REVIEW

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BRAF mutation in thyroid cancer

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Abstract

Genetic alteration is the driving force for thyroid tumorigenesis and progression, based upon which novel approaches to the management of thyroid cancer can be developed. A recent important genetic finding in thyroid cancer is the oncogenic T1799A transversion mutation of *BRAF* (the gene for the B-type Raf kinase, BRAF). Since the initial report of this mutation in thyroid cancer 2 years ago, rapid advancements have been made. *BRAF* mutation is the most common genetic alteration in thyroid cancer, occurring in about 45% of sporadic papillary thyroid cancers (PTCs), particularly in the relatively aggressive subtypes, such as the tall-cell PTC. This mutation is mutually exclusive with other common genetic alterations, supporting its independent oncogenic role, as demonstrated by transgenic mouse studies that showed *BRAF* mutation-initiated development of PTC and its transition to anaplastic thyroid cancer. *BRAF* mutation is mutually exclusive with *RET*/*PTC* rearrangement, and also displays a reciprocal age association with this common genetic alteration in thyroid cancer. The T1799A *BRAF* mutation occurs exclusively in PTC and PTC-derived anaplastic thyroid cancer and is a specific diagnostic marker for this cancer when identified in cytological and histological specimens. This mutation is associated with a poorer clinicopathological outcome and is a novel independent molecular prognostic marker in the risk evaluation of thyroid cancer. Moreover, preclinical and clinical evaluations of the therapeutic value of novel specific mitogen-activated protein kinase pathway inhibitors in thyroid cancer are anticipated. This newly discovered *BRAF* mutation may prove to have an important impact on thyroid cancer in the clinic.

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Introduction

Thyroid cancer is the most common endocrine malignancy. It can be classified histologically into follicular epithelial cell-derived papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC), and para-follicular C-cell-derived medullary thyroid cancer (MTC), which account for approximately 80, 15, 2, and 3% of all thyroid malignancies, respectively (Hundahl *et al*. 1998). Thyroid cancer harbors several highly prevalent genetic alterations, some of which are seen only in this cancer. The classical oncogenic genetic alterations commonly seen in thyroid cancer include *Ras* mutations (Fagin 2002, Bongarzone & Pierotti 2003), *RET*/*PTC* rearrangements (Nikiforov 2002, Santoro *et al*. 2002, Tallini 2002), and *PAX8*-peroxisome proliferator-activated receptor γ (*PPARγ*) fusion oncogene (Kroll *et al*. 2000, McIver *et al*. 2004). Various activating *Ras* mutations, widely seen in other cancers as well, occur mainly in FTC and the follicular variant of PTC (Vasko *et al*. 2003, Zhu *et al*. 2003). *RET*/*PTC* rearrangement represents a recombination of the promoter and N-terminal domain of a partner gene with the C-terminal region of the *RET* gene, resulting in a chimeric oncogene with a protein product containing a constitutively activated RET tyrosine kinase. At lease 10 types of *RET*/*PTC* rearrangement have been identified, which differ by their 5′ partner genes, with *RET*/*PTC*1, *RET*/*PTC*2, and *RET*/*PTC*3 being the most common and occurring mainly in PTC and some benign adenomas. The *PAX8*-*PPARγ* occurs both in FTC and benign thyroid adenoma (Cheung *et al*. 2003, Sahin *et al*. 2005). The recently discovered activating mutation in *BRAF* (the gene for the B-type Raf kinase, BRAF), the focus of this review, represents

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the most common genetic alteration in thyroid cancer. The *RET* and other mutations responsible for the less common and histologically distinct MTC, which are derived from parafollicular cells, are reviewed elsewhere (Koper & Lamberts 2000, Ichihara *et al.* 2004, Santoro *et al.* 2004). Most of the genetic alterations in thyroid cancer exert their oncogenic actions at least partially through the activation of the RET/PTC→Ras→Raf→mitogen-activated protein kinase (MAP kinase)/extracellular-signal-regulated kinase (ERK) kinase (MEK)→MAP kinase/ERK pathway (referred as the MAP kinase pathway hereafter). Activation of this pathway is a common and important mechanism in the genesis and progression of human cancers through upregulating cell division and proliferation. When constitutively activated, the MAP kinase pathway leads to tumorigenesis (Peyssonnaux & Eychene 2001, Hilger *et al.* 2002).

The discovery of activating mutations of the gene for BRAF has expanded the array of the known genetic alterations that activate the MAP kinase pathway and underscores the importance of this pathway in human cancer (Davies *et al.* 2002). Among the three forms of Raf kinases, BRAF, with its gene located on chromosome 7, is the most potent activator of the MAK kinase pathway (Sithanandam *et al.* 1992, Mercer & Pritchard 2003). BRAF-activating missense point mutations in the kinase domain are clustered in exons 11 and 15 of the gene and the T1799A transversion mutation accounts for more than 80% of all the *BRAF* mutations (Davies *et al.* 2002). This mutation had been formerly called T1796A, based on the NCBI GenBank nucleotide sequence NM 004333, which missed a codon (three nucleotides) in exon 1 of the *BRAF* gene. With the correct version of the NCBI GenBank nucleotide sequence NT 007914 available, this *BRAF* mutation is now designated T1799A (Kumar *et al.* 2003), the term used in this review. The T1799A mutation results in a V600E (formerly designated V599E) amino acid substitution in the protein product and subsequent constitutive activation of the BRAF kinase. The V600E mutation is thought to mimic phosphorylation in the activation segment of BRAF by inserting a negatively charged residue adjacent to an activating phosphorylation site at Ser-599 (Davies *et al.* 2002). This is believed to cause the conversion of BRAF to a catalytically active form by disrupting the association of the activation segment with the ATP-binding P loop, which normally holds BRAF in an inactive confirmation (Dhillon & Kolch 2004, Hubbard 2004, Wan *et al.* 2004). The oncogenic and transforming function of the mutated V600E BRAF has been well demonstrated (Davies *et al.* 2002). Since its initial discovery, *BRAF* mutations have now been reported in numerous types of human cancer with various frequencies (Garnett & Marais 2004), being most prevalent in melanomas and nevi, present in 66 and 82% of these dermatologic lesion types, respectively (Davies *et al.* 2002, Pollock *et al.* 2003). Over the last 2 years, substantial work has also described *BRAF* mutations in thyroid cancer, with a prevalence second only to that in melanoma. Discovery of this genetic alteration has created the opportunity to develop novel clinical strategies for the management of thyroid cancer. This review summarizes recent achievements in this exciting research area and highlights the clinical implications of this mutation in thyroid cancer.

High prevalence, specificity and oncogenic role of the T1799A *BRAF* mutation in PTC

Numerous studies have consistently shown a high prevalence of *BRAF* mutation in thyroid cancer, ranging from 29 to 83% (Namba *et al.* 2003, Kim *et al.* 2004; more references are listed in Table 1). The *BRAF* mutation found in thyroid cancer is almost exclusively the T1799A transversion mutation in exon 15. This mutation is a somatic mutation in sporadic thyroid cancers (Kimura *et al.* 2003, Xu *et al.* 2003) and was found not to be a germ-line mutation in a large series of familial PTCs (M. Xing, unpublished results). The only other *BRAF* mutation reported in thyroid tumors was the K601E mutation found in two benign thyroid adenomas (Soares *et al.* 2003, Lima *et al.* 2004) and three follicular-variant PTCs (Trovisco *et al.* 2004). The mutations in exon 11 of the *BRAF* gene found in other human cancers were not found in thyroid cancer (Cohen *et al.* 2003, Fukushima *et al.* 2003, Kimura *et al.* 2003, Namba *et al.* 2003, Frattini *et al.* 2004, Perren *et al.* 2004, Puxeddu *et al.* 2004). A rare but interesting genetic alteration that can also cause constitutive activation of BRAF is the recently reported *in vivo* fusion of the *BRAF* gene with *AKAP9* gene through a paracentric inversion of the long arm of chromosome 7. This results in a recombinant *AKAP9-BRAF* oncogene, which appears to occur in PTCs induced by radiation exposure and results in the loss of the autoinhibitory regulatory domains of BRAF and hence constitutive activation of the kinase (Ciampi *et al.* 2005, Fusco *et al.* 2005).

The present review is focused on the T1799A *BRAF* mutation, and the term *BRAF* mutation hereafter specifically refers to the T1799A *BRAF* mutation. As

Table 1 Frequency of the T1799A transversion *BRAF* mutation in sporadic adult thyroid tumors

	Frequency (mutation/total (%))					
Report	PTC	FTC	ATC	MTC	Benign neoplasm	**Reference**
1	28/78 (36)	0/10 (0)	–	–	0/26 (0)	Kimura *et al*. 2003
2	24/35 (69)	0/16 (0)	–	0/3 (0)	0/20 (0)	Cohen *et al*. 2003
3	21/56 (38)	–	–	–	0/24 (0)	Xu *et al*. 2003
4	23/50 (46)	0/18 (0)	–	–	0/72 (0)	Soares *et al*. 2003
5	40/76 (53)	0/8 (0)	0/7 (0)	0/9 (0)	–	Fukushima *et al*. 2003
6	49/170 (29)	0/11 (0)	2/6 (33)	–	0/20 (0)	Namba *et al*. 2003
7	45/119 (38)	0/32 (0)	3/29 (10)	0/13 (0)	0/111 (0)	Nikiforova *et al*. 2003
8	18/30 (60)	0/12 (0)	–	–	0/9 (0)	Xing *et al*. 2004*a*
9	14/28 (50)	0/14 (0)	2/10 (20)	0/14 (0)	0/54 (0)	Xing *et al*. 2004*b*
10	8/16 (0)	0/6	–	–	0/21 (0)	Xing *et al*. 2004*c*
11	45/124 (36)	–	–	–	–	Trovisco *et al*. 2004
12	–	–	8/16 (50)	–	–	Begum *et al*. 2004
13	58/70 (83)	–	–	–	–	Kim *et al*. 2004
14	30/82 (37)	–	–	–	–	Nikiforova *et al*. 2004
15	36/95 (38)	0/2 (0)	2/2 (100)	0/1 (0)	0/32 (0)	Cohen *et al*. 2004
16	19/60 (32)	–	–	–	–	Frattini *et al*. 2004
17	18/56 (32)	0/5 (0)	0/4 (0)	–	0/1 (0)	Fugazzola *et al*. 2004
18	24/60 (40)	0/5 (0)	0/1 (0)	–	0/6 (0)	Puxeddu *et al*. 2004
19	–	–	6/17 (35)	–	–	Soares *et al*. 2004
20	97/232 (42)	–	–	–	–	Penko *et al*. 2004
21	26/69 (38)	–	–	–	0/27 (0)	Salvatore *et al*. 2004
22	13/46 (28)	–	–	–	–	Sedliarou *et al*. 2004
23	55/91 (60)	0/3 (0)	–	–	0/24 (0)	Vasil'ev *et al*. 2004
24	–	–	–		0/40 (0)	Krohn *et al*. 2004
25	–	–	–	–	0/10 (0)	Kimura *et al*. 2004
26	7/15 (47)	0/7 (0)	–	0/24 (0)	–	Perren *et al*. 2004
27	37/72 (51)	0/8 (0)	0/2 (0)	0/1 (0)	0/45 (0)	Hayashida *et al*. 2004
28	38/61 (62)	0/8 (0)	–	–	–	Porra *et al*. 2005
29	37/65 (57)	–	–	–	–	M Xing *et al*. unpublished results
Overall	810/1856 (44)	0/165 (0)	23/94 (24)	0/65 (0)	0/542 (0)	

shown in Table 1, in all the studies published to date *BRAF* mutation has been found only in PTCs and some apparently PTC-derived ATCs, but not in FTCs, MTCs, or benign thyroid neoplasms (adenoma or hyperplasia). The *BRAF* mutation-positive ATCs were likely derived from *BRAF* mutation-positive PTCs as suggested by the co-existence of PTC and ATC components in the same tumor, which both harbored the *BRAF* mutation (Nikiforova *et al*. 2003, Begum *et al*. 2004, Cohen *et al*. 2004). As summarized in Table 1, the pooled data on sporadic adult thyroid cancer patients from the 29 studies revealed an overall prevalence of *BRAF* mutation of 44% (810/1856) in PTC and 24% (23/94) in ATC. None of the 165 FTCs, 65 MTCs, or 542 benign neoplasms harbored the *BRAF* mutation. This association of PTCs with the *BRAF* mutation, demonstrated consistently in various studies with patients from different geographical and ethnic backgrounds, strongly supports a unique role of *BRAF* mutation in the pathogenesis of PTC. *BRAF* mutation is the most prevalent among the known common oncogenic genetic alterations in thyroid cancer, including the *ras* mutations, *RET*/*PTC* rearrangements, and *PAX8-PPAR*γ rearrangements. The high frequency and specificity of *BRAF* mutation suggest that this mutation may play a fundamental role in the initiation of PTC tumorigenesis. This idea was supported by the presence of *BRAF* mutation in micro PTC (Nikiforova *et al*. 2003, Sedliarou *et al*. 2004, Trovisco *et al*. 2004). The presence of *BRAF* mutation in both the differentiated PTC components and the undifferentiated components in ATC tumors suggest a role for *BRAF* mutation in disease progression (from well-differentiated PTC to undifferentiated ATC; Nikiforova *et al*. 2003, Begum *et al*. 2004, Cohen *et al*. 2004). Consistent with this concept, a study by Sedliarou *et al*. (2004) showed that when well-differentiated tumors contained less-differentiated

Table 2 T1799A *BRAF* mutation in the common subtypes of PTC

Report	Frequency (mutation/total (%))			Reference
	Conventional PTC	Follicular-variant PTC	Tall-cell PTC	
1	28/53 (53)	2/30 (7)	6/6 (100)	Nikiforova *et al*. 2003
2	28/42 (67)	6/51 (12)	–	Cohen *et al*. 2004
3	58/70 (83)	–	–	Kim *et al*. 2004
4	28/53 (53)	0/32 (0)	1/3 (33)	Trovisco *et al*. 2004
5	–	–	11/14 (79)	Frattini *et al*. 2004
6	18/47 (38)	0/6 (0)	–	Fugazzola *et al*. 2004
7	19/35 (54)	–	–	Puxeddu *et al*. 2004
8	16/35 (45)	3/22 (14)	5/9 (55)	Salvatore *et al*. 2004
9	36/52 (69)	2/9 (22)	–	Porra *et al*. 2005
10	15/24 (63)	8/25 (32)	14/16 (88)	M Xing *et al*. unpublished results
Overall	246/411 (60)	21/175 (12)	37/48 (77)	

components, the prevalence of *BRAF* mutation was increased significantly. The *BRAF* mutation is not the only driving force for the formation of ATC, as many ATC tumors do not harbor this mutation; this latter group of ATCs is likely derived from FTC, which is negative for *BRAF* mutation (Nikiforova *et al*. 2003, Cohen *et al*. 2004, Soares *et al*. 2004). The most convincing evidence to support a role of *BRAF* mutation in the initiation and progression of PTC comes from the demonstration (Knauf *et al*. 2004) that the formation of PTC could be induced in transgenic mice in which expression of the V600E BRAF mutant was targeted to thyroid cells. PTC formed in this mouse model transitioned to more aggressive undifferentiated PTC, recapitulating the clinical findings on the association of *BRAF* mutation with a poorer prognosis of PTC, as will be discussed below (Namba *et al*. 2003, Nikiforova *et al*. 2003, Kim *et al*. 2004; M. Xing *et al*. unpublished results).

PTC can be further classified into several histologically distinct subtypes, including the most widely accepted and commonly seen: conventional PTC, follicular-variant PTC, and tall-cell PTC (Chan 1990). The distribution of *BRAF* mutation in PTC shows a clear subtype-related pattern. As summarized in Table 2, from the nine reports that have provided data on PTC subtype distribution of *BRAF* mutation, the prevalence of this mutation is highest in tall-cell PTC (77%), second highest in conventional PTC (60%), and lowest in follicular-variant PTC (12%). As other subtypes of PTC are rare, *BRAF* mutation has not been generally studied in these thyroid cancers. The study by Trovisco *et al*. (2004) represents one attempt to examine *BRAF* mutation in a relatively high number of uncommon subtypes of PTC. In this study, the authors found *BRAF* mutation in six (40%) of 15 oncocytic-variant PTCs and six (75%) of eight Warthin-like PTCs, but not in two diffuse sclerosing PTCs, one columnar cell variant PTC, five hyalinizing trabecular thyroid tumors, or in five mucoepidermoid thyroid tumors. As these are rare thyroid tumors, *BRAF* mutation in these tumors has generally not been reported by other studies.

Different subtype compositions of PTC, when analyzed without subtype stratification in various reports, may partially explain the wide variation in the prevalence of *BRAF* mutation reported by different authors. It should be pointed out that different observers may sometimes define the histological types of thyroid cancer differently (Franc 2003, Lloyd *et al*. 2004), which may affect the accuracy in reporting the tumor-subtype pattern of *BRAF* mutation. However, the distribution pattern of *BRAF* mutation among the three most common subtypes of PTC – conventional PTC, follicular-variant PTC, and tall-cell PTC – most likely represents a true phenomenon as these histological types of PTC can usually be defined with relative ease and the *BRAF* mutation pattern described here has been consistently revealed in all the studies that reported PTC subtypes in the analysis of *BRAF* mutation (Table 2). Therefore, *BRAF* mutation appears to play a major role in the tumorigenesis of tall-cell PTC and conventional PTC. This may explain some of the common features seen in these two subtypes of PTC, such as their high tendency to undergo lymph node metastasis. As tall-cell PTC and conventional PTC are more aggressive than follicular-variant PTC, and as tall-cell PTC is known to be particularly aggressive (Merino & Monteagudo 1997, Akslen & LiVolsi 2000, Prendiville *et al*. 2000), the order of tall-cell variant > conventional variant $\gg$ follicular-variant PTC in the prevalence of *BRAF* mutation is consistent with the idea that *BRAF* mutation is a driving force behind thyroid cancer's

aggressivity. This will become more evident in the discussion regarding the prognostic value of *BRAF* mutation.

Mutual exclusivity between *BRAF* mutation and other common genetic alterations in thyroid cancer

Mutual exclusivity between *BRAF* mutation and *ras* mutation was seen in several types of human cancer, including, for example, colorectal cancer (Rajagopalan *et al*. 2002), melanoma (Omholt *et al*. 2003), and ovarian cancer (Singer *et al*. 2003). Mutual exclusivity between these two mutations was also seen in thyroid cancer (Fukushima *et al*. 2003, Kimura *et al*. 2003, Soares *et al*. 2003, Frattini *et al*. 2004). These and other studies (Kumagai *et al*. 2004, Lima *et al*. 2004, Nikiforova *et al*. 2004, Vasil'ev *et al*. 2004) similarly showed mutual exclusivity between *BRAF* mutation and *RET*/*PTC* rearrangements in thyroid cancer. In fact, no study showed more than one type of these three common genetic alterations in the same case of thyroid cancer, except one study showing the overlap of *BRAF* mutation with *RET*/*PTC* (Xu *et al*. 2003). In this study, however, immunohistocheminical staining was used to define the presence of *RET*/*PTC* using C-terminal-specific antibodies. The results may therefore be non-specific as the antibodies used may not reliably discriminate between the rearranged and the wild-type RET proteins. Expression of the wild-type *RET* or *RET* proto-oncogene was previously demonstrated in PTC, particularly in PTC that lack the major *RET*/*PTC* rearrangements (Bunone *et al*. 2000). In general, the data on *BRAF* mutation, *ras* mutation, and *RET*/*PTC* rearrangements in thyroid cancer support the idea that each of the three genetic alterations alone is sufficient to cause thyroid tumorigenesis. The mutual exclusivity among these common genetic alterations in thyroid tumor may not be surprising, though, as the signaling pathways of these activating genetic alterations share the common MAP kinase pathway, albeit at different steps. A single oncogenic alteration along this pathway is likely sufficient to drive thyroid cell transformation and tumorigenesis. The genetic data supporting *BRAF* mutation as an independent oncogenic event for PTC tumorigenesis is consistent with the results from the transgenic mouse studies mentioned above (Knauf *et al*. 2004).

Like various genetic alterations, loss of expression of the pro-apoptotic tumor suppressor Ras-associated factor 1 (RASSF1) through an epigenetic alteration, gene methylation, is another important mechanism in the tumorigenesis of many human cancers (Pfeifer *et al*. 2002). The three splice variants (A, B, C) of RASSF1 all possess a Ras-association domain (Dammann *et al*. 2000). Ras has been shown to be able to use RASSF1 as a direct effector in the downstream signaling (Vos *et al*. 2000). Therefore, RASSF1 may function through a Ras-like signaling pathway. Promoter methylation of *RASSF1A* was frequently found in thyroid tumors (Schagdarsurengin *et al*. 2002, Xing *et al*. 2004*a*) and this methylation silenced the expression of *RASSF1A* gene in thyroid tumor cells (Schagdarsurengin *et al*. 2002). Therefore, aberrant methylation of *RASSF1A* may represent another important oncogenic mechanism in thyroid tumorigenesis. Intriguingly, aberrant methylation of *RASSF1A* was recently found to be mutually exclusive with *BRAF* mutation in PTC (Xing *et al*. 2004*a*). High-level *RASSF1A* methylation occurred mostly in FTC (Xing *et al*. 2004*a*), similar to *ras* mutations that also occur frequently in FTC (Vasko *et al*. 2003). Among different PTC subtypes, *ras* mutations were highly prevalent in follicular-variant PTC, while *RET*/*PTC* rearrangements, like *BRAF* mutation, were more prevalent in conventional PTC (Zhu *et al*. 2003) and tall cell-variant PTC (Basolo *et al*. 2002). Therefore, it appears that PTC subtype-predilections may partially account for the mutual exclusivity of these genetic and epigenetic alterations recently reported in thyroid cancer. In most of these studies, analysis of all PTC for genetic alterations was conducted without stratification of histological subtypes. To be certain about the mutual exclusivity of these common genetic alterations and their respective roles in thyroid tumorigenesis in each specific subtype of PTC, it would be necessary to examine all of these genetic and epigenetic alterations simultaneously in each of the specific subtypes of PTC.

BRAF mutation and *RET*/*PTC* rearrangements may act at steps that are different but close in their shared oncogenic pathway, resulting in conventional PTC, whereas *ras* mutations and *RASSF1A* methylation may act at different but related steps along their shared oncogenic pathway resulting in FTC and follicular-variant PTC. Although thyroid tumorigenesis caused by these genetic and epigenetic alterations may all involve the MAP kinase pathway, each of these genetic and epigenetic alterations, particularly those that act in this pathway at a step proximal to Raf kinase, may involve additional signaling pathways. For example, the phosphoinositide 3-kinase/Akt pathway, which is known to also play an important role in thyroid tumorigenesis, can be activated by Ras (Gire *et al*. 2000, Cheng & Meinkoth 2001) or RET/PTC (Kim *et al*. 2003, Miyagi *et al*. 2004). This may partially

Table 3 Prevalence of *BRAF* mutation and *RET/PTC* rearrangements in PTC in radiation-exposed and non-exposed children

	Prevalence (genetic event/total (%))				
	BRAF mutation		*RET/PTC* rearrangements		
Report	Radiation-exposed	Non-exposed	Radiation-exposed	Non-exposed	**Reference**
1	–	–	29/38 (76)	11/17 (65)	Nikiforov *et al.* 1997
2	–	–	–	15/33 (45)	Fenton *et al.* 2000
3	4/34 (12)	1/17 (6)	14/34 (41)	–	Lima *et al.* 2004
4	2/55 (4)	–	32/55 (58)	–	Nikiforova *et al.* 2004
5	0/15 (0)	1/31 (3)	17/48 (35)	–	Kumagai *et al.* 2004
6	–	0/7 (0)	–	3/6 (50)	Penko *et al.* 2004
7	1/5 (20)	–	–	–	Xing *et al.* 2004*b*
Overall	7/109 (6)	2/55 (4)	92/175 (53)	29/56 (52)	

explain the distinct characteristics of different subtypes of thyroid cancer that harbor different genetic and epigenetic alterations.

Reciprocal age-association of *BRAF* mutation and RET/PTC rearrangements

It is well known that *RET/PTC* is particularly common in the pediatric PTC that occurred in the victims of the Chernobyl nuclear accident (Ito *et al.* 1994, Fugazzola *et al.* 1995, Klugbauer *et al.* 1995, Nikiforov *et al.* 1997). A similarly high prevalence of *RET/PTC* has also been found in non-radiation-exposed sporadic pediatric PTC (Nikiforov *et al.* 1997, Fenton *et al.* 2000, Penko *et al.* 2004). As *BRAF* mutation and *RET/PTC* are together responsible for the majority of conventional PTC, the most common subtype of PTC, and are mutually exclusive in adult sporadic PTC, their relationship in pediatric PTCs, particularly in those that occurred as a result of the Chernobyl nuclear accident, has drawn much interest (Kumagai *et al.* 2004, Lima *et al.* 2004, Nikiforova *et al.* 2004, Xing *et al.* 2004*b*). As summarized in Table 3, and consistent with previous reports (Ito *et al.* 1994, Fugazzola *et al.* 1995, Klugbauer *et al.* 1995, Nikiforov *et al.* 1997), these recent studies uniformly showed a high prevalence of *RET/PTC* in both radiation-exposed and sporadic pediatric populations. As may be expected from the mutual exclusivity of *RET/PTC* and *BRAF* mutation observed in sporadic adult PTC and from the known high frequency of *RET/PTC* in radiation-exposed PTC, the initial study on a small series of PTC from Chernobyl victims showed a low prevalence of *BRAF* mutation (Xing *et al.* 2004*b*). In several subsequent larger studies on Chernobyl victims, the prevalence of *BRAF* mutation in PTC was found consistently to be low in this special population, ranging from 0 to 12% (Kumagai *et al.* 2004, Lima *et al.* 2004, Nikiforova *et al.* 2004). As in sporadic adult PTC patients, mutual exclusivity of *BRAF* mutation and *RET/PTC* was also demonstrated consistently in this Chernobyl population. It would be interesting to know, in a large series, how frequent the recently discovered radiation-sensitive recombinant *AKAP9-BRAF* oncogene (Ciampi *et al.* 2005, Fusco *et al.* 2005) would truly be and whether it, like the *BRAF* mutation, is mutually exclusive with *RET/PTC* in Chernobyl- or radiation-related PTCs.

Interestingly, the study by Lima *et al.* (2004) on Chernobyl victims showed that the average age of the children at the time of radiation exposure was much higher for the group with *BRAF* mutation than the group with *RET/PTC*. In the study by Kumagai *et al.* (2004), when the Chernobyl radiation-exposed children were divided into two age groups, none (0%) of the 15 cases in the group at or younger than 15 years harbored the *BRAF* mutation, whereas eight (24%) of the 33 cases in the group older than 15 years harbored this mutation. Several of these studies (Kumagai *et al.* 2004, Lima *et al.* 2004, Penko *et al.* 2004) also showed the mutual exclusivity between *RET/PTC* and *BRAF* mutation and a low prevalence of the latter (ranging from 0 to 6%) in non-radiation-exposed sporadic PTC in the pediatric population. From these recent studies, the overall prevalence of *BRAF* mutation for radiation-exposed and non-exposed pediatric PTC is 6 and 4%, respectively, and the overall prevalence of *RET/PTC* rearrangements for radiation-exposed and sporadic pediatric PTC is 53 and 52%, respectively (Table 3). The adult PTC patients included in some of these studies (Nikiforova *et al.* 2004, Xing *et al.* 2004*b*) showed uniformly a low prevalence of *RET/PTC* and a high prevalence of *BRAF* mutation regardless of their history of radiation exposure. Although the prevalence of *RET/PTC* rearrangements is generally found to be low in adults

and high in children, and children are more susceptible to the effects of radiation, conflicting data do exist. For instance, a study by Elisei *et al*. (2001) on different groups of thyroid tumor patients with various ethnic and demographic backgrounds showed no association of the occurrence of *RET*/*PTC* with age at the time of radiation exposure, albeit with relatively low numbers of study subjects in the cancer groups. This study also showed no difference in the occurrence of *RET*/*PTC* in radiation-exposed and non-exposed adult patients.

Therefore, studies in general demonstrate a reciprocal age-association of *BRAF* mutation and *RET*/*PTC* in PTC. Beyond inciting factors, such as radiation, age is apparently an important factor in determining the dominance of the two genetic alterations in PTC. *BRAF* mutation tends to occur in adults and is a major somatic genetic alteration that drives the formation of PTC in this population, whereas *RET*/*PTC* tends to occur in children and is a major somatic genetic alteration that drives the formation of PTC in this population. It appears that young age itself, in addition to radiation, is an important predisposing factor for the development of *RET*/*PTC* and subsequent PTC. The concept that *RET*/*PTC* is an initiator of the formation of PTC in nuclear-accident victims is somewhat challenged by a recent study of Unger *et al*. (2004) on Chernobyl-associated PTC. In this study, using an interphase *in situ* hybridization technique, the authors found *RET*/*PTC* rearrangements in some cells of PTC tumors but not in other cells of the same tumor. This raises the possibility that these PTCs might have arisen from different clones or that *RET*/*PTC* is a late subclonal event, and thereby challenges the general belief that *RET*/*PTC* plays an initiating role in the development of radiation-associated PTC. However, the possibility of inaccurate scoring of, and therefore missing, tumor cells harboring *RET*/*PTC* rearrangement due to a technical limitation in this study has been raised (Fagin 2004). Ionizing radiation could induce the formation of *RET*/*PTC* in both transplanted human thyroid tissues in mice (Mizuno *et al*. 1997) and in cultured thyroid tumor cells (Ito *et al*. 1993). A high prevalence of *RET*/*PTC* was also observed in PTC that developed in patients who had external radiation treatment during childhood (Bounacer *et al*. 1997). The transgenic mouse model demonstrated clearly the ability of *RET*/*PTC*1, 2 and 3 to initiate the development of PTC (Jhiang *et al*. 1996, 1998, Santoro *et al*. 1996, Powell *et al*. 1998). Therefore, radiation must have played an important role in the development of *RET*/*PTC* and PTC in Chernobyl nuclear accident victims. However, it has long been known that childhood radiation exposure is associated with a higher incidence of thyroid cancer (Duffy & Fitzgerald 1950, Wood *et al*. 1969, Shore *et al*. 1985). Radioiodine exposure in fallouts from a thermonuclear test (Conard *et al*. 1970) and the Chernobyl accident (Kazakov *et al*. 1992) was followed by a significantly increased incidence of thyroid cancer and, as studied and revealed in the latter case, *RET*/*PTC* primarily in child victims. The finding that young age is a risk factor for the development of *RET*/*PTC*-positive PTC even in non-radiation-exposed children additionally supports the possibility that young age itself predisposes to *RET*/*PTC* development through an unidentified mechanism. It is possible that young age may predispose *RET*/*PTC*-harboring PTC to more rapid growth and progression so PTC harboring this genetic alteration may tend to be caught clinically early in life. It would be consistent with this idea to confirm, in a large series of tumors, that the tumor size of *RET*/*PTC*-positive PTC in the pediatric population is larger than that of *RET*/*PTC*-positive PTC in the adult population.

In contrast to the association of young age with *RET*/*PTC*, the studies on *BRAF* mutation in adult and pediatric populations summarized above clearly show that old age is a predisposing factor for the development of *BRAF* mutation and PTC harboring this mutation. The prevalence of *BRAF* mutation in PTC was similarly high in radiation-exposed and non-exposed adult patients (Xing *et al*. 2004*b*). In an adult population, Nikiforova *et al*. (2003) further showed a significant association of *BRAF* mutation with older age. The study on adult patients by Xu *et al*. (2003) also showed a clear tendency of association of *BRAF* mutation with older age, although no statistical significance was reached. Other studies on adult patients did not reveal a specific age predilection of *BRAF* mutation. In most of these studies, however, the number of study subjects was small or the age range of the study subjects was not sufficiently wide and evenly distributed to reveal a clear association between age and the *BRAF* mutation. The fundamental basis for this link between older age and the development of *BRAF* mutation remains unclear. It also remains uncertain whether *BRAF* mutation-harboring PTC is more slowly growing than *RET*/*PTC*-harboring PTC so that the former tends to be caught clinically later in life. If proven to be the case, this could at least partially explain the reciprocal age distribution of *BRAF* mutation and *RET*/*PTC* rearrangements, at least in the non-radiation-exposed population. Regardless of the underlying mechanism, there appears to be an age window below which *RET*/*PTC* tends to occur or to be identified and above which *BRAF* mutation tends to

Table 4 *BRAF* mutation in thyroid fine-Needle aspiration biopsy (FNAB) specimens

	Frequency (mutation/total (%))						
	Histological diagnosis of the nodule			Cytologically indeterminate			
Report	PTC	FTC	Benign	Cancer	Benign	Total	**Reference**
1	22/54 (41)	0/2 (0)	0/32 (0)	5/32 (16)	0/23 (0)	5/55 (9)	Cohen *et al.* 2004
2	26/69 (38)	–	0/27 (0)	4/15 (27)	0/19	4/34 (12)	Salvatore *et al.* 2004
3	8/16 (50)	0/6 (0)	0/21 (0)	2/14 (14)	0/12 (0)	2/26 (8)	Xing *et al.* 2004*c*
4	–	–	–	–	–	2/45 (4)	Baloch *et al.* 2004*
5	30/58 (51)	–	–	1/8 (13)	–	1/8 (13)	Hayashida *et al.* 2004
Overall	86/197 (44)	0/8 (0)	0/80	12/69 (17)	0/54 (0)	14/168 (8)	

*This report is an abstract without complete information at this time, and their data cannot be included fully for discussion in this review.

occur or to be identified. The data currently available suggest that in most patients, this age window is likely to occur around the late teenage years, but the definition of the precise age range will need a large series of patients with a wide and evenly distributed age range. Knowing this age window may help predict the type of genetic alteration that a patient's thyroid cancer may harbor.

The diagnostic value of *BRAF* mutation in thyroid cancer

Thyroid nodules are common, and are palpable in approximately 5% of normal adults (Vander *et al.* 1968) and visualized by sonography in one-third or more of normal adults (Brander *et al.* 1991, Bruneton *et al.* 1994). As about 5–8% of palpable thyroid nodules are cancerous, a major task of the initial evaluation of thyroid nodules is to rule out malignancy (Werk *et al.* 1984, Belfiore *et al.* 1989). Thyroid fine-needle aspiration biopsy (FNAB) with cytological analysis is a widely used initial diagnostic measure in thyroid nodule evaluation (Hegedus 2004). However, at least 20% of biopsies yield indeterminate cytological findings that cannot distinguish between thyroid cancer and benign tumors with certainty, leaving uncertain the optimal management for these patients (Gharib *et al.* 1984, Sclabas *et al.* 2003). As the T1799A *BRAF* mutation occurs exclusively in PTC with a high prevalence, but not in benign thyroid neoplasms (Table 1), it is a specific diagnostic marker for thyroid cancer. Several studies have been conducted to evaluate the diagnostic applicability of *BRAF* mutation detection on FNAB specimens (Baloch *et al.* 2004, Cohen *et al.* 2004, Hayashida *et al.* 2004, Salvatore *et al.* 2004, Xing *et al.* 2004*c*). Most of these studies were retrospective, in which *BRAF* mutation was analyzed on FNAB specimens retrieved from existing cytological slides and in which the *BRAF* mutation status was correlated with the pre-established histopathological diagnoses of the tumors. The study by Xing *et al.* (2004*c*) was a prospective one, in which FNAB was performed, *BRAF* mutation analyzed preoperatively, and the results then correlated prospectively with the post-operative histological diagnosis of the biopsied thyroid nodule. Regardless of the detection methods used, all these studies demonstrated excellent accuracy and simplicity of *BRAF* mutation detection on FNAB specimens. For *BRAF* mutation-positive PTC, the diagnostic specificity and sensitivity of *BRAF* mutation detection on FNAB specimens were 100% in these studies. Consistent with the studies on primary tumors, in FNAB specimens, *BRAF* mutation was found only in histologically-proven PTC, but not in FTC and benign thyroid tumors (Table 4). The overall prevalence of *BRAF* mutation in PTC in these FNAB studies was 44%, similar to the generally reported prevalence of this mutation (Table 1). It is therefore expected that, as demonstrated by these FNAB studies (Table 4), nearly half of patients with PTC can be diagnosed solely based on *BRAF* mutation analysis on FNAB specimens. If the diagnostic reliability of this *BRAF* mutation approach is confirmed in more studies, PTC diagnosed solely based on *BRAF* mutation detection will probably not need further diagnostic cytology studies. *BRAF* mutation detection is robust and low in cost (Xing *et al.* 2004*c*), particularly if it can be done in a centrally coordinated laboratory with appropriate methods. In view of the high prevalence of both *BRAF* mutation and PTC, the elimination of the need for cytology examination in nearly half of the patients with PTC undergoing FNAB evaluation could be substantially cost-saving. Moreover, *BRAF* mutation detection may allow for more specific diagnosis of PTC as inter-observer variations in interpreting the cytology patterns of

FNAB specimens do exist (Greaves *et al*. 2000, Al-Shaikh *et al*. 2001). In fact, this point is well illustrated by the study of Baloch *et al*. (2004), in which 13% (seven of 53) of FNAB specimens cytologically read as benign and 7% (one of 14) of FNAB specimens read as thyroiditis were positive for *BRAF* mutation and the diagnoses were able to be corrected to PTC by mutation analysis.

Nevertheless, *BRAF* mutation detection alone on FNAB specimens is unlikely to solve the diagnostic dilemma of indeterminate cytology on FNAB. As summarized in Table 4, 17% of thyroid cancers with indeterminate cytology can be diagnosed by *BRAF* mutation analysis. When all the cases with indeterminate cytology were evaluated as a whole, only a small portion (8%) of the patients could be diagnosed with *BRAF* mutation detection. This is because the majority of thyroid tumors with indeterminate cytology are benign thyroid neoplasms harboring no *BRAF* mutation and only about 15% of thyroid tumors with indeterminate cytology prove to be PTC (Sclabas *et al*. 2003). Given the overall prevalence of *BRAF* mutation of around 45% in PTC (Table 1), 15% as PTC of the cytologically indeterminate cases can be translated into about 7% that will be positive for *BRAF* mutation, consistent with the *BRAF* mutation rate found on indeterminate cytological specimens in the several recent reports (Table 4). Moreover, many of the thyroid cancers with indeterminate cytology, particularly those with follicular neoplasm patterns, are FTC and follicular-variant PTC, with the former harboring no *BRAF* mutation and the latter carrying the mutation at a very low prevalence (Tables 1 and 2). Obviously, a positive *BRAF* mutation has a perfect positive predictive value and can establish the diagnosis of PTC, but a negative result in a specific patient will not be of any diagnostic value. It remains to be demonstrated definitively how effective *BRAF* mutation analysis on thyroid FNAB can truly be in addressing the diagnostic dilemma of indeterminate cytology. Nearly 300 000 new thyroid nodules are detected annually in the United States (Castro & Gharib 2000). If all of these thyroid nodules are to be evaluated with FNAB, approximately 90 000 (assuming a 30% rate of indeterminate cytology) of them may yield indeterminate cytological findings. With a diagnostic sensitivity of 8% (Table 4) for *BRAF* mutation detection on cytologically indeterminate FNAB, about 7200 patients per year in the United States could be helped with a definitive diagnosis of PTC by this technique and the optimal management of these patients could be pursued. Practically, it may be worth testing *BRAF* mutation on readily retrievable FNAB specimens from cytology slides when conservative follow-up of a cytologically indeterminate thyroid nodule is clinically debatable in a patient. The combination of *BRAF* mutation with additional sensitive and specific molecular markers will likely be the next step in increasing the FNAB diagnostic sensitivity. This approach was tested recently by combined use of *BRAF* mutation with *RET/PTC* (Salvatore *et al*. 2004), a process which did indeed improve the diagnostic sensitivity. However, the diagnostic specificity of this approach needs to be further investigated on large studies as *RET/PTC* is sometimes found in benign thyroid tumors (Nikiforov 2002, Santoro *et al*. 2002, Tallini 2002). Combined use of *BRAF* mutation with *Ras* mutation in conjunction with FNAB to diagnose thyroid cancer is also being investigated (Baloch *et al*. 2004), but a similar diagnostic specificity limitation also potentially exists as *Ras* mutations are also frequently seen in benign thyroid neoplasms (Tallini 2002, Vasko *et al*. 2003).

As cancer cells can dislodge into the bloodstream, efforts have been made to establish sensitive methods to detect *BRAF* mutation that could potentially be used on serum DNA samples. The technique of single-stranded DNA conformation polymorphism was recently used to detect *BRAF* mutation in plasma DNA from thyroid cancer patients, but apparently failed to provide sufficient sensitivity (Vdovichenko *et al*. 2004). Real-time allele-specific amplification for detection of the *BRAF* mutation was tested, which allowed detection of 1% mutated allele in a DNA sample (Jarry *et al*. 2004), a sensitivity that is unlikely to be sufficient for detection of mutated *BRAF* allele in blood samples. Lilleberg *et al*. (2004) recently reported the use of mutant allele-specific PCR amplification followed by detection with a denaturing HPLC platform that uses post-separation fluorescence technology to detect mutated alleles that represent $<0.1\%$ of the total analyzed DNA. With this method, the authors were able to scan for *BRAF* mutation as well as various *ras* mutations in plasma DNA from patients with colon cancer with 100% sensitivity. It remains to be tested whether this method can also be applied to thyroid cancer patients. The gap ligase chain-reaction technique was demonstrated to be a more sensitive method and could detect point mutations in the presence of up to 10 000-fold excess of wild-type allele DNA (Abravaya *et al*. 1995). A modified version of this method specifically for *BRAF* point mutation was developed recently and, with its high sensitivity, was used to rule out *BRAF* mutation in primary biliary tract cancers (Goldenberg *et al*. 2004). It would be interesting to see whether this stable and sensitive

method could reliably detect *BRAF* mutation in the serum DNA of thyroid cancer patients or other *BRAF* mutation-positive cancer patients. Using an even more sensitive sequence-specific real-time PCR technique, Rosenberg *et al*. (2004) were able to detect one heterozygous *BRAF* mutation-positive cell mixed in 21 692 normal cells. When applying it to blood samples, the authors were able to identify circulating *BRAF* mutation in one of five PTC patients tested. This encouraging method needs to be validated in a larger study. It is hoped that a sensitive and specific method to detect *BRAF* mutation in the blood, which could simplify the diagnostic evaluation of a large number of patients undergoing thyroid nodule evaluation, will be established in the near future; a positive *BRAF* mutation test on the blood may spare the patient from FNAB and other diagnostic procedures and prompt direct surgical treatment.

Prognostic value of *BRAF* mutation in thyroid cancer

Because *BRAF* mutation plays an important role in PTC tumorigenesis, it is conceivable that this mutation is a determinant of clinical and pathological behaviors of PTC and could be a novel prognostic factor for this cancer. The relationship between this mutation and the clinicopathological outcomes of PTC have been investigated in several studies (Namba *et al*. 2003, Nikiforova *et al*. 2003, Xu *et al*. 2003, Fugazzola *et al*. 2004, Kim *et al*. 2004, Puxeddu *et al*. 2004; M. Xing *et al*. unpublished results). In a series of 104 PTCs, comprised mainly of American patients, Nikiforova *et al*. (2003) reported a significant association of *BRAF* mutation with extrathyroidal invasion [(16/38 (42%) with *BRAF* mutation versus 13/66 (20%) without mutation, $P=0.03$)] and advanced stages [(for stage III, 10/38 (26%) with *BRAF* mutation versus 2/66 (3%) without mutation, $P=0.006$; for stage IV, 7/38 (18%) with *BRAF* mutation versus 3/66 (4%) without mutation, $P=0.03$)] of the primary tumor at the time of initial surgery. In a Japanese series of 126 PTCs, Namba *et al*. (2003) found a significant association of *BRAF* mutation with advanced stages of the tumor and distant metastasis [(7/38 (18%) with *BRAF* mutation versus 5/88 (6%) without mutation, $P=0.033$)]. In a recent Korean study, *BRAF* mutation was found to be significantly associated with neck lymph node metastasis [(39/58 (67%) with *BRAF* mutation versus 4/12 (33%) without mutation, $P=0.048$] (Kim *et al*. 2004). In two Italian studies (Fugazzola *et al*. 2004, Puxeddu *et al*. 2004) and an American study by Xu *et al*. (2003), no significant association of *BRAF* mutation with any of the common high-risk pathological characteristics was revealed. The number of the cases examined in these latter three studies was much smaller, however, ranging from 56 to 60. In some of the studies (Nikiforova *et al*. 2003, Xu *et al*. 2003, Fugazzola *et al*. 2004), a trend in association between *BRAF* mutation and lymph node metastasis was observed but did not achieve statistical significance. In the study by Fugazzola *et al*. (2004), a higher but non-significant recurrence rate of thyroid cancer was found to be associated with *BRAF* mutation. Our recent study on a large series of PTCs demonstrated a significant association of *BRAF* mutation with extrathyroidal invasion, lymph node metastasis, advanced tumor stages, and cancer recurrence, which still existed on multivariate analysis even with adjustment for all the common confounding clinicopathological factors (M Xing *et al*. unpublished results). In this study, we found a thyroid cancer recurrence rate of 25% in *BRAF* mutation-positive patients versus 9% in *BRAF* mutation-negative patients ($P=0.004$). Interestingly, we also observed a significantly higher incidence of the loss of radioiodine avidity in the recurrent thyroid cancer when *BRAF* mutation was positive, suggesting that *BRAF* mutation may not only predict a higher incidence of thyroid cancer recurrence but also predict a poorer response of recurrent thyroid cancers to radioiodine treatment. Only one study (Xu *et al*. 2003) showed an association of *BRAF* mutation with (male) gender. None of the studies showed association of *BRAF* mutation with larger tumor size, suggesting that *BRAF* mutation increases the aggressiveness of PTC by promoting its invasiveness, metastasis, and recurrence, but not growth in size of the primary tumor.

Most of the studies on the relationship between *BRAF* mutation and the clinicopathological outcomes of PTC were conducted without subtype stratification of PTC. As discussed above, *BRAF* mutation occurs mostly in conventional and tall-cell PTC and uncommonly in follicular-variant PTC. Compared with conventional PTC, follicular-variant PTC is infrequently associated with high-risk pathological characteristics such as lymph node metastasis and extrathyroidal invasion. Therefore, the inconsistent results from different reports on the association of *BRAF* mutation with high-risk pathological characteristics could be partially due to different combinations of various subtypes of PTC that were included in the study. For example, a significant association of *BRAF* mutation with high-risk pathological factors could be shown on a series of PTC that is comprised of certain proportions

of follicular-variant PTC and conventional PTC, while this association may be lost on analysis within a specific subtype of PTC, particularly when the sample number is small. This illustrates the importance of the use of multivariate analysis with adjustment for various confounding factors, including histological subtypes of PTC, as we did recently to establish an independent prognostic role of *BRAF* mutation (M Xing *et al*. unpublished results). As the *BRAF* mutation is so prevalent in conventional or tall-cell PTC, a large series of such cases may be needed to reveal an association of *BRAF* mutation with poorer clinicopathological outcomes within these subtypes of PTC. A recent Korean study by Kim *et al*. (2004) focused specifically on conventional PTC and showed a significant association of *BRAF* mutation with lymph node metastasis. Overall, the data available to date support the idea that *BRAF* mutation is an independent prognostic factor that predicts a poorer prognosis of PTC. As mentioned above, the demonstration of BRAF mutant-induced development of PTC and its transition into ATC in transgenic mice (Knauff *et al*. 2004) is consistent with the clinical findings on the role of *BRAF* mutation in predicting a poor outcome of PTC.

Whether to treat a PTC patient with radioiodine, and how vigilantly and aggressively to guard against recurrence, are often questions without straightforward clinical answers. Use of *BRAF* mutation status may help clarify such clinical situations and assist clinical decision making. It is expected that *BRAF* mutation may also be useful in risk and prognostic evaluation of micro PTC. Although this type of thyroid cancer is generally thought to be indolent and associated with a relatively good prognosis, local and distant metastasis and recurrence do occur, and no specific independent prognostic clinicopathological factors were identified on multivariate analysis for this type of PTC (Chow *et al*. 2003). As *BRAF* mutation often occurs in micro PTC as well (Nikiforova *et al*. 2003, Sedliarou *et al*. 2004, Trovisco *et al*. 2004), it would be interesting to investigate *BRAF* mutation as an independent prognostic factor to help manage these patients more appropriately.

As *BRAF* mutation can be readily analyzed on FNAB specimens (Baloch *et al*. 2004, Cohen *et al*. 2004, Salvatore *et al*. 2004, Xing *et al*. 2004*c*), preoperative *BRAF* mutation analysis, in conjunction with routine FNAB cytology study, could help surgeons better tailor their surgical procedures by helping them choose, for instance, between vigilant exploration and resection of suspicious regional lymph node and no neck dissection, and between total thyroidectomy and lobectomy. The current standard prognostic evaluation of thyroid cancer is based largely on clinicopathological criteria, which is often incomplete, particularly preoperatively, when the pathological characteristics of the tumor are not known. *BRAF* mutation represents the first molecular marker that can be used, even preoperatively, for more efficient prognostic evaluation and clinical management of PTC. Therefore, it may be reasonable to examine *BRAF* mutation on preoperative FNAB specimens for every patient not only for diagnostic purposes, but also for risk evaluation. In this sense, *BRAF* mutation may be examined on FNAB specimens even if a diagnosis of PTC is already known based on cytological studies. This approach may assist clinicians in optimizing both the short-term (surgical) and long-term (medical) management of their thyroid cancer patients.

Therapeutic potential of inhibiting the MAP kinase pathway using novel inhibitors in thyroid cancer

Although thyroid cancer is usually indolent and curable with the current standard treatments of surgery, often followed by adjuvant radioiodine therapy, there remain many patients whose conditions are incurable, disabling, and even fatal. The most difficult cases, for which there is no effective current treatment, are those that are inoperable and have lost radioiodine avidity. This includes ATC, which is often positive for *BRAF* mutation (Table 1). A novel effective treatment is needed desperately for these patients (Sherman 2003). As activation of the MAP kinase pathway by various genetic alterations, including *BRAF* mutation, plays a pivotal role in thyroid tumor genesis and progression, efforts targeted at inhibiting this pathway may lead to development of novel effective therapy for thyroid cancer.

A therapeutic approach targeted at the Raf kinases has been tested for human cancers using specific inhibitors with encouraging results in *in vitro* cell studies and *in vivo* animal studies (Wilhelm & Chien 2002, Bollag *et al*. 2003, Dumas *et al*. 2004). Among these inhibitors, the Bay 43–9006 compound seems to be a promising one as it has excellent safety profile in human subjects and effectiveness in inhibiting Raf kinases (Bollag *et al*. 2003, Lee & McCubrey 2003). The Bay 43–9006 compound is in several clinical trials at various phases targeted at several types of human cancer (Lee & McCubrey 2003). Although this compound most potently inhibits the C-type Raf kinase, it also has excellent potency in inhibiting

wild-type and V600E mutant BRAF kinases (Karasarides *et al*. 2004, Wan *et al*. 2004). X-ray crystallography has recently demonstrated the binding of this inhibitor with the kinase domain in both the wild-type and V600E BRAF kinases (Garnett & Marais 2004, Wan *et al*. 2004). By binding with the kinase domain of BRAF, Bay 43–9006 locks the kinase in an inactive state. Treatment with this compound can block kinase signaling downstream of Raf kinase, inhibit BRAF-stimulated DNA synthesis and cell proliferation, induce apoptosis in melanoma cells harboring *BRAF* mutation, and delay the growth of melanoma tumor xenografts in mice (Karasarides *et al*. 2004). A recent preliminary study by Kumar *et al*. (2004) has shown that Bay 43–9006 can inhibit the growth and proliferation, and induce apoptosis, of KAT-5 cells, a PTC-derived cell line harboring the *BRAF* mutation. As BRAF is the predominant type of Raf kinase in follicular thyroid cells (Fagin 2004) and as *BRAF* mutation is highly prevalent in PTC (Table 1), strategies targeted at inhibition of BRAF may be particularly effective for the treatment of PTC. Several other MAP kinase pathway inhibitors acting at steps other than Raf kinases have also been developed, including MEK inhibitors (Sebolt-Leopold 2004). A good example is the MEK-specific inhibitor CI-1040, which is the first MEK-targeted drug candidate to undergo clinical trials, although monotherapy with this drug in some cancers did not clearly prove to be effective on a multicenter phase II study (Rinehart *et al*. 2004). It remains to be investigated whether these MAP kinase pathway inhibitors may have therapeutic effects in thyroid cancer patients.

Several earlier studies demonstrated that the transformation of thyroid cells with *ras* oncogene induced loss of expression of thyroid-specific proteins such as thyroid-stimulating hormone (TSH) receptor (TSHR) (Berlingieri *et al*. 1990) and thyroglobulin (Avvedimento *et al*. 1991). A recent study by Knauf *et al*. (2003) demonstrated that acute expression of RET/PTC3, H-Ras, or constitutively activated MEK-1 could all block TSH-induced expression of thyroglobulin and sodium-iodide symporter (NIS) in PCCL3 thyroid cells. This study also demonstrated that treatment of cells with MEK inhibitors could restore the expression of thyroglobulin and NIS. Interestingly, the transgenic mice in which development of PTC and its transition to ATC were induced by V600E *BRAF* mutation had absent or decreased expression of thyroglobulin and developed hypothyroidism (Knauf *et al*. 2004). Normal expression of these thyroid-specific molecules is essential for the unique function of thyroid cells to take up and metabolize iodide and synthesize thyroid hormones (Nilsson 2001). It therefore appears that silencing of thyroid-specific genes by aberrant activation of the MAP kinase pathway may be the basis for the loss of radioiodine avidity seen clinically in some thyroid cancer patients. Aberrant methylation was shown to be a mechanism for silencing some of the thyroid-specific genes involved in iodide metabolism, including those for NIS (Venkataraman *et al*. 1999), TSHR (Xing *et al*. 2003*a*), and pendirn (Xing *et al*. 2003*b*) in thyroid cancer. It is thus plausible to propose that inhibiting the MAK kinase pathway could reverse the aberrant methylation of these genes and restore their expression and the lost iodide-concentrating ability of thyroid cancer cells. In this sense, the MAP kinase pathway inhibitors could be particularly useful as a conjunction therapy with radioiodine treatment of those patients whose thyroid cancers have decreased or lost radioiodine avidity. These hypotheses need to be tested.

The MAP kinase pathway-activating *BRAF* mutation, *ras* mutations, *RET/PTC*, and *RASSF1A* methylation may together account for nearly all follicular epithelial cell-derived thyroid cancers (Xing *et al*. 2004*a*), and these common genetic alterations may all induce thyroid tumor genesis and progression through the MAP kinase pathway, either entirely or partially. Therefore, the MAP kinase pathway inhibitors may be effective in treating a wide range of thyroid cancers, irrespective of *BRAF* mutation status. With the proven safety profiles of the Raf kinase inhibitor Bay 43–9006 and the MEK inhibitor CI-1040 in clinical trials on other cancers, a well-designed phase II clinical trial on these novel MAP kinase pathway inhibitors is now needed for thyroid cancer patients, particularly for those with incurable disease. Before such a clinical trial is conducted, more preclinical studies on the anticancer effects of these compounds in thyroid cancer cell lines and tumor xenograft animal models will provide important implications and necessary support for such clinical trials.

Summary and future directions

The discovery of the *BRAF* mutation in thyroid cancer represents one of the most important recent advancements in thyroid cancer research and is of significant clinical potential in thyroid cancer medicine. Since the initial report on *BRAF* mutation in thyroid cancer nearly 2 years ago, rapid progress has occurred due to an explosion of research in this area. The T1799A *BRAF* mutation is the most common activating genetic alteration in thyroid cancer. Advancements have also been made in understanding the relationship between

BRAF mutation and other common genetic alterations in thyroid cancer, and of particular note is the discovery of *BRAF* mutation's mutual exclusivity with other well-established genetic alterations, a finding which points toward an independent role of *BRAF* mutation in thyroid tumorigenesis. The results from transgenic mouse studies have unequivocally established the role of *BRAF* mutation in the initiation and progression of PTC. The reciprocal age association between *BRAF* mutation and *RET/PTC* rearrangements is interesting, although it remains without a clear explanation at this time. More recent studies have been focused on the clinical significance of the *BRAF* mutation, particularly its diagnostic and prognostic values. As the T1799A *BRAF* mutation does not occur in benign thyroid tumors, it is a specific diagnostic marker for thyroid cancer when used in conjunction with FNAB, albeit with a low sensitivity for cases with indeterminate cytology. The association of *BRAF* mutation with poor clinicopathological outcomes, demonstrated by several relatively large studies, has established that this mutation is a novel prognostic molecular marker and may add a new dimension to the conventional risk evaluation of thyroid cancer. Preoperative knowledge of the *BRAF* mutation status of the thyroid tumor, through mutation analysis on FNAB cytological specimens, may be particularly valuable as it can assist clinicians in more efficiently planning and optimizing both the short- and long-term managements of thyroid cancer patients. Studies aimed at the therapeutic potential of novel inhibitors of the MAP kinase pathway for the treatment of thyroid cancer may yield important breakthroughs.

Further work is needed in the following several areas: (1) the elucidation of the specific molecular and cellular alterations and events that are caused by *BRAF* mutation and MAP kinase pathway activation in thyroid cancer; (2) the possible restoration of the ability of thyroid cancer cells to metabolize iodide by interfering with *BRAF* mutation-initiated aberrant signaling; (3) the improvement of the diagnostic utility of *BRAF* mutation, possibly through combination with other specific molecular markers for thyroid cancer in conjunction with FNAB, and through the establishment of a *BRAF* mutation-based blood test; (4) the clinical application of the prognostic value of *BRAF* mutation in guiding the optimal short- and long-term managements of thyroid cancer patients and (5) further preclinical and clinical studies on the therapeutic potential of novel inhibitors of MAP kinase pathway. It is anticipated that rapid advancements in these areas will occur in the next few years.

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