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BRAF Mutation in Papillary Thyroid Cancer: Pathogenic Role, Molecular Bases, and Clinical Implications

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In recent years, the T1799A B-type Raf kinase *(BRAF)* mutation in thyroid cancer has received enthusiastic investigation, and significant progress has been made toward understanding its tumorigenic role and clinical significance. Among various thyroid tumors, this mutation occurs uniquely in papillary thyroid cancer (PTC), the most common endocrine malignancy, and some apparently PTC-derived anaplastic thyroid cancers. Many studies have found this mutation to be associated with those clinicopathological characteristics of PTC that are conventionally known to predict tumor progression and recurrence, including, for example, old patient age, extrathyroidal invasion, lymph node metastasis, and advanced tumor stages. Direct association of *BRAF* mutation with the clinical progression, recurrence, and treatment failure of PTC has also been demonstrated. The *BRAF* mutation has even been correlated with PTC recurrence in patients with conventionally low-risk clinicopathological factors. Some molecular mechanisms determining *BRAF* mutation-promoted progression and the aggressiveness of PTC have recently been uncovered. These include the down-regulation of major tumor suppressor genes and thyroid iodide-metabolizing genes and the up-regulation of cancer-promoting molecules, such as vascular endothelial growth factor, matrix metalloproteinases, nuclear transcription factor κB, and c-Met. Thus, *BRAF* mutation represents a novel indicator of the progression and aggressiveness of PTC. Significant advances have also occurred in the preclinical testing of new therapeutic strategies targeting the MAPK pathway aberrantly activated by *BRAF* mutation and other related mutations. New mitogen extracellular kinase (MEK) inhibitors developed recently are particularly promising therapeutic agents for thyroid cancer. With these advances, it has become clearer that *BRAF* mutation will likely have significant impact on the clinical management of PTC. (*Endocrine Reviews* 28: 742–762, 2007)

I. Introduction
  A. Thyroid cancer
  B. Clinicopathological risk evaluation of thyroid cancer
  C. MAPK pathway and its activating genetic alterations
  D. *BRAF* mutation in thyroid cancer
II. Association of *BRAF* Mutation with High-Risk Clinicopathological Characteristics of PTC
  A. Data supporting a positive association
  B. Association of *BRAF* mutation with the most aggressive clinicopathological characteristics of PTC
  C. Studies with negative results
III. Association of *BRAF* Mutation with Recurrence of PTC and Loss of Radioiodine Avidity in Recurrent Tumors
  A. Association with recurrence of PTC
  B. Association with loss of radioiodine avidity in recurrent PTC
IV. Molecular Bases for *BRAF* Mutation-Promoted Invasiveness and Progression of PTC
  A. Unique role of *BRAF* mutation in thyroid tumorigenesis
  B. *BRAF* mutation-associated aberrant methylation and silencing of tumor suppressor genes
  C. Up-regulation of tumor-promoting molecules by *BRAF* mutation
  D. Silencing of thyroid iodide-metabolizing genes in PTC
V. Testing of *BRAF* Mutation as a Potentially New Dimension to Risk Stratification and Clinical Management of PTC
  A. *BRAF* mutation as a novel prognostic molecular marker for PTC
  B. Potential utility of *BRAF* mutation in guiding medical management of PTC
  C. Value of preoperative testing of *BRAF* mutation on fine-needle aspiration biopsy in patients with PTC
  D. Special usefulness of *BRAF* mutation in the management of conventionally low-stage PTC
VI. *BRAF* Mutation and Related Signaling Pathways as Novel Therapeutic Targets for Thyroid Cancer
  A. MAPK pathway as a major therapeutic target
  B. MEK inhibitors as highly promising therapeutic agents for thyroid cancer
  C. Restoring the expression of thyroid iodide-metabolizing genes by suppressing MAPK pathway
  D. Targeting multiple signaling pathways in aggressive thyroid cancers
VII. Concluding Remarks

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Abbreviations: ATC, Anaplastic thyroid cancer(s); BRAF, B-type Raf kinase; CI, confidence interval; CIMP, CpG island methylator phenotype; FNAB, fine-needle aspiration biopsy; FTC, follicular thyroid cancer; MEK, mitogen extracellular kinase; MMP, matrix metalloproteinase; NF-κB, nuclear transcription factor κB; NIS, sodium/iodide symporter; PI3K, phosphoinositol-3 kinase; PTC, papillary thyroid cancer(s); siRNA, small interfering RNA; Tg, thyroglobulin; TIMP, tissue inhibitor of MMP; TPO, thyroperoxidase; TSHR, TSH receptor; VEGF, vascular endothelial growth factor.

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I. Introduction

A. Thyroid cancer

FOLLICULAR EPITHELIAL cell-derived thyroid cancer is the most common endocrine malignancy, and its incidence is rapidly rising in many areas of the world (1–4).

In the United States, the rise in the incidence of this cancer is the fastest among common human cancers, with a current incidence of 33,550 cases per year and a prevalence of 366,466 cases (4). The major histological types of thyroid cancer are papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and anaplastic thyroid cancer (ATC). The vast majority of thyroid cancers are PTC, which accounts for more than 80% of all thyroid malignancies (1, 2, 5). The rising incidence of thyroid cancer is almost entirely attributed to the increased diagnosis of PTC, particularly small PTC (1, 2). Differentiated thyroid cancer, including PTC, is relatively indolent and highly curable. However, a significant recurrence rate, about 20% at 10 yr and 30% at 30 yr of follow-up, is seen after initial treatment (6). The negative psychosocial and economic impact of such recurrence can be significant, and the quality of life for patients with recurrent thyroid cancer can be compromised. Although thyroid cancer-associated mortality is low—standard surgical treatment in conjunction with radioiodine ablation therapy is highly curative in most patients—some still die from this condition. The current mortality rate in the United Sates is 1,530 cases per year (4). Patients face an increased chance of death when the cancer becomes surgically inoperable and when it has lost radioiodine avidity. There is currently no curative treatment for this group of patients. Appropriate management of patients based upon accurate risk stratification and prognostic evaluation is therefore important for reducing the recurrence rate as well as the morbidity and mortality of thyroid cancer.

B. Clinicopathological risk evaluation of thyroid cancer

Conventional clinicopathological evaluation is currently the basis upon which risk stratification is pursued for patients with thyroid cancer (7, 8). There are several clinicopathological characteristics that are classical high-risk factors, including old patient age at the time of diagnosis, male gender, large tumor size, extrathyroidal invasion, lymph node metastasis, distant metastasis, and advanced disease stages (3, 6, 8–12). Each of these clinicopathological risk factors has been shown to be associated with an increased risk for the progression, recurrence, and even morbidity and mortality of thyroid cancer. For PTC, histological subtype is also an important factor in the risk evaluation of this cancer. These subtypes include mainly tall cell PTC, conventional PTC, and follicular variant PTC, which are associated with tumor aggressiveness in the order of tall cell PTC > conventional PTC > follicular variant PTC (13–17). Currently, risk stratification is the chief consideration in determining the aggressiveness with which to manage thyroid cancer, including both the extent of the initial treatment, such as whether to treat with radioiodine ablation after thyroidectomy, and the degree of vigilance in subsequent follow-up of the patient, such as how often to pursue surveillance testing. The reliability of this clinicopathological criteria-based approach, however, can be uncertain, particularly in patients with conventionally low clinicopathological stages (10, 18).

C. MAPK pathway and its activating genetic alterations

As illustrated in Fig. 1, the RET/PTC → Ras → Raf → mitogen extracellular kinase (MEK) → MAPK/ERK pathway (hereafter referred to as "MAPK pathway") is a classical conserved intracellular signaling pathway that plays a fundamental role in cell functions such as proliferation, differentiation, apoptosis, and survival (19–21), and, when aberrantly activated, tumorigenesis (22–25). Physiological activation of this pathway is triggered by a large array of growth factors, hormones, and cytokines through their receptors on the cell membrane. In normal cells, the activation of the Raf kinase occurs through direct interaction with GTP-bound Ras, a membrane-bound small G protein. Activated Raf, a serine/threonine protein kinase, phosphorylates and activates the immediate down-stream MEK, which, also a serine/threonine protein kinase, in turn phosphorylates and activates ERK. The activated ERK phosphorylates regulatory protein molecules in the nucleus and ultimately alters gene expression with consequent changes in the biological activities of the cell.

Aberrant activation of the MAPK pathway, through activating genetic alterations, has long been seen in many human cancers (26). RET/PTC, with more than 10 types known to exist mainly in thyroid cancer, represents a recombinant protein product of a chromosomal rearrangement with the combination of the 3′ portion of the *RET* gene and the 5′ portion of an unrelated gene (27, 28). This recombination confers ligand-independent activation of the tyrosine kinase contained in the *RET/PTC* protein product. It has been recently established that RET/PTC signaling in thyroid cells utilizes the MAPK pathway (29–31). Activating *Ras* mutation is a classical and common cause of aberrant activation of the MAPK pathway in human cancers (24, 32). The B-type Raf kinase (*BRAF*) mutation was recently discovered to be another major cause of aberrant activation of the MAPK pathway in human cancers (23, 33–35). There are three Raf kinases, A-Raf, B-Raf (BRAF), and C-Raf (36). Among the three, BRAF is the most potent activator of the MAPK pathway in many cells (23). Therefore, activating mutation of the *BRAF* gene is of particular importance in human cancers, especially in those that show a high prevalence of this mutation, such as thyroid cancer (13), the focus of this review.

D. BRAF mutation in thyroid cancer

Since the initial discovery of *BRAF* mutation in human cancers (33), there have been more than 40 mutations identified in the *BRAF* gene, among which the T1799A point *BRAF* mutation is the most common and accounts for more than 90% of all the mutations found in the *BRAF* gene (35). This mutation has been found to occur frequently in thyroid cancer (13, 37–42). The T1799A *BRAF* mutation causes a V600E amino acid change in the BRAF protein, resulting in the constitutive and oncogenic activation of the mutated BRAF kinase (33, 43). A few other activated BRAF mutants are only rarely found in thyroid cancer, such as the BRAF K601E (44), AKAP9-BRAF (45), BRAF V599ins (46), BRAF V600E+K601del (47, 48), and a recently characterized novel BRAF mutant, V600D+FGLAT601–605ins, resulting from an insertion of 18 nucleotides at nucleotide T1799 of the *BRAF* gene (47). Thus, the T1799A mutation is virtually the only *BRAF* mutation identified in thyroid cancer (hereafter referred to as "*BRAF* mutation"). *BRAF* mutation represents a

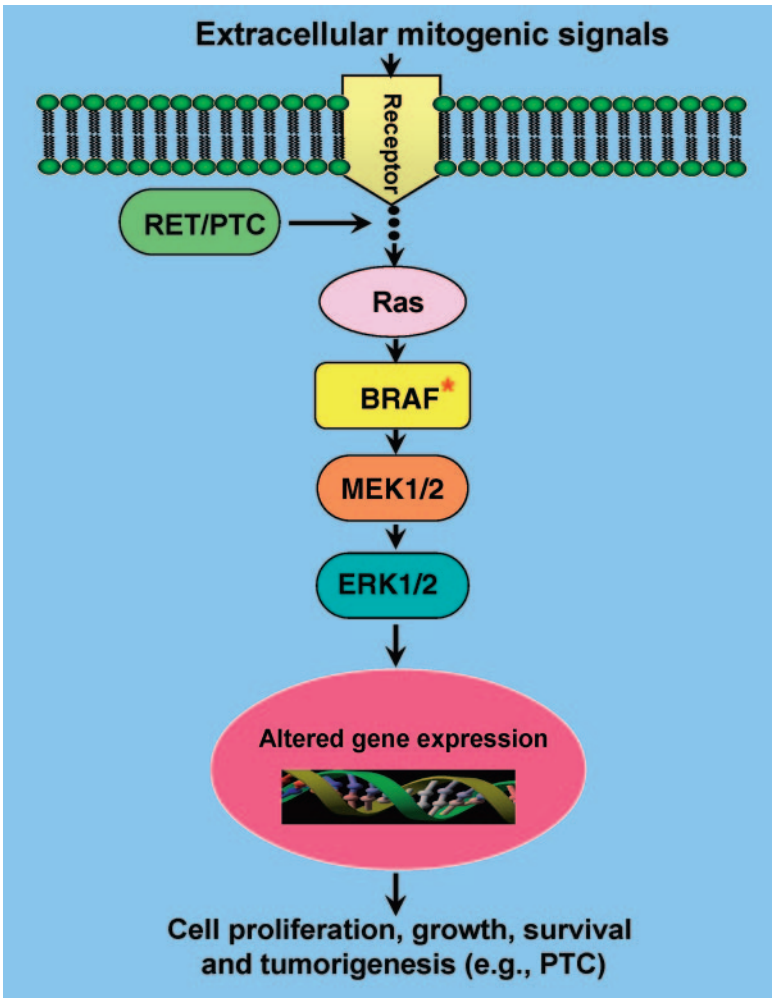

FIG. 1. Schematic illustration of the MAPK pathway. The signaling starts at the cell membrane receptor upon stimulation by extracellular mitogenic signals (*e.g.*, growth factors). Upon activation by binding with GTP, the Ras protein interacts with and activates Raf protein kinase. The B-type Raf kinase, or BRAF, is the most abundant and potent in the Raf family in many cells and is shown here in the figure. Activated BRAF phosphorylates and activates two MEKs, MEK1 and MEK2. Activated MEK1/2 in turn phosphorylates and activates the two immediately downstream ERKs, ERK1 and ERK2. ERK1/2 subsequently phosphorylates downstream proteins, many of which are kinases themselves, ultimately leading to alterations in the expression of various genes in the nucleus involved in cell proliferation, growth, survival, and tumorigenesis. Unique to some PTC is also the occurrence of RET/PTC, a recombinant protein consisting of the tyrosine kinase domain of the RET, a membrane receptor tyrosine kinase, and a portion of an unrelated protein. RET/PTC can activate the MAPK pathway through a step upstream of Ras. *, The gene for BRAF is a common site for mutations in this pathway, with the activating T1799A mutation commonly seen in PTC, which is the focus of this review.

somatic genetic alteration and is not a germline mutation in familial thyroid cancer (49, 50). A striking finding on *BRAF* mutation in thyroid cancer is its exclusive occurrence in PTC and PTC-derived ATC, with an average prevalence of 44% in the former and 24% in the latter, and it does not occur in FTC or other types of thyroid tumors (13). The high prevalence and high specificity of *BRAF* mutation for PTC suggest a unique and fundamental pathogenic role of this mutation in PTC, which has led to great enthusiasm in recent years over the potential clinical utility of this mutation as a novel prognostic molecular marker and as an effective target for the treatment of PTC. Several short reviews on *BRAF* mutation in thyroid cancer have been published recently (27, 51–57). This article is intended to provide a comprehensive review on this mutation in thyroid cancer, with an emphasis on its pathogenic role, underlying molecular bases, prognostic value, and potential as a novel therapeutic target.

II. Association of *BRAF* Mutation with High-Risk Clinicopathological Characteristics of PTC

A. Data supporting a positive association

Many studies have investigated the relationship of *BRAF* mutation with clinicopathological characteristics of PTC (40, 42, 58–85) (Table 1). Although the results are not entirely consistent, most of the studies from various ethnic and geographical backgrounds demonstrate a significant association of *BRAF* mutation with one or more conventional high-risk clinicopathological characteristics of PTC (40, 59, 61, 63, 64, 66–69, 71, 72, 74, 76, 79, 80, 82, 83, 85). For example, in a large series of PTC cases consisting of mainly American patients, a significant association of *BRAF* mutation with extrathyroidal invasion and advanced disease stages III and VI was reported by Nikiforova *et al.* (76). In a Japanese study, an association of *BRAF* mutation with advanced disease stages of PTC was observed by Namba *et al.* (40). Three Korean studies (67, 68, 72) reported a significant association of *BRAF* mutation with lymph node metastasis or extrathyroidal invasion. One of them showed an independent association of *BRAF* mutation with lymph node metastasis even in further multivariate analysis with adjustment for confounding factors (67). A Mayo Clinic study by Jin *et al.* (64) also reported a significant association of *BRAF* mutation with lymph node metastasis and extrathyroidal invasion. In a large comprehensive international multicenter study, Xing *et al.* (83) reported a close association of *BRAF* mutation with extrathyroidal invasion, lymph node metastasis, and advanced disease stages. Similarly, three recent studies—from the

TABLE 1. Correlation of *BRAF* mutation with clinicopathological characteristics of PTC

Study no.	Extrathyroidal invasion			Lymph node metastasis			Disease stages III and IV			First author, year (Ref.)
	BRAF +	BRAF −	*P* value[a]	BRAF +	BRAF −	*P* value[a]	BRAF +	BRAF −	*P* value[a]	
1	16/38 (42)	13/66 (20)	0.014	23/38 (61)	29/66 (44)	0.103	17/38 (45)	5/66 (8)	<0.001	Nikiforova, 2003 (76)
2	14/38 (37)	24/88 (27)	0.283	21/38 (55)	54/88 (61)	0.522	26/38 (68)	46/88 (52)	0.093	Namba, 2003 (40)
3				7/21 (33)	6/35 (17)	0.165	4/21 (19)	7/35 (20)	0.931	Xu, 2003 (42)
4	10/22 (45)	10/29 (34)	0.427				8/16 (50)	9/22 (41)	0.578	Puxeddu, 2004 (78)[b]
5				12/16 (75)	13/23 (57)	0.237	7/18 (39)	9/29 (31)	0.581	Fugazzola, 2004 (62)[b]
6				39/58 (67)	4/12 (33)	0.028				Kim, 2004 (68)
7				3/13 (23)	8/33 (24)	0.933				Sedliarou, 2004 (81)
8	44/107 (41)	18/112 (16)	<0.001	58/107 (54)	24/112 (21)	<0.001	31/107 (29)	16/112 (14)	0.008	Xing, 2005 (83)
9	7/21 (33)	10/53 (19)	0.182	11/21 (52)	18/53 (34)	0.143				Trovisco, 2005 (82)[c]
10	15/31 (48)	11/29 (38)	0.414	21/31 (68)	15/29 (52)	0.206	13/31 (42)	11/29 (38)	0.752	Kim, 2005 (70)
11				24/64 (38)	7/15 (47)	0.513				Kim, 2005 (69)
12	26/47 (55)	26/54 (48)	0.472	17/47 (36)	24/54 (44)	0.398	19/47 (40)	17/54 (31)	0.349	Liu, 2005 (73)
13	16/40 (40)	8/57 (14)	0.004	21/40 (53)	29/57 (51)	0.875	16/40 (40)	7/57 (12)	0.002	Adeniran, 2006 (59)
14	18/28 (64)	11/39 (28)	0.003	9/28 (32)	9/39 (23)	0.409	23/28 (82)	16/39 (41)	<0.001	Riesco-Eizaguirre, 2006 (79)
15	107/149 (72)	31/54 (57)	0.052	116/149 (78)	37/54 (69)	0.173	62/149 (42)	17/54 (31)	0.191	Kim, 2006 (71)
16				26/34 (76)	12/69 (17)	<0.001	5/34 (15)	4/69 (6)	0.152	Kim, 2006 (67)
17	32/58 (55)	14/42 (33)	0.031	14/58 (24)	10/42 (24)	0.970	19/58 (33)	7/42 (17)	0.070	Lee, 2006 (72)
18	8/31 (26)	2/27 (7)	0.087	15/31 (48)	7/27 (26)	0.106				Jin, 2006 (64)[d]
19	34/53 (64)	5/8 (62)	0.928	21/53 (40)	2/8 (25)	0.426				Park, 2006 (77)
20	68/102 (67)	31/59 (53)	0.076	49/102 (48)	27/59 (46)	0.780	23/102 (23)	11/59 (19)	0.559	Jo, 2006 (65)
21				0/18 (0)	7/19 (37)	0.004	1/18 (6)	6/19 (32)	0.043	Sapio, 2006 (84)
22	12/24 (50)	7/20 (35)	0.317	10/24 (42)	11/20 (55)	0.378				Abrosimov, 2007 (58)
23							8/21 (38)	11/37 (30)	0.514	Mitsiades, 2007 (75)
24	14/56 (25)	12/36 (33)	0.386	16/55 (29)	12/37 (32)	0.733	15/43 (35)	7/32 (22)	0.221	Durante, 2007 (60)
25	82/214 (38)	47/286 (16)	<0.001	34/214 (16)	19/286 (7)	<0.001	81/214 (38)	46/286 (16)	<0.001	Lupi, 2007 (74)
26				23/88 (26)	19/126 (15)	0.045				Rodolico, 2007 (80)
27	24/111 (22)	20/98 (20)	0.830	53/111 (48)	32/98 (33)	0.027	39/111 (35)	12/98 (12)	<0.001	Kebebew, 2007 (66)[e]
28	30/54 (56)	18/54 (33)	0.020	18/54 (33)	16/54 (30)	0.679	16/54 (30)	7/54 (13)	0.034	Wang, 2007[f]
Overall	577/1224 (47)	318/1211 (26)	<0.001	661/1513 (44)	451/1515 (30)	<0.001	433/1188 (36)	271/1281 (21)	<0.001	
	OR, 2.50; 95% CI, 2.11–2.97			OR, 1.83; 95% CI, 1.58–2.13			OR, 2.14; 95% CI, 1.79–2.56			

Data represent number/total (percent). OR, Odds ratio.

[a] *P* value per χ^2 test.

[b] Because many, but an unknown specific number of, cases from these two studies were overlapped with the cases in a recent study of the same authors [Fugazzola *et al.*, 2006 (63)], the latter study is not included in this table.

[c] This represents combined data on both conventional and follicular variant PTC from Table 3 of the study of Trovisco *et al.* (82).

[d] These stratified data were provided by Dr. Ricardo V. Lloyd through a personal communication, which was collectively reported in the original publication [Jin *et al.*, 2006 (64)].

[e] The raw data were provided by Dr. Electron Kebebew through a personal communication, which was not directly reported in the original publication [Kebebew *et al.*, 2007 (66)].

[f] Unpublished data.

United States (59), Spain (79), and Italy (61)—have all demonstrated a significant association of *BRAF* mutation with extrathyroidal invasion and advanced stages. Association of *BRAF* mutation with lymph node metastasis was also reported in the Italian study (61). Consistent with the role of *BRAF* mutation in lymph node metastasis of PTC, several studies observed a high prevalence of *BRAF* mutation in lymph node-metastasized PTC (48, 66, 67, 80, 86, 87). Interestingly, in these studies, *BRAF* mutation was sometimes found to be in the lymph node-metastasized PTC but not in the primary tumors, raising the possibility that this mutation could occur *de novo* in PTC cells metastasized to lymph nodes. To further support a role of *BRAF* mutation in lymph node metastasis of PTC, Rodolico *et al.* (80) recently demonstrated that metastatic PTC lesions in lymph nodes harboring *BRAF* mutation were larger in size than those harboring wide-type alleles. This study also showed a higher prevalence of extracapsular invasion of metastasized lymph nodes with *BRAF* mutation than metastasized lymph nodes without the mutation. A recent short meta-analysis on selected reports was published during the revision of this review and revealed a significant association of *BRAF* mutation with extrathyroidal invasion, lymph node metastasis, and advanced stages of PTC (54). Lupi *et al.* (74) recently reported their results of a study on 500 cases of PTC in a homogenous Italian cohort from a single institution, which represented the largest study ever on the relationship between *BRAF* mutation and clinicopathological outcomes. A strong association of *BRAF* mutation with extrathyroidal invasion, lymph node metastasis, and advanced tumor stages was demonstrated in this study. Interestingly, *BRAF* mutation was also found to be significantly associated with lack of tumor capsule in this study. It has been shown that PTC tumors that lack the capsule are associated with a higher risk for metastasis and recurrence (74, 88, 89). A recent large American study on PTC also confirmed some of the previous findings, including the association of *BRAF* mutation with lymph node metastasis and advanced tumor stages III and IV (66). Because distant metastasis of PTC is uncommon, particularly in adult patients, few studies had a sufficient number of cases to look at its relationship with *BRAF* mutation. Two large studies that looked at this issue showed a significant association of *BRAF* mutation with distant metastasis of PTC (40, 66). Among various subtypes of PTC, *BRAF* mutation occurred most commonly in the aggressive subtype, tall cell PTC; second most commonly in conventional PTC; and least commonly in follicular variant PTC (66, 74, 76, 79, 82, 83, 86, 90), with an average prevalence of 77, 60, and 12%, respectively (13). Association of *BRAF* mutation with larger tumor size is reported in several studies (65, 67, 71). In a larger study,

however, *BRAF* mutation was seen to be associated with a somewhat smaller tumor size in PTC (83). It therefore appears that *BRAF* mutation promotes the aggressiveness of PTC mainly by promoting its invasiveness and metastasis. *BRAF* mutation is an adult-associated mutation and is rarely seen in pediatric populations (13, 27). Even in adult populations, an association of *BRAF* mutation with older age was demonstrated in several studies (59, 63, 66, 69, 76, 80, 82, 85). A significant association of *BRAF* mutation with male gender has also been observed in a few studies (42, 71). The predilection of *BRAF* mutation for old and male patients may partially explain the known association of old age and male gender with thyroid cancer progression and aggressiveness.

B. Association of BRAF mutation with the most aggressive clinicopathological characteristics of PTC

Among the various clinicopathological risk factors discussed above, extrathyroidal invasion, lymph node metastasis, and advanced clinicopathological stages III and IV most reliably predict thyroid cancer progression, recurrence, aggressiveness, and, ultimately, higher morbidity and mortality (6, 8–11). Interestingly, among the various clinicopathological characteristics of PTC, many studies have found that *BRAF* mutation is also most commonly associated with these three risk predictors (Table 1 and Fig. 2). When all the published studies that provide sufficient information to calculate the number of analyzed cases are pooled, a significant association of *BRAF* mutation with extrathyroidal invasion, lymph node metastasis, and stages III and IV is clearly shown (Table 1), with overall odds ratios being 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), respectively. These relationships between *BRAF* mutation and the three clinicopathological characteristics from various studies are more clearly depicted in Fig. 2, A–C. The association of *BRAF* mutation with lymph node metastasis appears to be less uniform, probably reflecting a fact that the extent of neck dissection often varied in different patients and studies. When *BRAF* mutation distribution among PTC with different disease stages is analyzed across studies that report such information, a significantly higher prevalence is seen in the advanced stages III and IV than in stages I and II (Table 2). This pattern still exists when the Korean series, which have in general reported an unusually high prevalence of *BRAF* mutation, up to 80–90% in some studies (68, 71, 91), are excluded from this analysis (Table 2). It is also important to note that *BRAF* mutation is most commonly associated with the subtypes of PTC that are classically known to be particularly aggressive. This issue was specifically examined in the Xing *et al.* study (83), which showed clearly an order of tall cell PTC $>$ conventional PTC $\gg$ follicular variant PTC when ranked according to the prevalence of aggressive clinicopathological features of the tumor, such as extrathyroidal invasion, lymph node metastasis, advanced tumor stages III and IV, and tumor recurrence. Correspondingly, the same order of tall cell PTC $>$ conventional PTC $\gg$ follicular variant PTC held for the prevalence of *BRAF* mutation in these subtypes of PTC (83). This close association of *BRAF* mutation with aggressive subtypes of PTC is itself strong evidence for the role of *BRAF* mutation in determining the aggressiveness of PTC. This evidence may, in fact, suggest that *BRAF* mutation is an important, perhaps a primary, factor for the development of tall cell PTC. A recent study showed that, in comparison with conventional PTC, tall cell PTC was more aggressive (with more metastasis and recurrence) even when confounding factors were matched, including patient age, gender, tumor size, extrathyroidal extension status, therapy type, and follow-up length (92). This could be well explained by an independent pathogenic role of the *BRAF* mutation that occurs most commonly in tall cell PTC. Some studies also found a correlation of *BRAF* mutation with high-risk clinicopathological characteristics of PTC within specific subtypes, such as conventional PTC (66, 68, 71) and follicular variant PTC (74). Several studies showed that PTC components often existed in *BRAF* mutation-harboring ATC tumors and, in such cases, the PTC and ATC components usually both harbored *BRAF* mutation (76, 93–95). This suggests that *BRAF* mutation may play a role in promoting the progression of PTC to ATC. Thus, *BRAF* mutation is a driving force behind the aggressive pathological characteristics of PTC and, as will become more evident later in the discussion, predicts a poorer prognosis for patients with PTC.

C. Studies with negative results

Although most of the studies, as discussed above, support an association between *BRAF* mutation and the conventional high-risk clinicopathological factors, some studies have failed to reveal a significant association between them (58, 60, 62, 65, 70, 73, 75, 77, 78, 81, 84) (Table 1 and Fig. 2). There is no definitive explanation for these inconsistent results, but the relatively small number of cases in most of the "negative" studies seems to be one explanation. In fact, a clear tendency of association of *BRAF* mutation with poor clinicopathological characteristics was seen in some of these studies although no statistical significance was reached (Table 1 and Fig. 2). Variations in the extent of the disease at the time of the initial diagnosis (for example, PTC at an early stage with small tumor is less likely to be associated with aggressive pathological features), variations in the completeness of the pathological description of the tumor in the patient records, and variations in the criteria and protocols used for data collection may all exist among different studies and may be at least partially responsible for the inconsistent reports. Possible variations in the diagnostic criteria used by different pathologists, particularly the criteria for defining the various subtypes of PTC, could also contribute to some of the inconsistent reports. This may be true particularly given the fact that the prevalence of *BRAF* mutation varies greatly among the different subtypes of PTC, each of which is associated with different levels of aggressiveness as discussed above. In fact, in the Xing *et al.* study (83), when PTC subtypes were included in the multivariate logistic regression analysis, the association previously seen of *BRAF* mutation with some of the clinicopathological characteristics was cancelled. This result, however, may be well expected if *BRAF* mutation is a major pathogenic factor that drives the development of high-risk pathological characteristics of the aggressive subtypes, such as the tall cell PTC.

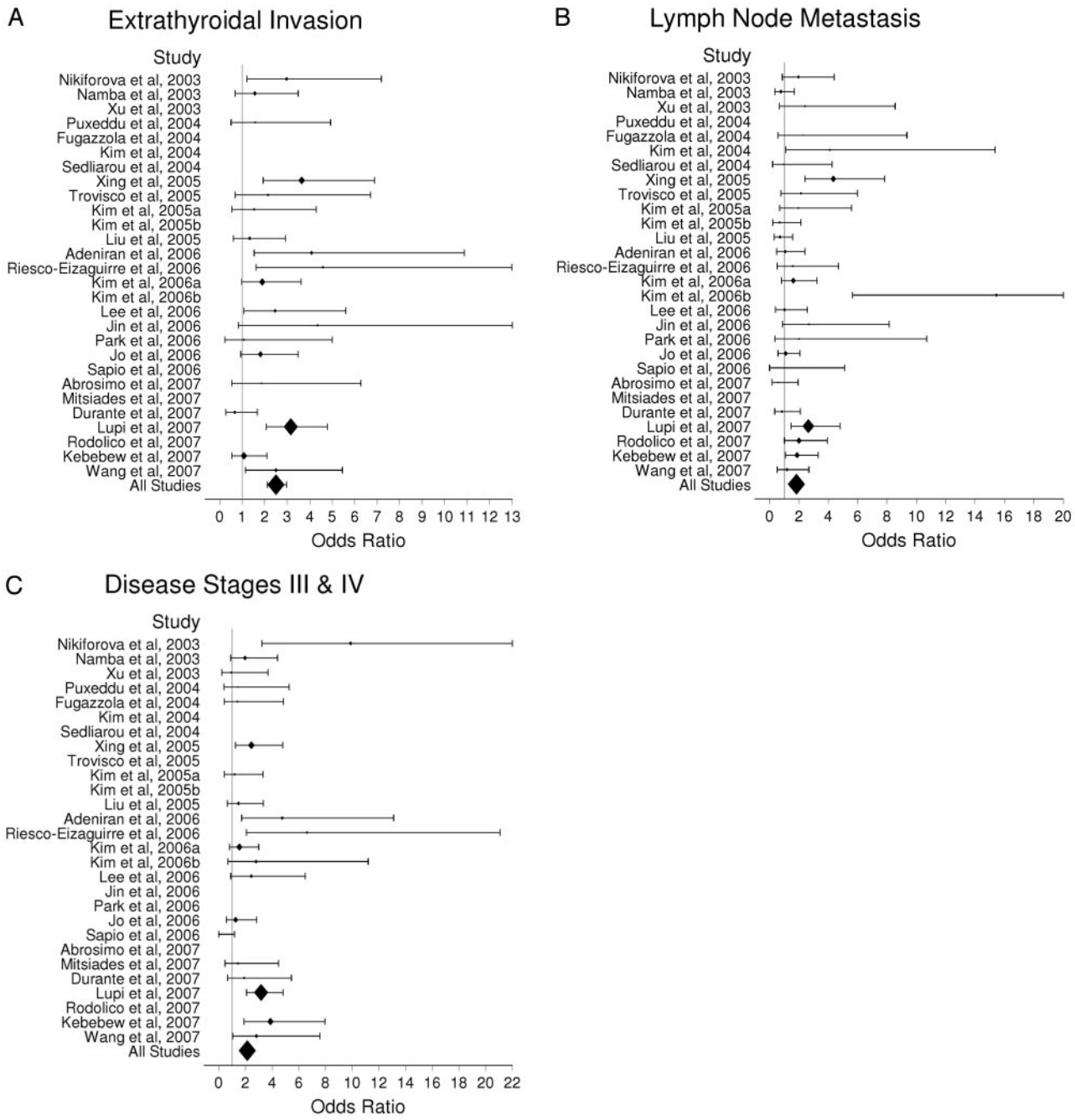

FIG. 2. Relationship of *BRAF* mutation (odds ratios) with extrathyroidal invasion (A), neck lymph node metastasis (B), and advanced stages III and IV (C) in PTC. Plotted are the data for each of the individual reports summarized in Table 1. Some reports did not provide the related data and are left blank in the figure. Shown at the *bottom* of each panel are the overall data added up from the individual reports that provided the related information. Kim *et al.* 2005a, Kim *et al.* 2005b, Kim *et al.* 2006a, and Kim *et al.* 2006b refer to Refs. 70, 69, 71, and 67, respectively.

III. Association of *BRAF* Mutation with Recurrence of PTC and Loss of Radioiodine Avidity in Recurrent Tumors

A. Association with recurrence of PTC

The predictive value of the *BRAF* mutation for PTC recurrence has been specifically investigated in several recent studies that directly examine the relationship between *BRAF* mutation and PTC recurrence. The first large study by Xing *et al.* (83), a multicenter investigation on 219 PTC patients, retrospectively analyzed the relationship of *BRAF* mutation in primary PTC with tumor recurrence. In addition to demonstrating the association of *BRAF* mutation with several aggressive pathological characteristics, such as extrathyroidal invasion, lymph node metastasis, advanced tumor stages, and aggressive histological subtypes, a close association of *BRAF* mutation with PTC recurrence was also demonstrated over a median clinical follow-up of 15 months. An

TABLE 2. Prevalence of *BRAF* mutation in different stages of PTC

Study no.	Stage						First author, year (Ref.)
	I	II	I + II	III	IV	III + IV	
1	20/74 (27)	1/8 (13)	21/82 (26)	10/12 (83)	7/10 (70)	17/22 (77)	Nikiforova, 2003 (76)
2	5/27 (19)	7/27 (26)	12/54 (22)	20/61 (33)	6/11 (55)	26/72 (36)	Namba, 2003 (40)
3	8/19 (42)	0/2 (0)	8/21 (38)	1/1 (100)	7/16 (44)	8/17 (47)	Puxeddu, 2004 (78)
4	10/30 (33)	1/1 (100)	11/31 (35)	6/12 (50)	1/4 (25)	7/16 (44)	Fugazzola, 2004 (62)
5			13/46 (28)[a]				Sedliarou, 2004 (81)[a]
6	44/83 (53)	30/87 (34)	74/170 (44)	29/44 (66)	2/3 (67)	31/47 (66)	Xing, 2005 (83)
7	18/36 (50)	0/0 (-)	18/36 (50)	11/22 (50)	2/2 (100)	13/24 (54)	Kim, 2005 (70)
8	25/57 (44)	3/8 (38)	28/65 (43)	19/33 (58)	0/3 (0)	19/36 (53)	Liu, 2005 (73)
9	20/62 (32)	1/7 (14)	21/69 (30)	9/12 (75)	7/11 (64)	16/23 (70)	Adeniran, 2006 (59)
10			5/28 (18)			23/39 (59)	Riesco-Eizaguirre, 2006 (79)
11	84/120 (70)	3/4 (75)	87/124 (70)	48/60 (80)	14/19 (73)	62/79 (78)	Kim, 2006 (71)
12	14/62 (23)	15/32 (47)	29/94 (31)	1/3 (33)	4/6 (67)	5/9 (56)	Kim, 2006 (67)
13	36/65 (55)	3/9 (33)	39/74 (53)	19/26 (73)		19/26 (73)	Lee, 2006 (72)
14			33/132 (25)[a]				Ugolini, 2007 (159)[a]
15	10/29 (35)	3/10 (30)	13/39 (33)	6/12 (50)	2/7 (29)	8/19 (42)	Mitsiades, 2007 (75)
16	25/47 (53)	3/6 (50)	28/53 (53)	4/5 (80)	11/17 (65)	15/22 (68)	Durante, 2007 (60)
17	124/357 (35)	9/15 (60)	133/372 (36)	81/127 (64)		81/127 (64)	Lupi, 2007 (74)
18			88/214 (41)[a]				Rodolico, 2007 (80)[a]
19	6/17 (35)	32/68 (47)	38/85 (45)			16/23 (70)	Wang, 2007[b]
Overall (1)[c]	449/1085 (41)	111/284 (39)	699/1789 (39)	264/430 (61)	63/109 (58)	366/601 (61)	$P < 0.001$[e]
Overall (2)[d]	297/802 (37)	90/239 (38)	526/1461 (36)	185/319 (58)	43/82 (52)	267/463 (58)	$P < 0.001$[e]

Data represent number/total (percent).

[a] These were cases of micro-PTC and, for convenience of analysis, are included in the category of stages "I + II" because they would usually fall into these stages.

[b] Unpublished data.

[c] Overall (1), overall values of all the studies.

[d] Overall (2), overall values after exclusion of the Korean series (studies 6 and 10–12).

[e] *P* value for comparison between "I + II" and "III + IV" per χ^2 test.

odds ratio of 4.0 (95% CI, 1.1–14.1; $P = 0.03$) for cancer recurrence with *BRAF* mutation was obtained on multivariate analysis with adjustment for all the classical confounding clinicopathological factors, including tumor subtypes and a history of radioiodine treatment. Interestingly, such an association of *BRAF* mutation with PTC recurrence was found even in a subgroup of patients with low-grade initial clinicopathological stages I and II, which are known to be generally associated with a low risk for recurrence. This association also remained significant on multivariate analysis with the adjustment for all the known confounding clinicopathological risk factors. A subsequent Korean study by Kim *et al.* (71) similarly demonstrated a close association of *BRAF* mutation with tumor recurrence in a series of 203 patients with conventional PTC. Kaplan-Meier analysis of disease recurrence-free probability showed similar overall patterns in the two studies (Fig. 3). The Kim *et al.* study (71) displayed smoother survival curves, probably because it consisted of a homogeneous subject population of only Korean patients, whereas the study by Xing *et al.* (83) consisted of a less homogeneous population from four different international medical centers. Also, the Kim *et al.* study consisted of only

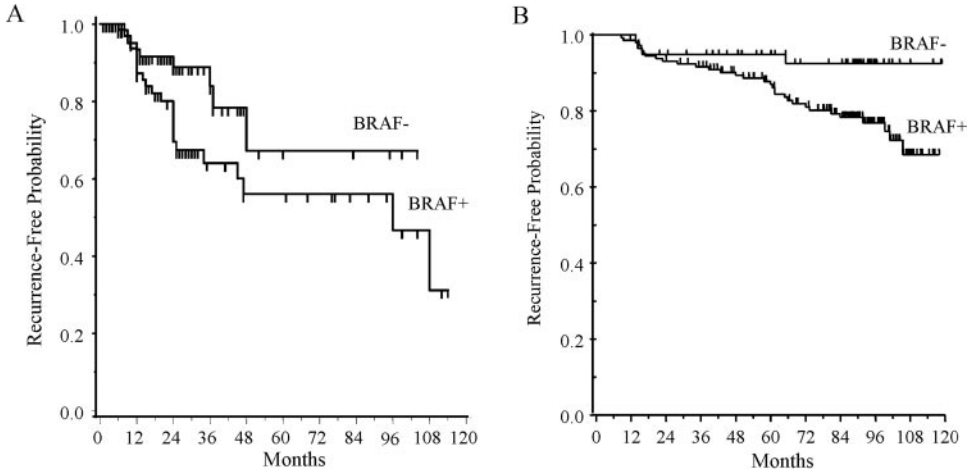

FIG. 3. Kaplan-Meier estimate of recurrence-free probability of PTC in patients with (+) or without (−) *BRAF* mutation. A, Analysis of a multicenter series consisting of 219 cases, mainly Caucasian patients. Log-rank test: $\chi^2 = 4.0$, $P = 0.04$. [Adapted from Xing *et al.*, 2005 (83), with permission from The Endocrine Society.] B, Analysis of a Korean series consisting of 203 patients. Log-rank test: $\chi^2 = 4.60$, $P = 0.037$. [Adapted from Kim *et al.*, 2006 (71), with permission from Wiley–Blackwell.]

conventional PTC, whereas the Xing *et al.* study consisted of three different PTC subtypes: conventional, tall cell, and follicular variant PTC. The disease-free probability curves declined more slowly in the Kim *et al.* study than in the Xing *et al.* study, reflecting the overall lower disease extent of the patients in the former study. A recent Spanish study by Riesco-Eizaguirre *et al.* (79) also demonstrated a strong association of *BRAF* mutation with the recurrence of PTC. Even in a relatively small Italian study on 47 cases of PTC, a tendency of association of *BRAF* mutation with PTC recurrence was shown, although it did not reach statistical significance (62) (Table 3). More recently, in a large American study on PTC, Kebebew *et al.* (66) also demonstrated a significant association of *BRAF* mutation with recurrence or disease persistence of PTC. Similar to the Kim *et al.* study (71), the Kebebew *et al.* study (66) could show a significant role of *BRAF* mutation in PTC recurrence when only conventional PTC were examined. Confirming the findings by Xing *et al.* (83), Kebebew *et al.* (66) also showed an independent association of *BRAF* mutation with PTC recurrence on multivariate analysis with adjustment for the classical clinicopathological confounding factors. As demonstrated in the Xing *et al.* study (83), Kebebew *et al.* (66) also showed a significant association of *BRAF* mutation with PTC recurrence even in low-stage (stage I) PTC. Although the median follow-up time, geographical regions, and ethnic backgrounds of the patients were very different among these studies, the pattern of the relationship of *BRAF* mutation with PTC recurrence was very similar (Table 3). Therefore, regardless of the risk level associated with the preexisting clinicopathological, geographical, and ethnic factors, *BRAF* mutation seems to add an incremental risk for disease recurrence of PTC. The overall odds ratio from five studies for this risk of PTC recurrence associated with *BRAF* mutation is 2.65 (95% CI, 1.77–3.96; $P < 0.001$) (Table 3).

B. Association with loss of radioiodine avidity in recurrent PTC

The mainstay of current medical treatment for PTC after thyroidectomy is radioiodine ablation therapy (7, 8). Effective medical treatment of recurrent PTC is also largely confined to radioiodine therapy. However, thyroid cancer may lose radioiodine avidity, a major cause for radioiodine treatment failure and associated increased morbidity and mortality (7, 8, 96). This can be illustrated by the case of a PTC patient who has initially been treated with radioiodine ablation therapy for apparently radioiodine-sensitive primary PTC after thyroidectomy and later develops a recurrent tumor that becomes radioiodine-resistant and continues to progress. Although various clinicopathological factors are known to be associated with increased risk for recurrence of thyroid cancer, no factor has been known to predict loss of radioiodine avidity in the recurrent tumor. The study by Xing *et al.* (83) showed an interesting association of *BRAF* mutation in the primary PTC with loss of radioiodine avidity in the recurrent tumor. The recurrent PTC in the patients with primary tumors harboring *BRAF* mutation required more aggressive treatments, including the need for surgeries and external radiation therapy, than the recurrent tumors in the patients without *BRAF* mutation, which mostly only required repeated radioiodine treatment (83). In many cases in the former group the treatment for the recurrent tumor failed and the patients continued to have persistent disease, whereas the patients in the latter group were all cured for their recurrent tumor, usually with only one repetition of the radioiodine therapy. A tendency of association of *BRAF* mutation in the primary or recurrent PTC with loss of radioiodine avidity in the recurrent tumor was observed in two more recent studies, although it did not reach statistical significance, probably due to the relatively small number of cases studied (79, 97).

Pediatric PTC is highly curable and rarely recurs if appropriate surgery with adjunctive radioiodine ablation is used, even if the initial disease is extensive and associated with extrathyroidal invasion, lymph node metastasis, or even lung metastasis (98, 99). This is largely because pediatric thyroid cancer is virtually always differentiated and sensitive to radioiodine treatment. Interestingly, this echoes the fact that *BRAF* mutation rarely occurs in pediatric PTC, a population in which *RET/PTC* rearrangement is the major genetic alteration in PTC (13, 27, 28). It appears that when *BRAF* mutation does occur in pediatric PTC, the tumor tends to be de-differentiated and the prognosis is potentially poorer (100). Therefore, *BRAF* mutation-promoted loss of radioiodine avidity may be an important cause for the failure or inefficiency of radioiodine treatment and, hence, a cause for both the progression and recurrence of PTC, although other mechanisms may also exist. Strong molecular bases have now been revealed for this phenomenon. These include the silencing of thyroid iodide-metabolizing genes, which occurs more profoundly with *BRAF* mutation than with *RET/PTC* as will be discussed in *Section IV*.

TABLE 3. Recurrence of PTC with (+) or without (−) *BRAF* mutation

Primary patient group	*BRAF* mutation (+)	*BRAF* mutation (−)	Median follow-up time (months)	OR (95% CI)	*P* value[a]	First author, year (Ref.)
Italian	5/18 (28)	5/29 (17)	?	1.85 (0.45–7.57)	0.39	Fugazzola, 2004 (62)
American	23/92 (25)	9/96 (9)	15	3.37 (1.47–7.74)	0.004	Xing, 2005 (83)
Korean	32/149 (21)	4/54 (7)	88	3.42 (1.15–10.18)	0.02	Kim, 2006 (71)
Spanish	9/28 (32)	3/39 (8)	36	5.68 (1.37–23.52)	0.01	Riesco-Eizaguirre, 2006 (79)
American	38/111 (34)	18/98 (18)	72	2.31 (1.21–4.41)	0.01	Kebebew, 2007 (66)[b]
Overall	107/398 (27)	39/320 (12)		2.65 (1.77–3.96)	<0.001	

Data represent number/total (percent), unless specified otherwise. OR, Odds ratio.

[a] *P* value per χ^2 test.

[b] The raw data were provided by Dr. Electron Kebebew through a personal communication, which was not directly reported in the original publication [Kebebew *et al.*, 2007 (66)].

IV. Molecular Bases for *BRAF* Mutation-Promoted Invasiveness and Progression of PTC

A. Unique role of BRAF mutation in thyroid tumorigenesis

The tumorigenic ability of *BRAF* mutation was elegantly demonstrated in transgenic mice that developed PTC upon targeted expression of the BRAF V600E protein in the thyroid gland (101). PTC developed in these animals progressed naturally into poorly differentiated tumors that showed a high frequency of invasion into blood vessels and surrounding tissues, recapitulating the clinicopathological findings on this mutation in human PTC. Studies in rat thyroid cell lines showed that BRAF V600E promoted matrigel invasion of thyroid cells (30, 102). Matrigel matrix used in this assay is a polymerized basement membrane which is rich in extracellular matrix proteins and mimics physiological conditions for cells to grow on. Migration of cancer cells across matrigel membrane is a commonly used measure to test the invasiveness of cancer cells. BRAF inhibitors could inhibit the growth and proliferation of cells harboring BRAF V600E (103). In *BRAF* mutation-harboring human PTC-derived cells, transient transfection with small interfering RNA (siRNA) to knock down BRAF inhibited cell growth and proliferation (104). Stable siRNA transfection of *BRAF* mutation-harboring PTC cells resulted in 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 (105). These results all strongly support the idea that *BRAF* mutation not only can initiate tumorigenesis of PTC but is also required to maintain and promote the progression of PTC.

The activating genetic alterations in the MAPK pathway, including *RET/PTC* rearrangement, *Ras* mutation and *BRAF* mutation, are mutually exclusive in PTC (13, 39, 41, 90, 106). *BRAF* mutation is also mutually exclusive with aberrant hypermethylation and the silencing of a major tumor suppressor gene, *RASSF1A* (ras-associated factor 1), in PTC (107). These data suggest that each of these genetic alterations in PTC may be sufficient on its own to drive PTC tumorigenesis. It remains, then, an apparently confusing issue why *BRAF* mutation is associated with increased aggressiveness and recurrence of PTC when compared with other genetic alterations related to the MAPK pathway, such as *RET/PTC*, the second most common genetic alteration in PTC. The answer may lie in the difference in the oncogenic strength of these genetic alterations and the molecular events coupled to them in the cell. For example, induced expression of BRAF V600E in rat thyroid cells uniquely caused genetic instability, but the expression of RET/PTC did not (108), suggesting that secondary genetic alterations may occur after *BRAF* mutation and that these alterations may play a special role in determining the progression and aggressiveness of *BRAF* mutation-induced PTC. Markedly increased matrigel cell invasion was also seen with induced expression of BRAF V600E, but not RET/PTC, in these cells (102). A previous study demonstrated that conditional expression of RET/PTC induced only a weak oncogenic drive in thyroid PCCL3 cells (109). In microarray gene expression analyses, different gene expression patterns were seen with induced expression of BRAF V600E and *RET/PTC* in rat thyroid cells (30, 102), suggesting a difference in the genes affected by the two genetic alterations. These cell line studies also suggest that BRAF mutant is probably a stronger activator of the MAPK pathway than RET/PTC. In human PTC tumor tissues, different gene expression profiles were also found to be differentially associated with *BRAF* mutation, *RET/PTC*, and *Ras* mutation (110). From all these studies it is convincing that molecular events that are coupled to BRAF V600E and are not shared or not sufficiently driven by other genetic alterations may be responsible for the tumor aggressiveness uniquely associated with *BRAF* mutation in PTC. *Ras* mutation was shown to be associated with poorly differentiated or undifferentiated thyroid cancers in one study on patients (111) and one study on a transgenic mouse model (112), but no comparison could be made with *BRAF* mutation in these studies. In fact, in a study by Adeniran *et al.* (59) comparing the relationships of different genetic alterations with clinicopathological characteristics of PTC, *BRAF* mutation was far more commonly seen than *Ras* mutation to be associated with high-risk clinicopathological characteristics, such as extrathyroidal invasion, lymph node metastasis, and advanced tumor stages III and IV. The *Ras* mutation occurs most commonly in FTC and follicular variant PTC and is virtually absent in nonfollicular variant PTC (59, 113–115). Therefore, *Ras* mutation may naturally not have a major impact on the tumorigenesis and prognosis of nonfollicular variant PTC.

B. BRAF mutation-associated aberrant methylation and silencing of tumor suppressor genes

Recent studies have demonstrated a close association of *BRAF* mutation with aberrant methylation of several important tumor suppressor genes in PTC, including the genes for tissue inhibitor of matrix metalloproteinase-3 (TIMP3), death-associated protein kinase (DAPK), SLC5A8, and retinoic acid receptor β2 (RARβ2) (116–118). Expression level of SLC5A8 was particularly examined in PTC in some studies and found to be inversely related to *BRAF* mutation (60, 118). Hypermethylation-induced silencing of these genes is associated with tumor progression and aggressiveness in many human cancers (119–124). Interestingly, *BRAF* mutation-associated methylation of these tumor suppressor genes was also correlated with several high-risk clinicopathological characteristics of PTC, including extrathyroidal invasion, lymph node metastasis, and advanced stages III and IV (117). The association of *BRAF* mutation with methylation of tumor suppressor genes was selectively concurrent in several genes in PTC (117), reminiscent of the phenomenon of "CpG island methylator phenotype (CIMP)" proposed for colorectal and other cancers, in which hypermethylation of CpG islands in specific groups of genes distinguishes specific phenotypes of the tumor (125, 126). As in PTC, this CIMP phenomenon was found to be tightly associated with *BRAF* mutation in colorectal cancers (126). It has been recently proposed that the silencing of major tumor suppressor genes in a CIMP-like manner may play an important role in *BRAF* mutation-promoted tumorigenesis and progression of PTC (127). TIMP3 is a particularly interesting tumor suppressor in this regard because it suppresses tumor growth, angiogenesis, invasion,

and metastasis both by preventing the interstitial matrix destruction promoted by matrix metalloproteinase (MMP)-3 (128) and by blocking the binding of vascular endothelial growth factor (VEGF) to the VEGF receptor (129). VEGF is a strong angiogenic protumor molecule that plays a critical role in human cancer progression and invasion (130). Therefore, methylation-mediated silencing of the *TIMP3* gene may play a unique role in *BRAF* mutation-promoted invasiveness and progression of PTC. Recently, *BRAF* mutation was reported to be associated with alterations in the expression of various micro-RNAs in PTC (131, 132). Micro-RNAs are short noncoding single-stranded RNA molecules that regulate gene expression and cell growth and, by functioning as oncogenes or tumor suppressor genes, play an important role in tumorigenesis (133). Of particular interest is the *BRAF* mutation-associated down-regulation of certain micro-RNAs in PTC that appeared to have tumor-suppressor function (132). This newly emerging area awaits to be explored further in thyroid cancer, which may lead to discovery of novel molecular mechanisms in *BRAF* mutation-promoted thyroid tumorigenesis. It would be particularly interesting to see whether alteration in the expression of micro-RNAs in association with *BRAF* mutation is related to the change in genomic DNA methylation.

C. Up-regulation of tumor-promoting molecules by BRAF mutation

Interestingly, a recent study demonstrated overexpression of VEGF in association with *BRAF* mutation in PTC (65). Therefore, the dual angiogenic effects of BRAF V600E through promoting methylation-induced silencing of *TIMP3* and VEGF overexpression in PTC represent a unique molecular mechanism underlying *BRAF* mutation-induced progression and invasiveness of PTC. This mechanism may be particularly involved in *BRAF* mutation-promoted extrathyroidal invasion and metastasis of PTC, which involves vigorous angiogenesis and tissue invasion. This is consistent with the fact that extrathyroidal invasion, most commonly and closely associated with *BRAF* mutation in PTC (Table 1 and Fig. 2A), is one of the most important clinicopathological characteristics associated with a poorer prognosis for patients with PTC.

Overexpression of several MMPs, including MMP3, was induced upon expression of BRAF V600E in thyroid cell lines (30, 102, 134), providing further support for a role of the MMP system in mediating *BRAF* mutation-promoted progression of PTC. MMP molecules, as exemplified by MMP3, promote tumor progression and metastasis by facilitating angiogenesis and the destruction of interstitial matrix (135). In the study by Palona *et al.* (134) that demonstrated induction of MMPs by V600E in thyroid cells, the authors also showed that BRAF V600E promoted activation of the nuclear transcription factor κB (NF-κB)-coupled signaling, which in turn promoted matrigel invasion of thyroid cancer cells. Many studies have shown that the NF-κB pathway promotes apoptosis resistance, cell proliferation, angiogenesis, invasion, and metastasis of human cancers (136). Thus, the NF-κB system may be another important pathway involved in *BRAF* mutation-mediated progression and aggressiveness of PTC. Interestingly, in the study by Palona *et al.* (134), BRAF V600E-promoted activation of NF-κB system seemed to take place through signaling directly from BRAF, independently of the down-stream MEK/MAPK/ERK signaling.

In comparison with *RET/PTC* or *Ras* mutation, *BRAF* mutation was also shown to be associated with markedly up-regulated expression of c-Met in PTC (110). The c-Met protein is a cell surface receptor tyrosine kinase that, through activation by binding with hepatocyte growth factor, stimulates mitogenesis of a wide range of cells and promotes oncogenesis, tumor progression and aggressiveness, and metastasis in several human cancers (137). Increased expression of c-Met was previously demonstrated in PTC (138). In fact, expression of c-Met in tall cell PTC was shown to be significantly higher than that in conventional PTC or follicular variant PTC (139), consistent with the patterns of *BRAF* mutation distribution and aggressiveness of the three subtypes of PTC (83). Expression of c-Met was also highly associated with extrathyroidal invasion and lymph node metastasis of PTC (139), mimicking the association pattern of *BRAF* mutation with these high-risk pathological characteristics of PTC (Table 1 and Fig. 2). Therefore, c-Met may be involved in *BRAF* mutation-promoted aggressiveness of PTC.

D. Silencing of thyroid iodide-metabolizing genes in PTC

The efficacy of radioiodine treatment for thyroid cancer depends on the ability of cancer cells to take up and accumulate radioiodine, which in turn relies on the integrity of the iodide-metabolizing system of the thyroid cell (140). The process involves the sodium/iodide symporter (NIS) in the basal membrane of the thyroid cell that transports iodide into the cell from the blood stream. Iodide is in turn transported into the thyroid follicle through the putative iodide transporter SLC26A4 (pendrin) (141) and possibly other undefined transporters in the apical membrane of the thyroid cell. In the follicle, iodide is oxidized by thyroperoxidase (TPO) and incorporated into tyrosine residues in the thyroglobulin (Tg) molecule to form thyroid hormone. TSH receptor (TSHR) plays a pivotal role in regulating this process, including up-regulation of the molecules involved in this process. Expression of these thyroid iodide-metabolizing genes is often impaired or lost in thyroid cancer (142–145). Interestingly, *BRAF* mutation was found to be associated with decreased expression of TPO (60, 97, 110, 146), NIS (60, 79), Tg (60), and pendrin (97) in primary or recurrent PTC tumors. Conditional expression of BRAF V600E in rat thyroid cell lines led to silencing of all these thyroid-specific iodide-metabolizing genes (79, 108, 147). Cessation of expression of BRAF V600E or inhibition of the MAPK pathway using inhibitors or BRAF siRNA could restore the expression of these and other thyroid genes (79, 147). Methylation was shown to be a mechanism mediating the silencing of some of these thyroid genes. For example, two previous studies demonstrated that the *TSHR* gene was silenced in a promoter methylation-dependent manner in rat thyroid cell lines (148, 149). This was similarly demonstrated in human thyroid cancer cells (150, 151). It was recently demonstrated that in human thyroid cancer cells, BRAF V600E-induced silencing of *TSHR* gene involved aberrant promoter methylation of this gene

partially through increased expression of DNA methyltransferases (147). Interestingly, expression of BRAF V600E impaired not only the expression of NIS protein but also its targeting to the cell membrane (60, 79). Thus, these studies provide a clear molecular explanation for the clinically observed association of *BRAF* mutation with the loss of radioiodine avidity in recurrent PTC (83). Because these thyroid-specific molecules are well-known differentiation markers of thyroid cells, their loss represents progression of thyroid cancer toward dedifferentiation. In this sense, the results on *BRAF* mutation-associated silencing of thyroid-specific genes are consistent with the notion that *BRAF* mutation promotes progression and aggressiveness of PTC.

Overexpression of RET/PTC or activating Ras mutant could also decrease the expression of thyroid iodide-metabolizing genes in thyroid cell lines (29). These oncoproteins, like the BRAF mutant, all lie in the MAPK pathway. It may then appear to be puzzling why *BRAF* mutation is preferentially associated with impaired radioiodine avidity in PTC tumors (83). As discussed in *Section IV.A* for the differential role of *BRAF* mutation among different genetic alterations in PTC aggressiveness, the answer may again lie in different oncogenic strength and molecular events coupled to these genetic alterations. For example, compared with *RET/PTC* and *Ras* mutation, *BRAF* mutation was far more profoundly associated with decrease in TPO expression in primary PTC tumors (110). It is also important to note that the correlation of *BRAF* mutation with abnormalities of the thyroid iodide-metabolizing machinery in PTC tumors, such as decreased expression of iodide-metabolizing genes (60, 79, 97, 110, 146) or decreased radioiodine avidity (79, 83, 97), was established by comparing two groups of PTC: *BRAF* mutation-negative and *BRAF* mutation-positive. The *BRAF* mutation-negative group of PTC in these studies conceivably harbored other relatively common MAPK pathway-related genetic alterations, such as *RET/PTC*, albeit showing less severe abnormalities, consistent with a weaker effect of these genetic alterations than *BRAF* mutation on iodide-metabolizing genes.

V. Testing of *BRAF* Mutation as a Potentially New Dimension to Risk Stratification and Clinical Management of PTC

A. BRAF mutation as a novel prognostic molecular marker for PTC

As evidenced by the strong data discussed above, from both the clinicopathological and the molecular biological perspectives, it is convincing that *BRAF* mutation is intrinsically associated with increased progression and aggressiveness of PTC. This mutation, therefore, may represent a novel and useful prognostic molecular marker for PTC. There has not been a useful molecular marker that, like *BRAF* mutation, has been extensively studied for the prognostic value in the management of thyroid cancer. Like several conventional clinicopathological factors, particularly extrathyroidal invasion, lymph node metastasis, and disease stages III and IV (6), *BRAF* mutation similarly has a high predictive value for PTC recurrence (66, 71, 79, 83) (Table 3). It is important to note that multivariate analysis with adjustment for all the conventional confounding clinicopathological factors showed an independent predicting power of *BRAF* mutation for PTC recurrence (66, 83). Therefore, *BRAF* mutation is not simply a good surrogate marker for the conventional clinicopathological factors, but may actually add an incremental risk to that associated with the conventional factors for PTC recurrence. Because *BRAF* mutation is highly associated with extrathyroidal invasion and advanced tumor stages III and IV (Table 1), which are themselves associated with a higher mortality of PTC (6), *BRAF* mutation is likely to be associated with an increased mortality of PTC. This possibility is consistent with the ability of *BRAF* mutation to promote the silencing of several major tumor suppressor genes, the up-regulation of several protumor and proangiogenesis molecules, and the dedifferentiation and loss of radioiodine avidity of PTC. Given the generally indolent nature of PTC, however, a long-term analysis, either retrospectively or prospectively, is needed to directly address the impact of *BRAF* mutation on PTC-associated mortality. Even given the lack of such data on mortality at this time, the high predicting value of *BRAF* mutation for PTC recurrence alone makes it tempting to apply this novel prognostic molecular marker to the clinic, where it would add a new dimension to the standard risk stratification of PTC, which is currently solely based on clinicopathological criteria.

B. Potential utility of BRAF mutation in guiding medical management of PTC

With this new dimension added to the current risk assessment and stratification of PTC, it is possible to medically manage patients with PTC more appropriately. Although more data, and perhaps a consensus on how specifically to use *BRAF* mutation in planning the treatment of thyroid cancer, may be needed, it can be expected that this novel prognostic factor may affect the current medical management of patients with PTC principally in two ways. In the first, it may assist in deciding how aggressive the initial treatment of the patient should be. Patients with *BRAF* mutation may need to be treated more aggressively, perhaps with more extensive thyroid surgeries (to be discussed further in *Section V.C*) followed by a more liberal (both in terms of patient selection and dosage) use of radioiodine ablation therapy. Because the *BRAF* mutation is associated with decreased expression of iodide-metabolizing genes and impaired radioiodine avidity of PTC, perhaps a higher initial dose of radioiodine should be used to treat *BRAF* mutation-positive patients. This "initial hard-hit" approach guided by *BRAF* mutation status may eliminate PTC cells more efficiently in the first place and therefore reduce the chance of later recurrence. In the second, information on *BRAF* mutation may assist in deciding how vigilantly and aggressively patients should be managed after the initial treatment. PTC patients with *BRAF* mutation may need to be more closely monitored by a more liberal battery of diagnostic tests, such as more aggressive use of imaging methods. Due to the impairment or loss of radioiodine avidity in recurrent PTC associated with *BRAF* mutation, a higher dose of radioiodine

might be needed for diagnostic imaging studies or ablation treatments for recurrent PTC in these patients. The presence of *BRAF* mutation may also help physicians to be prepared for the greater likelihood of radioiodine treatment failure in these patients. In this context, in an appropriate clinical setting, it may be reasonable to use imaging studies such as positron emission tomography scan more liberally to monitor a *BRAF* mutation-positive PTC patient who has a negative diagnostic radioiodine body scan. This idea is supported by the recent demonstration that *BRAF* mutation was associated with increased expression of GLUT-1 in PTC (60, 97). GLUT-1 is a glucose transporter whose function to transport radiotracer-labeled glucose into cancer cells is a basis for positron emission tomography scan. Knowledge of a patient's *BRAF* mutation status, in conjunction with other clinical factors, may also be helpful in determining whether radioiodine treatment should be pursued at all and whether other treatment modalities, such as surgery or external beam radiation, should be opted for at an early stage instead.

C. Value of preoperative testing of BRAF mutation on fine-needle aspiration biopsy in patients with PTC

As a stable DNA molecular marker, *BRAF* mutation can be easily detected on common DNA specimens, even in low quantities, such as those obtained from thyroid fine-needle aspiration biopsy (FNAB) (13, 64, 91, 152, 153). The diagnostic sensitivity of *BRAF* mutation testing alone is low for cytologically indeterminate thyroid nodules, which consist mainly of follicular thyroid tumors, including follicular adenoma, follicular thyroid cancer, and follicular variant PTC, which do not or only rarely harbor *BRAF* mutation (13). However, the accuracy of detection of *BRAF* mutation for PTC is high on FNAB specimens (13). Therefore, although *BRAF* mutation testing alone may not be a sufficiently sensitive diagnostic tool for the evaluation of thyroid nodules in general, it is tempting to propose that, for prognostic purpose, perhaps all patients with cytologically diagnosed PTC should be preoperatively tested for *BRAF* mutation on their FNAB specimens. Preoperative knowledge of *BRAF* mutation may be helpful to surgeons in defining the appropriate surgical strategy, such as lobectomy *vs.* total thyroidectomy and lymph node dissection *vs.* no dissection. It has been well demonstrated that aggressive surgery with careful neck lymph node examination and dissection can effectively decrease the chance of cancer recurrence (154, 155). On the other hand, however, the risk of surgical complications may also rise with aggressive neck surgeries. Because *BRAF* mutation is particularly associated with extrathyroidal invasion, lymph node metastasis, and local recurrence in the neck, preoperative knowledge of *BRAF* mutation may help surgeons better balance benefit and risk in determining the aggressiveness of neck dissection. This approach may help reduce the recurrent rate of PTC while avoiding an increase in complications. Preoperative knowledge of *BRAF* mutation may also be helpful to endocrinologists in planning the aggressiveness of medical management at an early stage, even before surgery. It will have to be seen whether this preoperative testing of *BRAF* mutation on FNAB specimens can prove to be sufficiently useful to become a routine clinical practice in treating PTC patients.

D. Special usefulness of BRAF mutation in the management of conventionally low-stage PTC

As discussed above, thyroid cancer stages III and IV are associated with a high rate of recurrence and increased morbidity and mortality (6–8). In contrast, PTC patients with stages I and II mostly have small tumors, which are generally associated with a good prognosis. However, even in this low-grade group of PTC patients, high-risk pathological characteristics, such as neck lymph node metastasis, are associated with a higher incidence of recurrence (156). PTC in a subgroup of these patients seems to be bound to recur and progress, and conventional clinicopathological evaluation cannot identify this risk (3, 18). Because the rising incidence of thyroid cancer in recent years is largely attributed to the increased diagnosis of small PTC (1, 2), it has become increasingly challenging to tailor the aggressiveness of treatment for thyroid cancer patients with low stages solely on the basis of clinicopathological criteria (3, 18). Testing of *BRAF* mutation may thus have a special value in helping identify those patients from this group that are likely to have a recurrence. This notion is strongly supported by the finding that *BRAF* mutation was independently associated with a higher rate of recurrence of PTC even in patients with low-grade stage I and II disease (66, 83). In fact, the Xing *et al.* (83) study showed the odds ratio for PTC recurrence with *BRAF* mutation on the multivariate analysis in this conventionally low-risk group of patients to be 11.6 (95% CI, 2.2–62.6; $P = 0.004$), representing an even more significant predictive power of *BRAF* mutation for PTC recurrence within this subgroup than was seen in the analysis of the whole series (odds ratio of 4.0; 95% CI, 1.1–14.1; $P = 0.03$). The recent Korean study by Kim *et al.* (71) that showed a significant predicting power of *BRAF* mutation for PTC recurrence consisted of patients mostly with low-risk clinicopathological stages. These results on clinical outcomes are consistent with the recent pathological data in a large study by Lupi *et al.* (74) showing that even in micro-PTC *BRAF* mutation was significantly associated with extrathyroidal invasion, high tumor stage, and lack of tumor capsule. It is thus highly probable that, if *BRAF* mutation proves to be a clinically useful prognostic marker in PTC, it will have a special value in helping appropriately manage PTC patients with low-grade stages. To put this in a practical perspective, perhaps one could use *BRAF* mutation to help determine the need for radioiodine ablation treatment and more vigilant follow-up. Because *BRAF* mutation is associated with impairment of iodide metabolism in PTC, a relatively aggressive dose of radioiodine for ablation therapy might be reasonable for *BRAF* mutation-positive patients even with disease of low stages. The benefit of TSH suppression therapy is often controversial in low-risk thyroid cancer patients (7, 8, 157). Although TSH suppression therapy may not have significant impact on the overall recurrence of thyroid cancer in patients with stages I and II (158), it would be interesting to see whether for the subgroup of these patients that are positive for *BRAF* mutation it is beneficial to pursue aggressive TSH

suppression. A low *BRAF* mutation prevalence of about 30% was reported in most series on micro-PTC or PTC with stages I and II (Table 2). An even lower prevalence (17%) of *BRAF* mutation was reported in micro-PTC of several millimeters in size that were incidentally found on histological examination of the thyroid gland (159). This relatively low prevalence of *BRAF* mutation was seen with stages I and II PTC in most regions in the world except for certain regions, such as Korea, where *BRAF* mutation is generally reported to be extremely prevalent (68, 71, 91) (Table 2). Therefore, it appears to be reasonable and perhaps economically affordable to more aggressively manage only the one third of PTC patients with stages I and II that are positive for *BRAF* mutation in regions where the prevalence of this mutation is not too high (Table 2).

VI. *BRAF* Mutation and Related Signaling Pathways as Novel Therapeutic Targets for Thyroid Cancer

A. MAPK pathway as a major therapeutic target

Because *RET/PTC*, *Ras* mutation, and *BRAF* mutation are all oncogenic activators of the MAPK pathway and together occur in the majority of cases of PTC (39, 41, 83, 90, 106), the MAPK pathway plays a major role in tumorigenesis and progression of PTC, as evidenced in the clinicopathological and molecular studies discussed above. Consequently, the MAPK pathway is a potentially effective therapeutic target for PTC (160, 161). Much work has been done in recent years to investigate the therapeutic potential of suppressing this pathway in human cancers (22, 24, 25). For thyroid cancer, recent effort has been particularly directed toward testing the therapeutic potential of knocking down the MAPK pathway signaling aberrantly activated by *BRAF* mutation. One such example is the demonstrated inhibition of proliferation of *BRAF* mutation-harboring thyroid cancer cells by Raf kinase inhibitors AAL-881 and LBT-613 (103). This inhibition was associated with G_1 arrest and induction of cell death and was seen both in cell lines and in xenograft tumors. Although these BRAF inhibitors may be reasonably toxic enough to prevent their clinical development (51), the data provide implications that specific targeting of BRAF itself may be therapeutic for *BRAF* mutation-harboring thyroid cancers. This idea is also supported by the demonstration that specific knockdown of BRAF by transient siRNA transfection inhibited the proliferation of thyroid cancer cells harboring *BRAF* mutation (104). Stable knockdown of BRAF using stable siRNA transfection persistently suppressed the proliferation of *BRAF* mutation-harboring thyroid cancer cells even after long-term culture (105). Stable BRAF siRNA transfection also suppressed the transformation of thyroid cancer cells and the growth of xenograft tumors harboring *BRAF* mutation (105). Differentiation of thyroid cancer cells harboring *BRAF* mutation as reflected by reexpression of some thyroid-specific genes was induced by stable siRNA knockdown of BRAF as well (147). Thus, these studies all strongly support the therapeutic potential of suppressing BRAF/MAPK signaling for *BRAF* mutation-harboring PTC. The widely studied BAY 43-9006 compound was initially developed as a Raf kinase inhibitor (162) and has been recently demonstrated to have effective antitumor activity in renal cancers in both phase II and phase III clinical trials (163). However, a recent phase II clinical trial on this drug showed little or no antitumor activity in melanoma (164), which harbors *BRAF* mutation with the highest prevalence among all human cancers (33). In particular, this study showed no correlation between *BRAF* mutation and disease stability. This may reflect the fact that BAY-43-9006 is now known to be a multikinase inhibitor, with high potency particularly for C-Raf and various tyrosine kinases (162, 165). As an inhibitor of tyrosine kinases, which are usually associated with the cell membrane upstream of BRAF, Bay-43-9006 may not have sufficient impact on mutant BRAF-activated MAPK pathway signaling. However, a recent preclinical study did show inhibitory effect of this compound on *BRAF* mutation-harboring thyroid cancer cell lines and tumor xenografts (104). It is possible, though, that these cells harbored aberrantly activated signaling pathways, aside from BRAF, in which tyrosine kinases played a significant role. This possibility could explain the responsiveness of the cells to Bay-43-9006. From the current understanding of the molecular mechanisms involved in *BRAF* mutation-promoted PTC tumorigenesis, it cannot be predicted whether Bay-43-9006 will be effective in treating *BRAF* mutation-harboring PTC as a single therapy. It seems to be more likely that BAY 43-9006, as a potent multityrosine kinase inhibitor, will be an effective drug for RET/PTC-harboring PTC because RET/PTC is a constitutively activated tyrosine kinase. This notion is supported by a recent study demonstrating potent inhibition of RET/PTC-transfected NIH3T3 cells and PTC cells that naturally harbored RET/PTC by this compound (104, 166). For *BRAF* mutation-harboring thyroid cancers, targeting the downstream MEK in the MAPK pathway with specific inhibitors may prove to be an effective alternative approach. Several specific and potent MEK inhibitors have been developed in recent years and are now available for clinical trials as will be discussed in *Section VI.B*.

B. MEK inhibitors as highly promising therapeutic agents for thyroid cancer

Great efforts have been made in recent years to develop strategies targeting MEK with anticancer drugs to suppress the MAPK pathway signaling (22, 25, 167–170). Much of what is known about the clinical potential of MEK inhibitors has been from the studies of CI-1040 compound, the first MEK inhibitor to enter clinical trials. CI-1040 is a potent small-molecule MEK-selective inhibitor, which inhibits both MEK-1 and MEK-2, and was first demonstrated to inhibit colon tumor growth in mice several years ago (171). Subsequent studies have demonstrated the inhibitory effects of this compound on human cancer cell lines and animal tumor models of diverse origins (172–175). Phase I (176) and phase II (177) clinical trials on CI-1040 have been recently completed on patients with various advanced cancers, including non-small-cell lung, breast, colon, and pancreatic cancers. Disappointingly, however, no significant antitumor activity of CI-1040 in these two clinical trials was demonstrated. Insufficient potency and bioavailability may have potentially contributed to the negative results but did not seem to ex-

plain completely the discrepancy in the results on CI-1040 between the preclinical and clinical studies. An interesting recent study demonstrated that MEK inhibitors, including the CI-1040 compound, could preferentially inhibit the growth and proliferation of cell lines or tumor xenografts derived from various human cancers that harbored *BRAF* mutation (175). It was concluded that *BRAF* mutation predicted sensitivity of human cancer cells to MEK inhibitors. This may at least partially explain the failure of CI-1040 to show significant antitumor effects in the two clinical trials because the cancers studied generally infrequently harbor *BRAF* mutation. In fact, disease stabilization with CI-1040 was observed in some patients in the two clinical trials (176, 177), and in one of them (177), a nearly significant association of disease stabilization by CI-1040 with baseline phosphorylation level of ERK in the tumor was observed ($P < 0.055$). This result raises the possibility that a high activity of the MAPK pathway, such as that achieved with *BRAF* mutation, may be a prerequisite for MEK inhibitors to exert therapeutic effects.

This *BRAF* mutation-sensitized effect of CI-1040 in other human cancers was recently reproduced in thyroid cancer cells (178). In this study, it was shown that CI-1040 inhibited the growth and proliferation of thyroid cancer cells harboring mutant BRAF but not wild-type BRAF. The study also showed that the CI-1040 compound preferentially induced the differentiation of some thyroid cancer cells that harbored *BRAF* mutation. Preferential inhibition of *BRAF* mutation-harboring thyroid cancer cells was in fact also shown with another MEK inhibitor, U0126, in a previous study by Namba *et al.* (40), which was confirmed in a recent study by Henderson *et al.* (179). This drug, however, cannot be developed clinically due to its limited solubility and bioavailability (22, 170). Thyroid cancer cells harboring the *Ras* mutation that was associated with activated MAPK pathway also responded well to the inhibitory effects of CI-1040 (178). This result is consistent with the finding that the BRAF inhibitors AAL-881 and LBT-613 could inhibit MAPK pathway signaling, promoted by conditional expression of either BRAF mutant or Ras mutant, in PCCL3 rat thyroid cells (103). Therefore, in thyroid cancer, MEK inhibitors may be effective in both *BRAF* and *Ras* mutation-harboring cancer cells. In ovary cancer cell lines, it was similarly found that CI-1040 preferentially inhibited the proliferation of cells harboring activating mutant BRAF or Ras but not wild-type BRAF or Ras (174). The data are therefore compelling that MEK is a potentially effective therapeutic target in thyroid cancer in which the MAPK pathway is activated by upstream mutations such as *BRAF* and *Ras* mutations. More potent and pharmaceutically superior second-generation MEK inhibitors are currently under development and clinical trial, such as the PD-0325901 compound (22, 25, 167, 168, 170) and the ARRY-142886 (AZD6244) compound (22, 170). The PD-0325901 compound is structurally similar to CI-1040 but has greatly improved potency with an IC_{50} of only 1 nM against both MEK1 and MEK2 (170). It also has great biopharmaceutical superiority over CI-1040, and preliminary results from early clinical studies on this drug in patients with solid tumors are promising (22, 170). Like CI-1040, PD-0325901 also showed strong *BRAF* mutation preferentiality in its potent inhibition on cancer cells and xenograft tumors (175). This *BRAF* mutation-selective effect of PD-0325901 was similarly seen in thyroid cancer cells (D. Liu and M. Xing, unpublished data). The ARRY-142886 (AZD6244) compound also displays great potency and showed promising preliminary results in clinical trials on other cancers (22, 170). This MEK inhibitor was shown to selectively inhibit *BRAF* and *Ras* mutation-harboring cancer cells (180). *BRAF* mutation-selective inhibition of thyroid cancer cells and xenograft tumors by this compound was also demonstrated recently (181). In contrast, the MEK inhibitors discussed here uniformly showed no or only a minimal effect on the proliferation of *RET/PTC*-harboring thyroid cancer cells in different studies (40, 178, 179, 181; D. Liu and M. Xing, unpublished data), consistent with their *BRAF* mutation preferentiality over *RET/PTC*. This interestingly echoes the preferential association of *BRAF* mutation with poorer clinicopathological outcomes of PTC discussed in previous sections. These new MEK inhibitors showed excellent patient tolerability and toxicity profiles in the early clinical trials (22, 170), but this needs to be confirmed in more extensive clinical studies. Given the high prevalence of *BRAF* and *Ras* mutations in thyroid cancer, MEK inhibitors hold great promise as novel and potentially effective therapeutic agents for this cancer. It is anticipated that clinical trials on some of these agents in thyroid cancer will occur in the near future, particularly on those that have been documented to be safe in clinical trials on other cancers. Such trials may be reasonably conducted first on progressive PTC and ATC that are positive for *BRAF* mutation and have lost radioiodine avidity.

C. Restoring the expression of thyroid iodide-metabolizing genes by suppressing MAPK pathway

The recently demonstrated correlation of *BRAF* mutation with the loss of or decreased expression of a number of thyroid iodide-metabolizing genes, including *NIS*, *TSHR*, *TPO*, *Tg*, and *pendrin* genes in PTC strongly suggests that suppression of the BRAF/MEK/MAPK signaling may be able to restore expression of these genes and therefore provide a novel therapeutic approach in conjunction with radioiodine therapy. As discussed in previous sections, several recent studies have demonstrated the restorability of these genes, to various extents, in thyroid cancer cells by inhibiting the BRAF and MAPK pathway using either BRAF siRNA or specific MEK inhibitors (79, 147, 178). The restorability of expression of NIS was particularly encouraging because it plays a central role in iodide uptake of thyroid cells. Because loss of radioiodine avidity is a major cause for failure of radioiodine treatment of thyroid cancer and is associated with *BRAF* mutation in PTC (79, 83, 97), therapy targeted at suppression of BRAF and MAPK pathway, such as the use of MEK inhibitors, in conjunction with conventional radioiodine ablation therapy may be particularly effective in treating PTC. Because aberrant methylation of some of the thyroid genes is an important mechanism in their silencing in thyroid cancer (116, 117, 147, 150, 151, 182), use of demethylating agents in conjunction with inhibitors of the MAPK pathway might be synergistically effective in restoring expression of thyroid genes, an attractive possibility that is to be tested. T_4

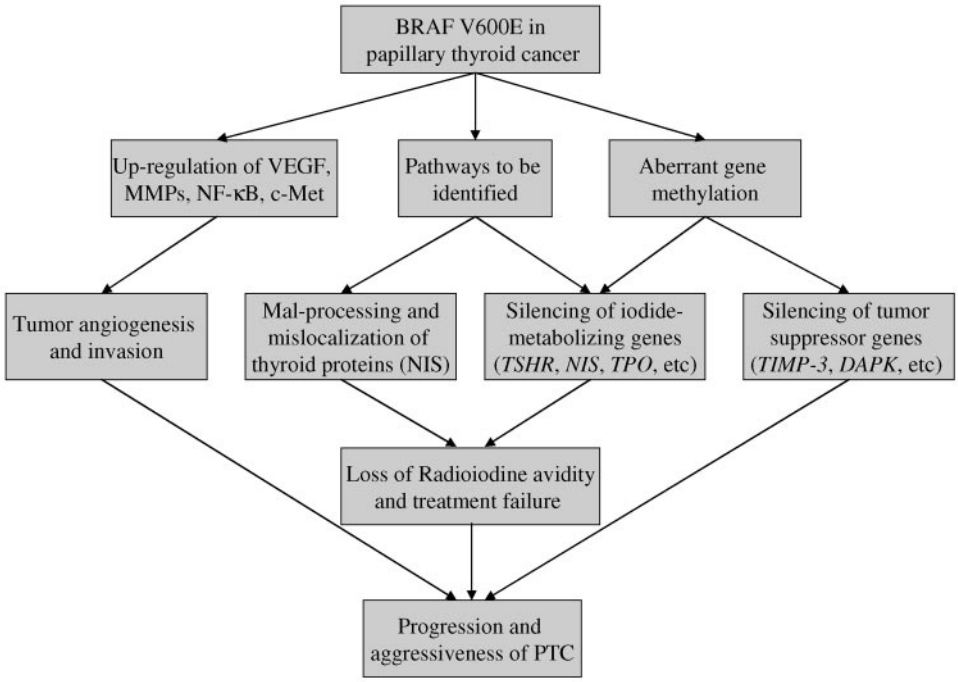

FIG. 4. Summary of the molecular events and pathways involved in *BRAF* mutation-promoted PTC aggressiveness and recurrence. These include overexpression of tumor-promoting molecules such as VEGF, MMPs, NF-κB, and c-Met, silencing of tumor suppressor genes, and silencing and malfunction of thyroid iodide-metabolizing molecules. Aberrant gene methylation plays an important role in some of these molecular events. Additional unidentified molecular events and pathways that are linked to *BRAF* mutation are also expected to exist as indicated. DAPK, Death-associated protein kinase.

withdrawal or the use of recombinant TSH to increase TSH level are routinely used in the radioiodine treatment of thyroid cancer to enhance the uptake of radioiodine and hence the efficacy of treatment. This strategy could be even more effective in enhancing the efficacy of radioiodine treatment if combined with the use of MEK inhibitors to increase the expression of TSHR, which is commonly decreased in thyroid cancer (142–145). It remains to be seen whether such therapeutic strategies directed both at inhibiting cell growth and at the restoration of iodide-metabolizing genes may be of particular benefit in those patients whose thyroid cancers have lost radioiodine avidity and are currently incurable.

D. Targeting multiple signaling pathways in aggressive thyroid cancers

ATC and poorly differentiated thyroid cancers are the most aggressive thyroid cancers and account for a major portion of thyroid cancer-associated mortality. Currently, there is virtually no cure for these cancers. In addition to *BRAF* mutation, ATC also frequently harbor one or more genetic alterations in the phosphoinositol-3 kinase (PI3K)/Akt pathway, including *PIK3CA* mutations and amplification, *PTEN* mutations, and *Ras* mutations (183–186; D. Liu and M. Xing, unpublished data). The PI3K/Akt pathway plays an important role in the pathogenesis and progression of human cancers (187, 188). Targeting this pathway is another important strategy in the current development of novel treatments for human cancers, including thyroid cancer. Overlap of *BRAF* mutation with genetic alterations in the PI3K/Akt pathway was common in ATC (183, 184; D. Liu and M. Xing, unpublished data). Interestingly, the rate of such overlap of *BRAF* mutation with PI3K/Akt pathway-related genetic alterations increased as thyroid tumor progressed from low grade to a more aggressive form (184). Therefore, simultaneous targeting of both the MAPK pathway and PI3K/Akt pathway using specific inhibitors may be a more effective, and perhaps necessary, approach in treating this lethal cancer. Inclusion of the multikinase inhibitor BAY 43-9006 in such a combination therapy would likely enhance the therapeutic efficacy. As discussed in previous sections, *BRAF* mutation is coupled to several other tumor-promoting molecular pathways, including the VEGF, MMP, NF-κB, and c-Met systems. It is likely that the PI3K/Akt pathway is also coupled to multiple signaling pathways that are yet to be identified. It is conceivable that targeting these pathways could also be therapeutically effective for thyroid cancer depending on their dominance level in the pathogenesis of thyroid cancer. As the molecular events and signalings coupled to MAPK and PI3K/Akt pathways become more clearly dissected, an effective combination therapy using combined inhibitors targeting at multiple molecular pathways may become possible for aggressive and lethal thyroid cancers.

VII. Concluding Remarks

From numerous clinicopathological and molecular studies, it is clear that *BRAF* mutation plays a fundamental role in the pathogenesis of PTC. Recent data support the notion that *BRAF* mutation not only initiates PTC, but also maintains and promotes the progression and aggressiveness of

PTC. Its unique predictive value for aggressive pathological characteristics and for clinical progression and recurrence of PTC may make *BRAF* mutation a useful prognostic marker that can be applied to managing patients with PTC. As illustrated in Fig. 4, several molecular bases of *BRAF* mutation-associated progression and aggressiveness of PTC have been revealed recently, which include down-regulation of major tumor suppressors and important thyroid-specific molecules as well as up-regulation of tumor-promoting molecules and pathways. This prognostic molecular marker may be a useful guide for the management of PTC even before thyroid operation and continue to be so in the posttreatment follow-up of the patient. Although there is not yet an agreement on how *BRAF* mutation as a prognostic marker can be specifically used, it will likely have an impact on the management of PTC patients in the clinic. A prospective multicenter study, involving both endocrinologists and surgeons, would best evaluate the value of this novel molecular marker in the surgical and medical management of PTC patients. The strong therapeutic potential of targeting the MAPK pathway represents another exciting dimension that *BRAF* mutation may add to thyroid cancer medicine. Clinical trials on related novel and promising agents for treating thyroid cancer are being eagerly awaited and may fundamentally affect the current treatment of thyroid cancer. In particular, potent MEK inhibitors may prove to be the first effective therapeutic agents for thyroid cancer. Perhaps the day when an effective treatment for aggressive and lethal thyroid cancer, such as PTC with advanced stages and aggressive ATC that have lost radioiodine avidity, will be available is not too far away.

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