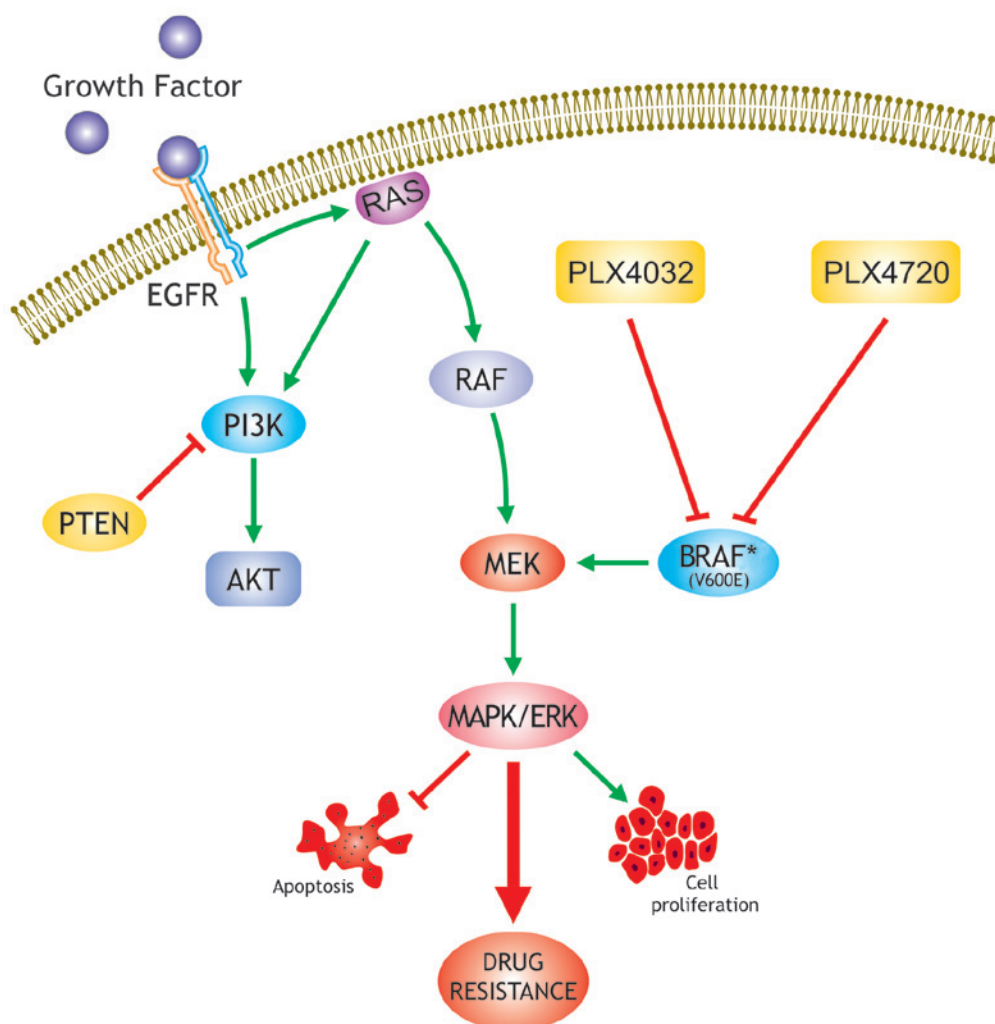


Figure 1. Scheme of the MAPK molecular pathway describing the role of the BRAF V600E mutation in tumor growth and how the BRAF inhibitors, PLX4032 and PLX4720, block cellular proliferation by inducing apoptosis.

and tissue invasion) and the mutation-mediated progression and aggressiveness of PTC.

The efficacy of radioiodine ablation therapy for the treatment of thyroid cancer depends on the ability of the cancer cells to absorb and accumulate radioiodine, which in turn relies on the integrity of the iodide-metabolizing system of the thyroid cell (77). The expression of the thyroid iodide-metabolizing genes is often impaired or lost in thyroid cancer (78,79). Intriguingly, the BRAF mutation has been found to be associated with the decreased expression of thyroperoxidase (62,80-82), sodium/iodide symporter (80,83), thyroglobulin (80) and pendrin (81) in primary or recurrent PTC tumors. The conditional expression of BRAF V600E in rat thyroid cell lines has led to the silencing of all these thyroid-specific iodide-metabolizing genes (60,83,84). The MAPK inhibitors or BRAF siRNA could re-establish the expression of these genes (83,84). Several studies have demonstrated that the thyroid-stimulating hormone (TSH) receptor gene is silenced in a promoter methylation-dependent manner in rat thyroid cell lines (85,86), as well as human thyroid cancer cells (87,88). Xing suggested that the silencing of thyroid-specific genes associated with the BRAF mutation supports the notion

that the BRAF mutation promotes the progression and aggressiveness of PTC (89).

4. Correlation of V600E mutation with PTC pathological features

A positive correlation between the BRAF V600E mutation with the clinicopathological characteristics of PTC, including extrathyroidal invasion, lymph node metastasis and advanced stages has been identified in a number of studies (83,90,93-97, 99,100,102,103,105,107,109-111,113,115,117). Accordingly, the metanalysis performed by Xing in 2007 confirmed such an association, displaying overall odds ratios of 2.50 [95% confidence interval (CI) 2.11-2.97], 1.83 (95% CI 1.58-2.13) and 2.14 (95% CI 1.79-2.56) (89). BRAF mutations have also been observed in 77, 60 and 12% of tall cell PTCs, conventional PTCs and follicular variant PTCs, respectively. These data support the notion that the V600E mutation detected in BRAF is associated with the aggressive variants of PTC (33,110). Furthermore, it has previously been suggested that the BRAF mutation renders PTC prone to progress into the more aggressive ATC (105,118-120).

As considered above, the association between BRAF mutation and the conventional high-risk clinicopathological factors in PTCs is supported by several studies; however, other studies have failed to reveal such an association (54,75,80,90,92,98,101,104,106, 108,112,114,115,121).

Conflicting data have also been generated regarding the correlation between BRAF mutations, old age, male gender, thyroid cancer progression and aggressiveness (32,91,92,95,96,100,105,107,109,111,114,115,117,122). This controversy may be supported by different hypotheses that have been properly considered by Xing in 2007, such as different diagnostic criteria and the small number of cases analyzed in some published studies (89).

5. Detection of V600E mutation in PTC recurrence

The association between BRAF mutation and PTC recurrence has been explored in several studies. Kim *et al* demonstrated a close association between the BRAF mutation and tumor recurrence in 203 patients with conventional PTC (99). A strong association between the BRAF mutation and the recurrence of PTC has also been confirmed by Riesco-Eizaguirre *et al* (83). In a large study by Xing *et al*, an odds ratio of 4.0 (95% CI 1.1-14.1; $P=0.03$) for cancer recurrence with the BRAF mutation was obtained by multivariate analysis with adjustment for all the confounding clinicopathological factors, including tumor subtypes and a history of radioiodine treatment. Interestingly, such an association was even established in a subgroup of patients with low-grade initial clinicopathological stages I and II, usually linked with a low risk of recurrence (110). Despite this evidence however, in 2011, Cañadas Garre *et al* found no association between BRAF mutation and the recurrence of PTC (114).

The core of the current medical treatment of PTC following thyroidectomy is radioiodine ablation therapy. The medical treatment of recurrent PTC is also largely confined to radioiodine therapy (8,9,123). Although various clinicopathological factors are known to predict an increased risk of recurrence of thyroid cancer, no factors have been associated with the loss of radioiodine avidity, mainly responsible for treatment failure. On this regard, Xing *et al* showed that patients harboring BRAF mutations in the primary PTC tumor developed a recurrence with an aggressive course of disease, to be treated with surgery and external radiation therapy. However, PTC patients with recurrent tumors that did not display any BRAF mutation in the primary tumor may be treated with radioiodine therapy, suggesting a better clinical behavior (110). The association between BRAF mutation in primary or recurrent PTC and the loss of radioiodine avidity in the recurrent tumor, was observed in two studies; however, such an association did not reach statistical significance (81,83).

6. Conclusion

From both the clinicopathological and molecular biological evaluations, it is evident that BRAF mutations are fundamentally associated with the increased progression and aggressiveness of PTC. Several studies have shown that BRAF mutations may be considered as powerful markers for PTC recurrence risk (83,95,99,110). BRAF mutations can be easily detected in tumor DNA from thyroid fine-needle aspiration

biopsy (FNAB). Surgical and medical treatments can be better applied if the BRAF mutation status is determined from thyroid FNAB (33,94,124-128).

It is evident that the MAPK pathway plays a major role in tumorigenesis and the progression of PTC and may be consequently considered as a potentially effective therapeutic target for PTC.

In recent years, the inhibition of MAPK pathway has been investigated in human cancers, generating interesting results (reviewed in refs. 34-38,42). The silencing of the MAPK pathway by Raf kinase inhibitors, such as AAL-881 and LBT-613, has been shown to result in the inhibition of BRAF-mutated thyroid cancer cell proliferation (57-59). Moreover, Liu *et al* showed that through a stable siRNA knockdown of BRAF, thyroid cells were able to differentiate as reflected by the restoration of the expression of some thyroid-specific genes (84).

The results from an advanced human thyroid cancer preclinical study suggest that PLX4720, an orally available BRAF V600E inhibitor, may be used in clinical trials for the treatment of patients with BRAF V600E-positive thyroid cancers, refractory to conventional therapy (129-131). MEK inhibitors are another class of highly promising therapeutic agents for thyroid cancer. Liu *et al* showed that CI-1040, a potent small molecule MEK-selective inhibitor, which inhibits both MEK-1 and MEK-2, inhibited the growth and proliferation of thyroid cancer cells harboring mutant BRAF but not wild-type BRAF. The study also demonstrated that the CI-1040 compound preferentially induced the differentiation of some thyroid cancer cells that harbored the BRAF mutation (132). Namba *et al* showed that another MEK-inhibitor, U0126, inhibited cell proliferation in thyroid cancer cells harboring the BRAF mutation (30) and these findings were confirmed in the study by Henderson *et al* (133). This chemically synthesized compound, however, cannot be developed clinically due to its limited solubility and bioavailability (134). More potent and pharmaceutically superior second-generation MEK inhibitors, such as the PD-0325901 compound and the ARRY-142886 (AZD6244) compound (134,135), are currently under development and are being used in clinical trials. Several clinical trials with BRAF inhibitors and MEK inhibitors are currently under evaluation, presenting a new perspective in PTC treatment. Therefore, it is evident that the detection of the BRAF V600E mutation is crucial in order to identify novel avenues for thyroid cancer treatment.

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