CLINICAL ASPECTS OF *BRAF* MUTATION IN THYROID CANCER

Mingzhao Xing

Laboratory for Cellular and Molecular Thyroid Research, Division of Endocrinology and Metabolism, the Johns Hopkins University School of Medicine, Baltimore, MD 21287

Concise Review invited by Sheue-Yann Cheng; Reviewing Editor: Luca Persani

The author received payment as a co-holder of the licensed patent for the discovery of BRAF mutation in thyroid cancer.

Correspondence to: Michael Mingzhao Xing, M.D., Ph.D. - Division of Endocrinology & Metabolism, Johns Hopkins University School of Medicine, 1830 E. Monument Street/ Suite 333 - Baltimore, MD 21287, USA.
Email: mxing1@jhmi.edu; Tel: 410-955-3663; Fax: 410-955-8172

ABSTRACT

The *BRAF*^{T1799A} mutation has been extensively studied in recent years in thyroid cancer, particularly for its aggressive role and clinical potential in papillary thyroid cancer (PTC). This mutation is the most common known genetic alteration in PTC, seen in 45% of cases on average, with a lower prevalence in low-stage disease. The mutation causes a change in amino acid at position 600 from valine to glutamic acid in the BRAF protein kinase, constitutively activating it and resulting in aberrant activation of the MAP kinase signaling pathway. Most of the studies have shown a strong association of *BRAF*^{T1799A} mutation with aggressive clinicopathological outcomes of PTC, including tumor extra-thyroidal extension, lymph node metastasis, advanced TNM stages, loss of radioiodine avidity, disease persistence/recurrence, and even patient death. The aggressive role of *BRAF*^{T1799A} mutation has also been demonstrated in low-stage and micro-PTC. This mutation is therefore generally viewed as a strong prognostic molecular marker for poorer prognosis of PTC. The *BRAF*^{T1799A} mutation can be tested for on thyroid fine needle aspiration biopsy specimens to assist diagnostic evaluation of thyroid nodules and preoperative risk stratification of PTC. Information from such testing can be helpful in guiding early decision making on surgical and medical treatments of thyroid nodules and PTC. The BRAF^{V600E} mutant has also been demonstrated to be an effective therapeutic target in thyroid cancer. Use of *BRAF* mutation as a diagnostic and prognostic molecular marker as well as a therapeutic target has shown an increasing impact on our current management of thyroid cancer.

Key Words: *BRAF* mutation, thyroid cancer, molecular marker, diagnostic marker, prognostic marker, therapeutic target.

Introduction

As the most common endocrine malignancy, thyroid cancer has caught considerable attention globally. An important, and often controversial, issue related to this cancer is how to optimally manage it. Giving the rapidly rising incidence of thyroid cancer in recent years (1-3), this issue becomes even more challenging. The controversy starts at the initial evaluation of thyroid nodule, the clinical "precursor" of thyroid cancer, where diagnostic and therapeutic dilemmas are a subject of frequent debate (4,5). It continues at surgical decision making on how to optimize the type and extent of surgery and subsequently at the medical decision making on how to optimize postoperative treatments, such as radioiodine ablation and follow-up surveillance. The fundamental goal in making these efforts in the management of thyroid cancer, from which controversies can arise, is to optimally balance the benefit against the harm of treatments. To this end, clinicopathological criteria are currently the main, and often the only, "gold standard" that is universally used for risk stratification and guidance of the management of thyroid cancer. This practice has been improved significantly in recent years by better understating of clinicopathological behaviors of thyroid cancers from numerous recent studies and the increasing use of certain imaging modalities, such as ultrasonography. Expert opinion- and evidence-based practice guidelines have also been greatly helpful in optimizing the management of thyroid cancer as exemplified by the stellar practice guidelines from the European Thyroid Association (6) and American Thyroid Association (7).

Nevertheless, there is still great room for further improvement in the efficiency and efficiency of today's practice of thyroid cancer medicine, particularly in those on-going controversial areas. It is unlikely that this can happen only based on optimizing the use of classical clinicopathological criteria. New promises lie in molecular medicine. Specifically, thyroid cancer molecular markers may have the best potential in helping improve the diagnostic, prognostic, and therapeutic efficiencies for thyroid cancer. In this regard, the widely investigated $BRAF^{T1799A}$ mutation, as a unique diagnostic and prognostic genetic marker and effective therapeutic target for thyroid cancer, has shown great promises. Its clinical potential has recently drawn considerable attention from clinicians around the world who manage patients with thyroid cancer. Summarized here are several major clinical aspects of $BRAF^{T1799A}$ mutation in thyroid cancer, which reflects how this marker can be practically useful in the management of this cancer.

BRAF Mutation in Thyroid Cancer

BRAF is one, and often the most potent one, of the three Raf kinases (A, B, C) that relay the signaling of the Ras → Raf → MEK→ MAP kinase/ERK pathway (MAPK pathway) (8,9). The diagram in Figure 1 illustrates this pathway. Numerous mutations have been discovered in the *BRAF* gene in human cancers, but the most common one is the $BRAF^{T1799A}$ point mutation that causes an amino acid change in codon 600 from valine to glutamic acid in BRAF. The resultant $BRAF^{V600E}$ is a

Online ISSN: 2075-2202

constitutively activated serine and threonine protein kinase, causing oncogenic over-activation of the MAPK pathway (10).

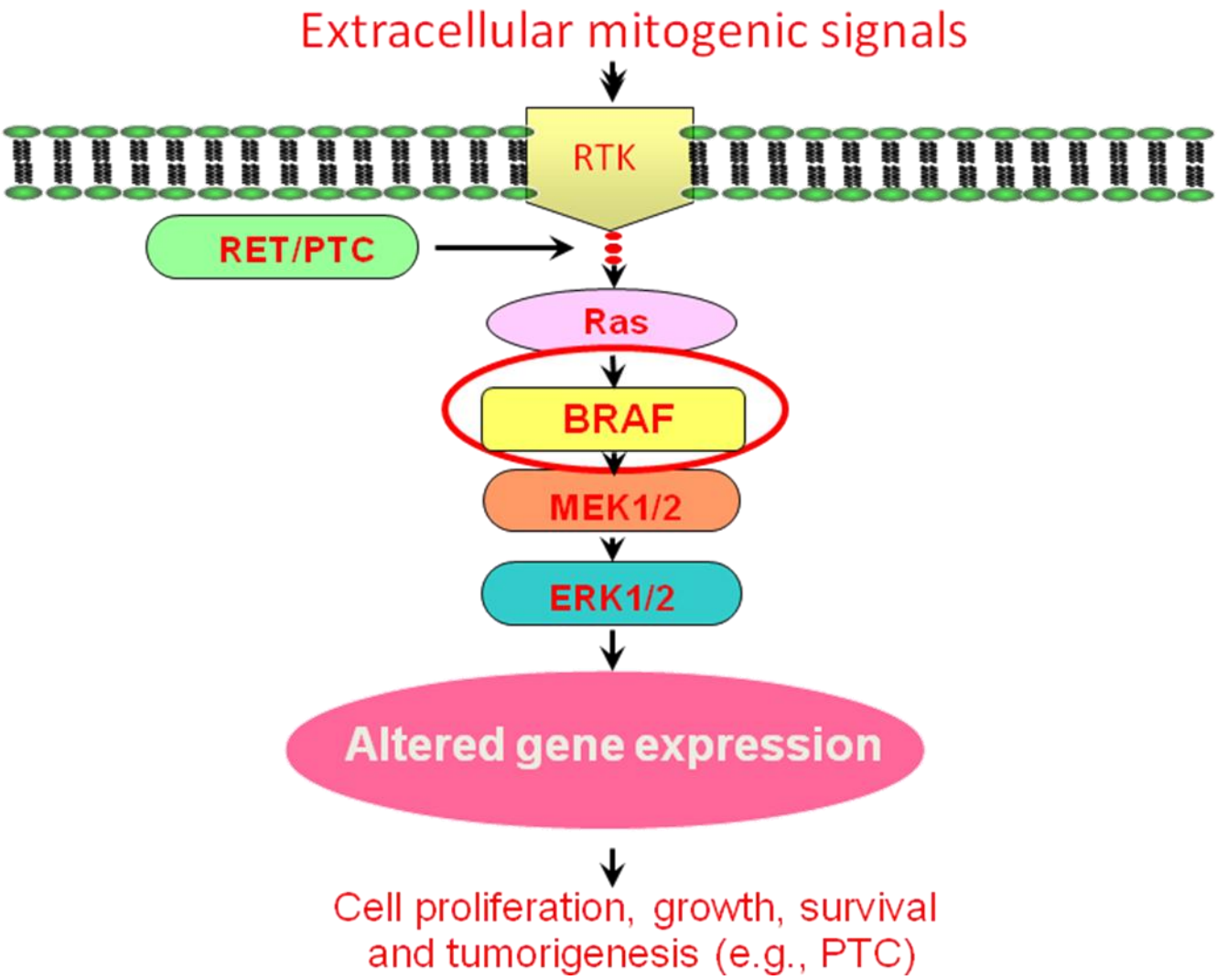

Figure 1. Illustration of the MAP kinase signalling pathway. *This figure is adapted from reference 13. RTK, receptor tyrosine kinase.*

This is now a well-known mechanism involved in the tumorigenesis of papillary thyroid cancer (PTC). Since the initial submission and acceptance of the first manuscript of a Johns Hopkins group on the discovery of the *BRAF*^{T1799A} mutation in thyroid cancer seven years ago (11), numerous studies have been published on this mutation in thyroid cancer. Among different thyroid tumors, the *BRAF*^{T1799A} mutation occurs exclusively in PTC and what appears to be PTC-derived anaplastic thyroid cancer (ATC), in 45% of the cases of the former and 25% the latter on average (12). The prevalence of *BRAF*^{T1799A} mutation in small thyroid cancers or cancers with low stages, such as TNM stages I and II, was generally lower, with a prevalence of about 35% (13). No *BRAF*^{T1799A} mutation has been found in benign thyroid tumors and follicular thyroid cancer. A Greek study reported a high prevalence of

BRAF^{T1799A} mutation in medullary thyroid cancer (14); no other studies demonstrated the presence of this mutation in this type of thyroid cancer. Although several other types of *BRAF* mutation have been discovered in PTC, they are uncommon and the *BRAF*^{T1799A} mutation is most important in the tumorigenesis of PTC (15-17). The clinical utility of *BRAF* mutation in PTC has therefore been investigated virtually exclusively for the *BRAF*^{T1799A} mutation, which, for simplicity, is termed *BRAF* mutation hereafter in this review.

Diagnostic Value of *BRAF* Mutation for Thyroid Cancer

Many investigators have been interested in the diagnostic value of *BRAF* mutation testing on fine needle aspiration biopsy (FNAB) specimens in the evaluation of thyroid nodules. As can be expected, the specificity of *BRAF* mutation as a diagnostic marker on FNAB specimens for PTC was exceptional as it uniformly achieved 100% in several early studies (12). However, the diagnostic sensitivity of *BRAF* mutation was poor when used in the general evaluation of thyroid nodules. Although about 45% of PTC harbored this mutation, only a few percent of thyroid nodules in general are PTC. Therefore, if applied to the general diagnostic evaluation of thyroid nodules, very few percents of thyroid nodule patients may be found to be positive for this mutation, giving a low diagnostic sensitivity. This diagnostic sensitivity was increased to around 8% on FNAB specimens that fall into the classical indeterminate category (12). This is still a low sensitivity that is unlikely to be generally applicable, if used alone, in most areas of the world where the *BRAF* mutation rate is around 45%. However, the diagnostic sensitivity of *BRAF* mutation was significantly increased when used in combination with cytological characteristics of a thyroid nodule, particularly when suspicious for PTC (18,19).

Vitale's group demonstrated that when combined with galectin-3, *BRAF* mutation detected on FNAB specimens showed a significantly increased diagnostic sensitivity (20). Nikiforov's and Pacini's groups have recently tested the diagnostic value of combinational use of *BRAF* mutation with *Ras* mutations, *RET/PTC* rearrangement, and *PPARγ/Pax8* rearrangement in assisting the evaluation of thyroid nodules and reported an increased diagnostic sensitivity (21,22). It remains to be investigated how these strategies could alter the current practice. Unlike *BRAF* mutation, which is a cancer-specific marker, the other three genetic alterations have been widely found also in benign thyroid conditions, including adenomas and even Hashimoto's thyroiditis, with relatively high prevalences (23). Therefore, the diagnostic specificity of using *BRAF* mutation in combination with these additional mutation markers could be potentially problematic although its diagnostic sensitivity is increased compared with the use of *BRAF* mutation alone. Good diagnostic utility of a molecular marker combination approach will likely rely on the discovery of new molecular markers that, like the *BRAF* mutation, are highly specific for thyroid cancer.

It should be noted that the diagnostic utility of testing for *BRAF* mutation alone on FNAB may have a special clinical place in regions that have a high prevalence of *BRAF* mutation, such as South

Korea, where PTC accounts for >90% of thyroid cancers and *BRAF* mutation is exceedingly prevalent, up to >80% in some series (24-26). The diagnostic sensitivity of *BRAF* mutation on FNAB in Korea is therefore exceedingly high, unlike most of the regions in the world where a modest prevalence of *BRAF* mutation in PTC has been generally reported (12,13). Aside from the diagnostic value, testing for *BRAF* mutation on FNAB has a special and more important clinical place for its high prognostic value in preoperative risk stratification of thyroid cancer as will be further discussed.

Prognostic Value of *BRAF* Mutation in Papillary Thyroid Cancer

There are few molecular markers that have been so extensively studied as *BRAF* mutation for its prognostic value. Previous meta analyses on a large number of studies clearly showed an association of *BRAF* mutation with high-risk clinic-pathological characteristics of PTC, including extrathyroidal extension, lymph node metastasis, and advanced TNM stages III and IV (13, 27). There have been also a few individual studies that failed to show significant association of *BRAF* mutation with high risk pathological characteristics (28, 29). Of these studies, the one by Fugazzola et al was a pooled multicenter analysis from Italy that failed to show a significant prognostic role of BRAF mutation (29). However, many other Italian investigators, including some co-authors of the Fugazzola et al paper, later were able to show a significant prognostic role of *BRAF* mutation for poor clinico-pathological outcomes of PTC when examining individual series from their institutions (13, 30, 31, 35). The reason for this discrepancy is not clear. Possible variation in pathological characterization of tumors and extent of thyroidectomy and neck dissection could potentially be among the explanations. Variation in diagnostic criteria of clinical disease progression, such as tumor persistence and recurrence, could be another contributing factor for the inconsistent findings on the prognostic value of *BRAF* mutations in these studies.

A recent updated meta analysis on numerous studies continued to show a strong relationship of *BRAF* mutation with aggressive clinicopathological characteristics of PTC (30). This relationship was even seen in papillary thyroid microcarcinomas (PTMC) in several studies (31-34). Importantly, *BRAF* mutation is also demonstrated to be associated with PTC persistence/recurrence in many studies (27,13,30) and even increased patient mortality (35). The findings from these numerous studies on the clinicopathological association for the *BRAF* mutation in PTC have confirmed, in virtually all aspects, the results of an early report of Xing et al on a comprehensive examination of the association of *BRAF* mutation with extrathyroidal extension, lymph node metastasis, advanced stages III and IV, and recurrence/persistence of PTC (36). The Xing et al study for the first time reported a strong association of *BRAF* mutation with persistence/recurrence of PTC, which was subsequently confirmed in a number of studies around the world (Fig. 2). The association of *BRAF* mutation with PTC persistence/recurrence was seen even in PTC of low TNM stages (I and II) in the Xing et al study, which was confirmed subsequently by Kebebew et al (37). In the Xing et al study, patients with

BRAF mutation in PTC required more aggressive treatments for recurrent diseases, including repeated surgical intervention and external beam radiation therapies (36).

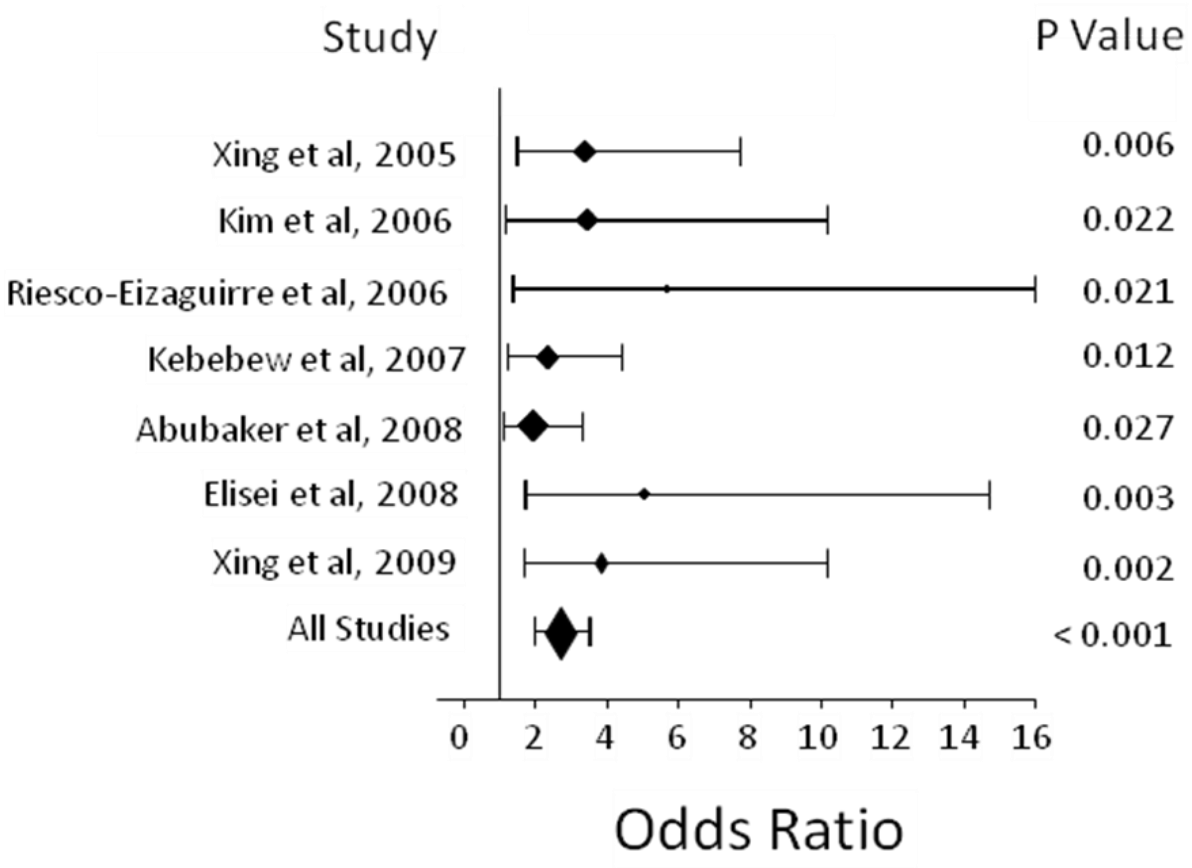

Figure 2. Meta-analysis of the association of BRAF mutation with the recurrence of papillary thyroid cancer. *Shown are odds ratios for tumor recurrence with BRAF mutation in the indicated studies. These studies reported specific information on how to define tumor recurrence, including the criteria used and the follow-up time to monitor the recurrence. Citations of these studies are detailed in reference 30, from which this figure is adapted.*

This finding has been confirmed in a recent study by Yip et al (38). Also an interesting and initial observation in the Xing et al study was the association of *BRAF* mutation with decreased or absent radioiodine avidity of recurrent PTC (36), which was confirmed in several subsequent studies (39-41). This phenomenon is an important cause of the failure of *BRAF* mutation-harboring PTC to respond to radioiodine treatment and hence disease persistence/recurrence. An underlying molecular mechanism is the aberrant silencing of the thyroidal iodide-handling genes, including those for the sodium-iodide symporter (NIS), thyroid-stimulating hormone receptor, thyroperoxidase and thyroglobulin as shown in numerous studies (13,30). An interesting recent study by the Santisteban's group demonstrated that the $BRAF^{V600E}$ promoted silencing of NIS through an autocrine loop that involved and required the release of transforming growth factor (TGF)β and the activation of the TGFβ signalling system (42). It remains to be investigated whether this is a mechanism generally involved in

Online ISSN: 2075-2202

$BRAF^{V600E}$-promoted silencing of thyroid iodide-handling genes in PTC. Other important molecular mechanisms underlying the aggressive role of *BRAF* mutation in PTC include over-expression of various tumor-promoting molecules, including proto-oncogenes, and aberrant silencing of various tumor suppressors promoted by the *BRAF* mutation (13, 30). Thus, the powerful prognostic value of *BRAF* mutation for poorer prognosis of PTC, as reflected by an odds ratio of 3-5 for disease persistence/recurrence (30), has both strong clinicopathological and molecular bases.

Use of *BRAF* Mutation in Risk Stratification for Conventionally Low-risk Papillary Thyroid Cancer

Given the strong association of *BRAF* mutation with aggressive clinicopathological outcomes in most studies, it has become increasingly recognized and anticipated that this mutation can be a useful prognostic genetic marker in the risk stratification of PTC. In fact, *BRAF* mutation is already used in many institutions, including the author's, as a prognostic marker to assist the management of patients with PTC. There are several clinical areas that could be particularly helped with this prognostic use of *BRAF* mutation. Among these is the management of conventionally low-risk PTC patients, such as those with TNM stage I disease and PTMC. Although patients with these cancers generally have a low recurrence and mortality rate, many of them seem to be bound to recurrence and some even to death. These patients may lack obvious conventional high-risk clinicopathological characteristics. Consequently, it is often impossible, based on the current risk criteria, to predict the clinical course of the disease in these patients. Dilemma then often exists with respect on how to optimally treat these patients to prevent disease progression and at the same time balance against the increased risk of aggressive treatment-associated adverse effects. For example, whether to pursue total thyroidectomy *vs* lobectomy, neck dissection *vs* no neck dissection, and radioiodine treatment *vs* no radioiodine treatment has been particularly controversial in the management of these patients. Given the relatively low risk of progression in these patients, the recent ATA guideline on the management of differentiated thyroid cancer holds a conservative stance on these patients; for example, it recommends lobectomy with no radioiodine treatment for PTMC in the absence of classical clinicopathological risk factors, such as lymph node metastasis and extrathyroidal extension (7). As *BRAF* mutation is a strong and independent predictor of PTC persistence/recurrence (35-37) with a power higher or at least equal to some of the conventional clinicopathological risk factors of PTC, such as extrathyroidal extension and lymph node metastasis (13, 30), it should be reasonable to consider total thyroidectomy for patients with *BRAF* mutation-positive PTMC and perhaps even prophylactic central neck dissection in selected patients by experienced surgeons. This seems to be justifiable particularly given the association of *BRAF* mutation with the loss of radioiodine avidity of PTC, which may make it difficult to eradicate residual disease or lymph metastases with radioiodine, rendering initial complete surgical eradication of the disease particularly important in *BRAF* mutation-positive patients. The current ATA guideline also recommends sparing radioiodine treatment for

PTMC in patients without classical clinicopathological risk factors (7). Given the established aggressive role of *BRAF* mutation in low-stage PTC and PTMC (13,34), it is reasonable in this clinical setting to regard *BRAF* mutation as an equivalent to a conventional high-risk factor and therefore treat these patients with radioiodine ablation if *BRAF* mutation is positive. This may help maximize the chance of eliminating thyroid cancer cells to prevent future recurrence. Importantly, and more realistically, radioiodine ablation treatment will facilitate the convenience and reliability of the use of serum thyroglobulin testing in these *BRAF* mutation-positive patients who have a higher risk for recurrence and may therefore need particularly vigilant and reliable surveillance during follow-up. It should be noted that an important rationale in this clinical practice using *BRAF* mutation as a risk factor is that its value is at least equal to some of the conventional risk factors that normally prompt relatively aggressive treatments of thyroid cancer (7).

Use of *BRAF* Mutation Testing on FNAB Specimens to Assist Preoperative Risk Stratification of Papillary Thyroid Cancer

BRAF mutation can be easily tested for on thyroid FNAB specimens as shown in numerous studies (12, 18-22, 25, 26, 43, 44). Xing et al recently demonstrated that preoperative testing for *BRAF* mutation on FNAB specimens had a high power in prospectively predicting lymph node metastasis and extrathyroidal extension of PTC as well as a higher recurrence rate when analyzed retrospectively (45). Therefore, although testing for *BRAF* mutation alone on FNAB specimens may have a limited diagnostic value in general evaluation of thyroid nodules, preoperative testing for *BRAF* mutation on FNAB specimens has a unique prognostic value in assisting risk stratification to guide early decision making for the appropriate treatment of PTC. Testing for *BRAF* mutation in conjunction with certain conventional imaging studies, such as ultrasonography, may be expected to be even more helpful in preoperatively assessing the risk level of PTC and guiding decision making for its optimal managements. *BRAF* mutation is the only molecular marker that has been demonstrated to have a significant preoperative prognostic value for PTC. Pathological risk factors, which are currently the main criteria for risk stratification of thyroid cancer, usually become known only postoperatively and therefore have limited value in preoperative surgical planning for thyroid cancer. Preoperative knowledge of *BRAF* mutation status of PTC would be particularly useful to surgeons in defining the initial type and extent of thyroid surgeries, such as total thyroidectomy vs. lobectomy and neck dissection vs. no dissection as discussed above. This knowledge of *BRAF* mutation will also be useful in helping physicians decide the need and extent of radioiodine treatment and the vigilance level of subsequent surveillance and follow up of the patient. Appropriate planning can thus be made at an early stage of the treatment course of the PTC patient. As a prognostic marker, *BRAF* mutation may not be of much value in guiding the management of advanced cases of thyroid cancer as aggressive treatments, including, for example, total thyroidectomy and neck dissection as well as radioiodine ablation, have been well shown to be beneficial and therefore their indication for aggressive treatment

is generally clear. However, it remains to be investigated whether use of *BRAF* mutation can better tailor the management of certain specific issues that may be affected by the presence of *BRAF* mutation in such patients. For example, since *BRAF* mutation is associated with increased resistance of PTC to radioiodine ablation treatment, a relatively high dose of radioiodine might be required to treat these patients. In contrast, a relatively low dose may be reasonable for *BRAF* mutation-negative cases since they are usually sensitive to radioiodine. In the same context, in a right clinical setting, a non-radioiodine treatment, such as external radiation therapy, and a non-radioiodine diagnostic testing, such as PET scan, should be preferred to radioiodine-based measures for PTC. In these clinical settings of PTC patients, the *BRAF* mutation status that has been documented early in the disease course of the patient, such as one obtained through preoperative testing on FNAB specimens, could be very helpful.

Therapeutic Potential of Targeting *BRAF* Mutation in Thyroid Cancer

Another area related to *BRAF* mutation in thyroid cancer that holds great clinical potential is *BRAF* mutation-based therapeutic targeting of this cancer. Using a large number of what were believed to be authentic thyroid cancer-derived cell lines, several studies demonstrated a clear *BRAF* mutation dependence of inhibition of cell proliferation by MEK inhibitors (46-49). Some earlier studies also demonstrated a *BRAF* mutation dependence of cell inhibition by MEK inhibitors although the cells used in these studies were later shown to be mostly non-thyroid cancer-derived cells (50, 51). A new class of recently developed $BRAF^{V600E}$ mutant-selective inhibitors, as represented by PLX4720, could potently and selectively inhibit melanoma cells that harbored *BRAF* mutation (52). Recently, Salerno et al (53) tested PLX4720 and another $BRAF^{V600E}$ inhibitor, PLX4032, in thyroid cancer cells and, as expected, *BRAF* mutation-selective sensitivity to these inhibitors was clearly demonstrated in these cells. Recent phase I clinical trials showed remarkable therapeutic effects of PLX4032 in melanoma (54,55). These exciting results suggest that BRAF mutant-selective inhibitors may be clinically effective for *BRAF* mutation-positive thyroid cancers. Interestingly, in a recent clinical trial, even a non-MAPK pathway-specific inhibitor, motesanib, which is a classical VEGF receptor tyrosine kinase inhibitor, could inhibit thyroid cancer tumor growth preferentially in patients that harbored the *BRAF* mutation (56). It is possible that the *BRAF* mutation-promoted MAPK pathway could enhance or synergize receptor tyrosine kinase-coupled signaling pathways and therefore potentiate the cell sensitivity to their inhibitors.

Combination of a MEK inhibitor with a PI3K inhibitor showed synergistic effects on the inhibition of thyroid cancer cell proliferation (47). Recent studies also demonstrated a synergistic inhibition of cell proliferation and xenograft tumor growth by MEK inhibitors in combination with mTOR inhibitors (49, 57). In the Liu et al study, a better synergism between MEK and mTOR inhibitors was seen in cells with *BRAF* mutation than cells harboring the wild-type *BRAF* gene (49). A potential drawback of the current inhibitors targeting the MAPK pathway is that they only showed anti-proliferative effects on

thyroid cancer cells in all these studies and no pro-apoptotic effects were shown. The lack of pro-apoptotic effects of MEK inhibitors was seen even when they were combined with mTOR inhibitors (49, 57). Therefore, therapeutic targeting of MAPK and mTOR pathways using these particular inhibitors may not be able to eliminate thyroid cancers. Alternative combinations that can promote thyroid cancer cell apoptosis need to be identified for effective treatment of thyroid cancer. Genetic-based targeting of major signaling pathways, such as *BRAF* mutation-based targeting of the MAPK pathway, will likely be an important component of such combination therapy. Liu et al recently demonstrated a dependence of the PI3K/Akt pathway inhibitors on genetic alterations in this pathway in the inhibition of thyroid cancer cells and in the induction of cell apoptosis (58). These results support the concept of genetic-based targeting of thyroid cancer (59).

In summary, *BRAF* mutation is the most common and important oncogenic genetic alteration in PTC. Numerous studies from recent years have clearly demonstrated its clinical potential in the management of PTC. In particular, its value as a diagnostic and prognostic molecular marker and a therapeutic target has been widely recognized. A significant impact, as it has already started to occur, of this genetic marker on the practice of thyroid cancer medicine is expected.

Acknowledgement

This work is partly supported by NIH grant RO1-CA113507 to the author.

References

1. Leenhardt L, Grosclaude P, Cherie-Challine L. Increased incidence of thyroid carcinoma in France: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee. Thyroid 14:1056–1060, 2004

2. Sprague BL, Warren Andersen S, Trentham-Dietz A. Thyroid cancer incidence and socioeconomic indicators of health care access. Cancer Causes Control 19:585–593, 2008

3. Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, et al (eds). SEER Cancer Statistics Review, 1975-2007, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2007/, based on November 2009 SEER data submission, posted to the SEER web site, 2010

4. Gharib H, Papini E, Paschke R. Thyroid nodules: a review of current guidelines, practices, and prospects. Eur J Endocrinol 159:493-505, 2008

5. Layfield LJ, Cibas ES, Gharib H, Mandel SJ. Thyroid aspiration cytology: current status. CA Cancer J Clin 59:99-110, 2009

6. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European Thyroid Cancer Taskforce. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 154:787-803, 2006

7. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, et al. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19:1167-1214, 2009

8. Sithanandam G, Druck T, Cannizzaro LA, Leuzzi G, Huebner K, Rapp UR. B-raf and a B-raf pseudogene are located on 7q in man. Oncogene 7:795–799, 1992

9. Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. Biochimica et Biophysica Acta 1653:25–40, 2003

10. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, et al. Mutations of the BRAF gene in human cancer. Nature 417:949–954, 2002
11. Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, Beller U, Westra WH, Ladenson PW, Sidransky D. BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst 95:625-627, 2003
12. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer 12:245-262, 2005
13. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 28:742-762, 2007
14. Goutas N, Vlachodimitropoulos D, Bouka M, Lazaris AC, Nasioulas G, Gazouli M. BRAF and K-RAS mutation in a Greek papillary and medullary thyroid carcinoma cohort. Anticancer Res 28:305-308, 2008
15. Riesco-Eizaguirre G, Santisteban P. Molecular biology of thyroid cancer initiation. Clin Transl Oncol 9:686-693, 2007
16. Xing M. Recent advances in molecular biology of thyroid cancer and their clinical implications. Otolaryngol Clin North Am 41:1135-1146, 2008
17. Sobrinho-Simões M, Máximo V, Rocha AS, Trovisco V, Castro P, Preto A, Lima J, Soares P. Intragenic mutations in thyroid cancer. Endocrinol Metab Clin North Am 37:333-362, 2008
18. Marchetti I, Lessi F, Mazzanti CM, Bertacca G, Elisei R, Coscio GD, Pinchera A, Bevilacqua G. A morpho-molecular diagnosis of papillary thyroid carcinoma: BRAF V600E detection as an important tool in preoperative evaluation of fine-needle aspirates. Thyroid 19:837-842, 2009
19. Zatelli MC, Trasforini G, Leoni S, Frigato G, Buratto M, Tagliati F, Rossi R, Cavazzini L, Roti E, degli Uberti EC. BRAF V600E mutation analysis increases diagnostic accuracy for papillary thyroid carcinoma in fine-needle aspiration biopsies. Eur J Endocrinol 161:467-473, 2009
20. Sapio MR, Guerra A, Posca D, Limone PP, Deandrea M, Motta M, Troncone G, Caleo A, Vallefuoco P, Rossi G, et al. Combined analysis of galectin-3 and BRAFV600E improves the accuracy of fine-needle aspiration biopsy with cytological findings suspicious for papillary thyroid carcinoma. Endocr Relat Cancer 14:1089-1097, 2007
21. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, Nikiforova MN. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 94:2092-2098, 2009
22. Cantara S, Capezzone M, Marchisotta S, Capuano S, Busonero G, Toti P, Di Santo A, Caruso G, Carli AF, Brilli L, et al. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab 95:1365-1369, 2010
23. Xing M. Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. Thyroid 20:697-706, 2010
24. Kim KH, Kang DW, Kim SH, Seong IO, Kang DY. Mutations of the BRAF gene in papillary thyroid carcinoma in a Korean population. Yonsei Med J 45:818-821, 2004
25. Chung KW, Yang SK, Lee GK, Kim EY, Kwon S, Lee SH, Park do J, Lee HS, Cho BY, Lee ES, et al. Detection of BRAFV600E mutation on fine needle aspiration specimens of thyroid nodule refines cyto-pathology diagnosis, especially in BRAF600E mutation-prevalent area. Clin Endocrinol (Oxf) 65:660-666, 2006
26. Kim SK, Kim DL, Han HS, Kim WS, Kim SJ, Moon WJ, Oh SY, Hwang TS.
Pyrosequencing analysis for detection of a BRAFV600E mutation in an FNAB specimen of thyroid nodules. Diagn Mol Pathol 17:118-125, 2008
27. Lee JH, Lee ES, Kim YS. Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: a meta-analysis. Cancer 110:38-46, 2007
28. Liu RT, Chen YJ, Chou FF, Li CL, Wu WL, Tsai PC, Huang CC, Cheng JT. No correlation between BRAFV600E mutation and clinicopathological features of papillary thyroid carcinomas in Taiwan. Clin Endocrinol (Oxf) 63:461-466, 2005
29. Fugazzola L, Puxeddu E, Avenia N, Romei C, Cirello V, Cavaliere A, Faviana P, Mannavola D, Moretti S, Rossi S, et al. Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature. Endocr Relat Cancer 13:455-464, 2006
30. Xing M. Prognostic utility of BRAF mutation in papillary thyroid cancer. Mol Cell Endocrinol 321:86-93, 2010

31. Lupi C, Giannini R, Ugolini C, Proietti A, Berti P, Minuto M, Materazzi G, Elisei R, Santoro M, Miccoli P, et al. Association of BRAF V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. J Clin Endocrinol Metab 92:4085-4090, 2007

32. Kwak JY, Kim EK, Chung WY, Moon HJ, Kim MJ, Choi JR. Association of BRAFV600E mutation with poor clinical prognostic factors and US features in Korean patients with papillary thyroid microcarcinoma. Radiology 253:854-860, 2009

33. Lee X, Gao M, Ji Y, Yu Y, Feng Y, Li Y, Zhang Y, Cheng W, Zhao W. Analysis of differential BRAF(V600E) mutational status in high aggressive papillary thyroid microcarcinoma. Ann Surg Oncol 16:240-245, 2009

34. Xing M. BRAF mutation in papillary thyroid microcarcinoma: the promise of better risk management. Ann Surg Oncol 16:801-803, 2009

35. Elisei R, Ugolini C, Viola D, Lupi C, Biagini A, Giannini R, Romei C, Miccoli P, Pinchera A, Basolo F. BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. J Clin Endocrinol Metab 93:3943-3949, 2008

36. Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab 90:6373-6379, 2005

37. Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, Shibru D, Bastian B, Griffin A. The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann Surg 246:466-470, 2007

38. Yip L, Nikiforova MN, Carty SE, Yim JH, Stang MT, Tublin MJ, Lebeau SO, Hodak SP, Ogilvie JB, Nikiforov YE. Optimizing surgical treatment of papillary thyroid carcinoma associated with BRAF mutation. Surgery 146:1215-1223, 2009

39. Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M, Santisteban P. The oncogene BRAF V600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of Na+/I- targeting to the membrane. Endocr Relat Cancer 3:257-269, 2006

40. Mian C, Barollo S, Pennelli G, Pavan N, Rugge M, Pelizzo MR, Mazzarotto R, Casara D, Nacamulli D, Mantero F, et al. Molecular characteristics in papillary thyroid cancers (PTCs) with no (131) I uptake. Clin Endocrinol (Oxf) 681:108-116, 2008

41. Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, Janakiraman M, Solit D, Knauf JA, Tuttle RM, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1.Cancer Res 69:4885-4893, 2009

42. Riesco-Eizaguirre G, Rodríguez I, De la Vieja A, Costamagna E, Carrasco N, Nistal M, Santisteban P. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Res 69:8317-8325, 2009

43. Xing M, Tufano RP, Tufaro AP, Basaria S, Ewertz M, Rosenbaum E, Byrne PJ, Wang J, Sidransky D, Ladenson PW. Detection of BRAF mutation on fine needle aspiration biopsy specimens: a new diagnostic tool for papillary thyroid cancer. J Clin Endocrinol Metab 89:2867-2872, 2004

44. Cohen Y, Rosenbaum E, Clark DP, Zeiger MA, Umbricht CB, Tufano RP, Sidransky D, Westra WH. Mutational analysis of BRAF in fine needle aspiration biopsies of the thyroid: a potential application for the preoperative assessment of thyroid nodules.Clin Cancer Res 10:2761-2765, 2004

45. Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, Kim M, Tufaro A, Ladenson P, Zeiger M, et al. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. J Clin Oncol 27:2977-2982, 2009

46. Leboeuf R, Baumgartner JE, Benezra M, Malaguarnera R, Solit D, Pratilas CA, Rosen N, Knauf JA, Fagin JA. BRAFV600E mutation is associated with preferential sensitivity to mitogen-activated protein kinase kinase inhibition in thyroid cancer cell lines. J Clin Endocrinol Metab 93:2194-2201, 2008

47. Liu D, Xing M. Potent inhibition of thyroid cancer cells by the MEK inhibitor PD0325901 and its potentiation by suppression of the PI3K and NF-kappaB pathways. Thyroid 18:853-864, 2008

48. Schweppe RE, Kerege AA, Sharma V, Poczobutt JM, Gutierrez-Hartmann A, Grzywa RL, Haugen BR. Distinct genetic alterations in the mitogen-activated protein kinase pathway dictate sensitivity of thyroid cancer cells to mitogen-activated protein kinase kinase 1/2 inhibition. Thyroid 19:825-835, 2009

Online ISSN: 2075-2202

49. Liu D, Xing J, Trink B, Xing M. BRAF mutation-selective inhibition of thyroid cancer cells by the novel MEK inhibitor RDEA119 and genetic-potentiated synergism with the mTOR inhibitor temsirolimus. Int J Cancer 2010 Mar 5. [Epub ahead of print]

50. Ball DW, Jin N, Rosen DM, Dackiw A, Sidransky D, Xing M, Nelkin BD. Selective growth inhibition in BRAF mutant thyroid cancer by the mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244. J Clin Endocrinol Metab 92:4712-4718, 2007

51. Liu D, Liu Z, Jiang D, Dackiw AP, Xing M. Inhibitory effects of the mitogen-activated protein kinase kinase inhibitor CI-1040 on the proliferation and tumor growth of thyroid cancer cells with BRAF or RAS mutations. J Clin Endocrinol Metab 92:4686-4695, 2007

52. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, Bremer R, Gillette S, Kong J, Haass NK, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A 105:3041-3046, 2008

53. Salerno P, De Falco V, Tamburrino A, Nappi TC, Vecchio G, Schweppe RE, Bollag G, Santoro M, Salvatore G. Cytostatic activity of adenosine triphosphate-competitive kinase inhibitors in BRAF mutant thyroid carcinoma cells. J Clin Endocrinol Metab 95:450-455, 2010

54. Chapman P, Puzanov I, Sosman J, et al. Early efficacy signal demonstrated in advanced melanoma in a phase I trial of the oncogenic BRAF-selective inhibitor PLX4032. European Journal of Cancer Supplements 7 (3): 5, abstract 6LB, 2009

55. Flaherty K, Puzanov I, Sosman J, Kim K, Ribas A, McArthur G, Lee RJ, Grippo JF, Nolop K, Chapman P. Phase I study of PLX4032: proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. J Clin Oncol 27 (suppl), abstract 9000, 2009

56. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, et al. Motesanib Thyroid Cancer Study Group. Motesanib diphosphate in progressive differentiated thyroid cancer. N Engl J Med 359:31-42, 2008

57. Jin N, Jiang T, Rosen DM, Nelkin BD, Ball DW. Dual inhibition of mitogen-activated protein kinase kinase and mammalian target of rapamycin in differentiated and anaplastic thyroid cancer. J Clin Endocrinol Metab 94:4107-4112, 2009

58. Liu D, Hou P, Liu Z, Wu G, Xing M. Genetic alterations in the phosphoinositide 3-kinase/Akt signaling pathway confer sensitivity of thyroid cancer cells to therapeutic targeting of Akt and mammalian target of rapamycin. Cancer Res 69:7311-7319, 2009

59. Xing M. Genetic-targeted therapy of thyroid cancer: a real promise. Thyroid 19:805-809, 2009

Online ISSN: 2075-2202