

B-Raf mutation and papillary thyroid carcinoma patients (Review)

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Abstract. Thyroid carcinoma is the most prevalent endocrine neoplasm globally. In the majority of thyroid carcinoma cases, a positive prognosis is predicted following administration of the appropriate treatment. A wide range of genetic alterations present in thyroid carcinoma exert their oncogenic actions partially through the activation of the mitogen-activated protein kinase pathway, with the B-Raf mutation in particular being focused on by experts for decades. The B-Raf gene has numerous mutations, however, V600E presents with the highest frequency. It is believed that the existence of the V600E mutation may demonstrate an association with the clinicopathological characteristics of patients, however, inconsistencies remain in the literature. A number of explanatory theories have been presented in order to resolve these discrepancies. Recently, it has been suggested that the V600E mutation may function as a target in a novel approach that may aid the diagnosis and prognosis of thyroid carcinoma, with a number of vying methods put forward to that effect. The current review aims to assist researchers in further understanding the possible association between B-Raf mutations and thyroid carcinoma.

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1. Introduction

Thyroid cancer accounts for 1% of all epithelial malignancies worldwide, and it is currently understood to be the most frequently occurring endocrine neoplasm (1). The incidence of the disease continues to rise, with papillary thyroid cancer (PTC) being the most prevalent histological subtype of thyroid malignancy (2). It is currently unknown as to whether the increase in PTC occurrence is a valid result or a false-positive result possibly occurring due to improved diagnostic techniques and other procedures, or due to increased screening methods for small nodules (3,4).

The majority of thyroid cancers have a positive prognosis following administration of the appropriate treatment. Such treatments may include surgery, and adjuvant radioactive iodine and thyroid-stimulating hormone (TSH) suppression therapy. However, in the literature, following initial treatment, the recurrence rate of differentiated thyroid cancer increased to 30% and the mortality rate was 8% at 30 years of follow-up (5,6).

The Ras/Raf/mitogen-activated protein kinase (MAPK) kinase/MAPK/extracellular signal-regulated kinase (Ras/Raf/MEK/MAPK/ERK) signaling pathway serves a fundamental role in cell cycling, proliferation and survival, and if there is constitutive activation of the MAPK/ERK pathway, it functions in the upregulation of cell division and proliferation, and the subsequent tumorigenesis (7-9). The MAPK/ERK pathway is an important mechanism in the initiation and progression of human cancer, with mutagenic variations of Ras, B-Raf and rearranged during transfection (RET)/PTC all reported to be associated with papillary thyroid tumorigenesis (9).

2. B-Raf and B-Raf^{V600E} mutation

B-Raf belongs to the Raf kinase family, with a specific mutation, V600E, typically resulting in the constitutive activation of the MAPK/ERK pathway and the subsequent development of PTC. V600E mutation, however, has not yet been identified in other histological subtypes of thyroid cancer, including medullary thyroid cancer and follicular thyroid cancer (10-13).

Mutations of B-Raf, a serine-threonine kinase and downstream signaling molecule of Ras and RET, are potent activators of the MAPK/ERK pathway (9,14). B-Raf somatic mutations have previously been reported in a broad range of human cancers, with the highest prevalence observed in melanoma

and thyroid cancer (14). One mutation in particular, located on chromosome 7, occurs in the kinase domain of B-Raf and is a T1799A transversion mutation, which results in a single amino acid substitution of valine to glutamic acid (V600E). This V600E mutation accounts for 25-85% of B-Raf activating mutations, dependent on the patient population and histological subtype (15). The B-Raf^{V600E} mutation potentially increases the kinase activity of B-Raf by evoking the phosphorylation of ERK1/2 at 480-fold higher than the B-Raf wild-type (16). This mutant stimulates constitutive signaling, which may then bypass the requirement for extracellular growth factors. The phosphorylation of downstream MEK1/2 and ERK1/2 results in the expression of a number of genes that are involved in cell proliferation, differentiation, survival, tumorigenesis and the promotion of epithelial-mesenchymal transition (17,18).

The results of the study by Schweppe *et al* (19), amongst others, are consistent with the possibility that the B-Raf^{V600E} mutant, which lies downstream from Ras and RET/PTC, serves a more significant function than RET/PTC and Ras mutants in the activation of the MAPK signaling pathway (19).

3. Association of B-Raf mutation with clinicopathological PTC characteristics

A previous study reported that in comparison with Ras or RET/PTC mutations, the B-Raf^{V600E} mutation was frequently more potent in promoting aggressive pathogenesis and poorer clinicopathological outcomes in patients with PTC (20).

In thyroid cancer, the B-Raf^{V600E} mutation has been demonstrated to be associated with tumor recurrence, high-risk clinicopathological characteristics and reduced sensitivity to radioiodine therapy. Conventional factors that indicated high-risk clinicopathological characteristics included the male gender, an increased age, a larger tumor size, the presence of extrathyroidal invasion, local lymph node metastasis, distant metastasis and advanced disease stages (21). Studies have demonstrated a significant correlation between the B-Raf^{V600E} mutation and reliable prognostic predictors, including extrathyroidal invasion, lymph nodal metastasis and an advanced tumor-node-metastasis (TNM) stage (22-24). Lupi *et al* (23) reported that PTC patients who presented with a B-Raf^{V600E} mutation achieved a 1.5 to 2.1-fold increase in extrathyroidal extension, lymph node metastasis and advanced TNM stages when compared with those who exhibited wild-type B-Raf. Frasca *et al* (25) observed that the aggressive features of PTC correlated independently with the B-Raf^{V600E} mutation when the general data of patients, including gender, age, tumor size, residence, multifocality and histological subtype, were selected for multivariate logistic regression analysis. There is partial agreement over the aforementioned conclusions (26), however, conflicting results have also been reported, stating that no significant association was observed between the B-Raf^{V600E} mutation and poorer clinicopathological factors (27,28). Despite this, in a Finish cohort of TNM stage I or II PTC patients following a 16-year follow-up, the presentation of a similar outcome suggested that there was no association between B-Raf^{V600E} mutation and PTC recurrence following primary treatment with total thyroidectomy and radioiodine remnant ablation (29). However, the question as to why there are so many differing arguments remains. It

has been suggested that the number of cases, the enrollment criteria and the tumor classification involved in these studies may be the reason (30). In B-Raf^{V600E} mutation research, it has been reported that B-Raf overexpression or downregulation is a protective event. B-Raf expression serves an important role in benign and malignant thyroid disease, by delaying their development and progression in the absence of activating mutations by at least 10 years (31).

Regarding PTC with a diameter <10 mm (PTMC), typically exhibiting a satisfactory prognosis, a B-Raf mutation is suggested to be more likely to manifest with aggressive clinicopathological characteristics. This may be useful in the evaluation and estimation of the risk stratification and management of PTMC (32). It has also been observed that the B-Raf^{V600E} mutation strongly correlates with radioiodine resistance in patients with PTC due to the reduced expression of sodium iodide symporter (NIS) and TSH receptor (TSHR), which results in a reduced capacity for iodine uptake (33).

Subsequently, several alternative published risk factors for the occurrence of thyroid cancer have included radiation exposure, diets low in iodine and certain hereditary conditions, such as multiple endocrine neoplasia type 2A (MEN-2A), MEN-2B and familial medullary thyroid carcinoma (34).

4. BRAF mutation in association with other factors

Shimamura *et al* concluded that B-Raf^{V600E} itself may not be sufficient for PTC development. This, however, does not mean that B-Raf^{V600E} is not the driver mutation, but rather that additional genetic and/or epigenetic changes may be required for full transformation (35). Several other studies also agreed with this theory, stating that the increased expression of several tumor-promoting molecules, including vimentin (36), matrix metalloproteinase (25,37-39), nuclear factor- κ B (39), Ki-67 (40), prohibitin (41), vascular endothelial growth factor (42) and hepatocyte growth factor receptor (43), is associated with BRAF mutations in carcinoma.

In one previous study, hypermethylation was observed to occur in 33% (76/231) of PTCs, and was also linked with multifocality (40%) and extrathyroidal invasion (40%) (44). It is believed that hypermethylation occurs during the later stages of PTC and is specific to the classical variant; thus, this epigenetic event may be secondary to alternative genetic modifications, and be dependent on tumor type (45). Furthermore, a strong association has been observed between low solute carrier family 5 member 8 (SLC5A8) expression and the presence of the B-Raf^{V600E} mutation (45,46) or advanced clinicopathological features (44), suggesting that there is an association with the progression to aggressive PTC.

Huetal(44)demonstratedthatmethylationofseveraltumor suppressor genes, including SLC5A8, retinoic acid receptor b2, tissue inhibitor of metalloproteinase-3 and death-associated protein kinase, were closely associated with B-Raf mutation in PTC. The inhibition of these particular tumor suppressor genes represented an important molecular mechanism of the B-Raf mutation-induced invasiveness and progression of PTC (44), including lymph node metastasis, extrathyroidal invasion and an advanced tumor stage at diagnosis, and their epigenetic silencing may be an important mechanism by which B-Raf mutation promotes aggressive progression (47,48).

NIS is located in the basal membrane, functioning to absorb and accumulate radioiodine in the cells from the blood stream. The B-Raf mutation in PTC has been frequently associated with the aberrant silencing or decreased expression of thyroid iodide-handling genes, including the genes for NIS thyroid peroxidase, pendrin, thyroglobulin and TSHR (43,49-51).

5. Detection of B-Raf^{V600E} as a powerful marker in PTC

Thyroid nodules are common, and with the increasing use of diagnostic imaging, the number of thyroid nodules that are detected is growing, as is the number that require further diagnostic evaluation via fine-needle aspiration biopsy (FNAB). Diagnostic imaging is an office-based, straightforward, safe and sensitive procedure that represents an accepted standard of practice (52).

Only 5% of all thyroid nodules are malignant; therefore, the presurgical determination as to whether nodules are benign or malignant is imperative (52). In the aspirate test of nodules following FNAB, 60-70% are considered benign and 5% are considered malignant, whilst 10-30% are considered as uncertain or suspicious. The indeterminate thyroid nodules on FNABs are grouped into three subcategories: Follicular lesions of undetermined significance, follicular and oncocytic neoplasms, and suspicious nodules for malignancy (53). However, this presents its own limitation. In practice, the use of FNAB cytopathological categorization of indeterminate nodules is required as a definitive diagnosis of malignancy; it necessitates a morphological finding of vascular and/or capsular invasion by the tumor that is solely identifiable in a resected thyroid sample (52).

Recently, FNAB has been utilized in conjunction with molecular testing to improve the accuracy of cytological diagnosis. The current challenge is to develop a highly accurate diagnostic test for early-stage thyroid cancer, and also to produce effective molecular-directed therapies for advanced thyroid cancer. The early detection of cancer, prior to the occurrence of metastasis, is crucial for patients and clinicians, as it improves prognosis and patient quality of life, and may provide additional treatment options. One of the most promising tools for the early detection of cancer are biomarkers (54). Tumor markers may include functional subgroups of proteins (glycoproteins, enzymes and receptors), hormones, molecular markers (genetic mutations, translocations or amplifications) (55), and epigenetic markers (tumor suppressor gene hypermethylation) (56). Beyond their screening and diagnostic value, biomarkers may also be utilized for the estimation of tumor volume, the evaluation of response to treatment or as prognostic indicators of disease progression. Biomarkers therefore represent a non-invasive approach that may provide important information during the diagnosis and follow up of PTC (55,57-59). Among the nodules suspected of being cancerous, 20-25% eventually exhibit thyroid cancer following surgery, and therefore, 75-80% of patients in this subgroup undergo a redundant thyroidectomy (53,60-62). Following extensive research, particularly in genetic alterations, a number of biomarkers have been used to improve diagnostic accuracy in the cases of undetermined or suspected cytological tests, and mutation detection for B-Raf, RAS, RET/PTC and PAX8/PPAR γ mutations in clinical

FNAB samples from thyroid nodules may contribute to the cancer diagnosis (63). B-Raf may also be utilized as a tool for the diagnosis and follow-up of PTC. Pupilli *et al* (64) observed that patients with a histopathological diagnosis of PTC demonstrated a higher percentage of circulating B-Raf^{V600E}, as cell-free DNA, compared to those with benign tumors.

Techniques that are utilized for the detection of B-Raf^{V600E} mutation include the colorimetric gene detection method, direct DNA sequencing, PCR-based single-strand conformation polymorphism, pyrosequencing, immunohistochemical detection, dual-priming oligonucleotide (DPO)-based multiplex PCR analysis and restriction fragment length analysis, which identifies B-Raf mutations in 2-20% of cells with a wild-type background (30). The liability and diversity of such techniques may contribute to the variability of the outcomes. It has been suggested that the pre-operative acquaintance of B-Raf mutations of the thyroid nodules may aid surgical decisions regarding the extent of surgery required, i.e., a subtotal or total thyroidectomy, and whether neck dissection is necessary. An appropriate surgical plan prior to surgery appears to be possible, particularly for patients presenting with an early stage of thyroid carcinoma, and may subsequently reduce any post-operative complications. The testing of B-Raf mutations has been identified as an independent predictor of treatment failure, and may also be used to estimate the risk of tumor recurrence (22,65,66).

6. Treatments for PTC patients with B-Raf mutation

Based on the evidence that B-Raf is involved in the development of papillary carcinoma, and also the progression to anaplastic cancer, B-Raf is an appealing target in thyroid cancer. B-Raf^{V600E} has been observed to induce thyroid cancer *in vivo* and thyroid cell transformation *in vitro*, verifying that this mutation functions as an oncogene for thyroid cancer (67,68).

Since the B-Raf^{V600E} mutation is highly prevalent in PTC, particularly in aggressive subtypes (9), recurrent PTC (69) and radioiodine-refractory PTC (22,33,51,70), B-Raf mutation-directed targeting of the MAPK pathway using MEK and Raf inhibitors will be likely to have wide applicability for thyroid cancer (71).

Numerous studies have demonstrated the role of the MAPK signaling pathway in thyroid tumorigenesis, and therefore, B-Raf has become an inviting target (72). Several molecule inhibitors of B-Raf have been developed, including sorafenib, PLX4032, PLX4720, XL281 and RAF265, each with varying selectivity (73). Encouraging results regarding the B-Raf inhibitors PLX4032 and sorafenib have been reported in clinical trials treating malignant melanoma, a disease that has a high frequency of B-Raf mutations (74,75). These drugs exhibit a marked inhibition of cell proliferation, motility, survival and invasion *in vitro* and *in vivo* (76,77).

Potent B-Raf^{V600E} specific inhibitors are currently available as a novel treatment against melanoma (78). Therapeutic drugs that have been thoroughly tested in various clinical trials and have been approved by the Food and Drug Administration are dabrafenib, vemurafenib and trametinib, all proving to be highly specific for the B-Raf kinase, particularly for the V600E mutant. During phase III clinical trials comparing

vemurafenib/dabrafenib and dacarbazine in the treatment of V600E mutant melanomas, each drug demonstrated significant activity in terms of response rate, however, the survival benefits were limited by the rapid acquisition of resistance (79,80). It is likely that B-Raf mutation-targeted treatment using such inhibitors, similar to MEK inhibitors, will be particularly effective for thyroid cancer. However, it has been reported that B-Raf mutation-directed therapy lacks the ability to induce the significant apoptosis of thyroid cancer cells (81-84). Consistent with this, Schweppe *et al* (19) observed that MEK inhibitors inhibited cell proliferation, but not apoptosis. Analogous to MEK inhibitors, these types of inhibitors are also inclined to have a similar limitation, namely a lack of pro-apoptotic effects. Cancer cells are arrested only at the G₀/G₁ stage of the cell cycle if they do not undergo apoptosis, and tumor growth will persist once the administration of the inhibitors has ceased (85).

Despite a lack of additional supporting studies, it was observed that 38% of B-Raf-positive PTCs demonstrated RET/PTC rearrangement, contrary to previous reports stating that the B-Raf^{V600E} mutation does not occur alongside RET/PTC or Ras mutations in cancer. Concurrent RET/PTC and B-Raf mutations have also been reported in PTC. This phenomenon suggests that mutations occurring in the joint pathway of B-Raf and RET/PTC1 may cooperate in PTC development (86,87).

Schweppe *et al* (19) demonstrated that MEK inhibitors only inhibited cell proliferation and not apoptosis. Such results are consistent with the possibility that the targeting of a limited number of pathways, including the MAPK pathway, may not be sufficient enough to induce apoptosis, based on evidence from several studies (81-84). Therefore, given the frequent disturbance in multiple major signaling pathways in thyroid tumorigenesis, this may be an effective therapeutic strategy for thyroid cancer, as previously proposed (20).

Despite an increasing number of clinical trials with selective pathway inhibitors demonstrating promising results in patients with B-Raf^{V600E} mutations, acquired resistance to such agents is an emerging problem. It is clear that primary or acquired resistance to B-Raf inhibitors is a clinical problem. Based on previous study data, acquired resistance to B-Raf inhibitors in melanoma has been reported to occur through a number of different mechanisms, resulting in reactivation of the MAPK pathway and/or upregulation of the phosphoinositide 3-kinase pathway (88). The current mechanistic explanations are as follows: i) Intratumoral heterogeneity in the B-Raf genotype, with wild-type and mutant B-Raf within the same tumor; ii) a tumor microenvironment in which stromal cell secretion of hepatocyte growth factor (HGF) results in the activation of the HGF receptor, reactivation of the MAPK and phosphoinositide 3-kinase-AKT signalling pathways and resistance to B-Raf inhibition; iii) the upregulation of specific tyrosine kinase receptors, which has been demonstrated to be a mechanism of primary and acquired resistance to B-Raf inhibitors; and iv) rapid upregulation and activation of the epidermal growth factor receptor caused by B-Raf inhibition (89-92).

The clinical effectiveness and safety of the inhibitors tested is typically limited, raising the question of the effectiveness of inhibiting only the MAPK pathway to target resistant and aggressive tumors presenting with B-Raf mutations (21).

Therefore, novel concepts are being analyzed, including the combined treatment with dabrafenib and the MEK inhibitor trametinib, delaying the emergence of resistance and resulting in more positive outcomes (93).

There are a number of uncertainties that require further study. Firstly, the association between B-Raf mutations and PTC clinical clinicopathological characteristics does not produce a clear correlation. Secondly, the use of B-Raf inhibitors is a specific treatment for patients presenting with mutations, particularly those with incurable thyroid cancer; however, when and how to use two or more B-Raf inhibitors simultaneously, and the possibility of primary or acquired resistance, are difficult tasks to overcome. Thirdly, the mechanisms of how B-Raf interacts with other signal networks requires further investigation. Finally, how to apply testing for B-Raf mutations in clinical settings, and how to use this as a guideline for surgeons to draft personal treatment strategies are questions that require prolonged future investigation and discussion.

The identification of B-Raf mutations, particularly V600E, is a promising revolution for the research into human cancer. The identification of associations between mutations and cancer may be taken advantage of in order to aid the investigation of thyroid cancer tumorigenesis and possibly the development of appropriate therapies in the future.

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