**Molecular and Cellular Pathobiology**

**Mutationally Activated BRAF**<sub>V600E</sub>** Elicits Papillary Thyroid Cancer in the Adult Mouse**

Roch-Philippe Charles<sup>1,2</sup>, Gioia Iezza<sup>3</sup>, Elena Amendola<sup>4</sup>, David Dankort<sup>1,2</sup>, and Martin McMahon<sup>1,2</sup>

**Abstract**

Mutated BRAF is detected in approximately 45% of papillary thyroid carcinomas (PTC). To model PTC, we bred mice with adult-onset, thyrocyte-specific expression of BRAF<sup>V600E</sup>. One month following BRAF<sup>V600E</sup> expression, mice displayed increased thyroid size, widespread alterations in thyroid architecture, and dramatic hypothyroidism. Over 1 year, without any deliberate manipulation of tumor suppressor genes, all mice developed PTC displaying nuclear atypia and marker expression characteristic of the human disease. Pharmacologic inhibition of MEK1/2 led to decreased thyroid size, restoration of thyroid form and function, and inhibition of tumorigenesis. Mice with BRAF<sup>V600E</sup>-induced PTC will provide an excellent system to study thyroid tumor initiation and progression and the evaluation of inhibitors of oncogenic BRAF signaling. *Cancer Res; 71(11); 1–9. ©2011 AACR.*

**Introduction**

Thyroid malignancies are the most common tumors of the endocrine system with approximately 45,000 newly diagnosed cases estimated in the United States in 2010 (1). Although thyroid cancer is often indolent, there is concern about its rapidly increasing incidence, especially among women (2). Of the various histologic subtypes, papillary thyroid carcinoma (PTC) represents approximately 80% of all cases. Although surgery combined with radioiodine therapy is often curative, a better understanding of how thyroid cancer genetics influences the pathophysiology and therapy of this disease is required.

Of the somatic genetic alterations detected in PTC, mutational activation of BRAF is most common (~45%) and often associated with more aggressive disease (3). As observed in melanoma and colon cancer, the most common mutation is a transversion in exon 15 that encodes BRAF<sup>V600E</sup> (4). Once mutationally activated, the BRAF<sup>V600E</sup>→MEK→ERK MAP kinase signaling pathway elicits alterations in gene expression that contribute to the aberrant behavior of the cancer cell. Moreover, recent data suggest BRAF<sup>V600E</sup> is required for PTC maintenance because pharmacologic inhibition of BRAF<sup>V600E</sup> by PLX-4032 in thyroid cancer patients led to tumor regression (5).

We have previously described the utility of Braf<sup>CA</sup> mice carrying a Cre-activated allele of *Braf* to model lung cancer (6) and melanoma (7). By using mice with thyrocyte-specific expression of a conditional Cre recombinase (CreER<sup>T2</sup>) under the control of the thyroglobulin promoter (*Thyroglobulin::CreER<sup>T2</sup>*), we explored the consequences of induced BRAF<sup>V600E</sup> expression in adult thyroid. Shortly after BRAF<sup>V600E</sup> expression, mice displayed signs of hypothyroidism accompanied by striking alterations in the size and architecture of thyroid follicles. Over a 6- to 9-month period, all of these mice developed PTC, displaying phenotypic characteristics of the cognate human disease. Moreover, treatment of the mice with a pharmacologic MEK inhibitor elicited a striking reduction in thyroid size, restoration of thyroid hormone production, and inhibition of tumorigenesis. Importantly, because of leaky activity of CreER<sup>T2</sup>, untreated *Thyroglobulin::CreER<sup>T2</sup>*; Braf<sup>CA</sup> mice developed PTC without displaying hypothyroidism, albeit with delayed kinetics compared with tamoxifen-treated mice. These data suggest that, unlike in the lung and skin in which BRAF<sup>V600E</sup> induces a clearly defined stage of benign tumorigenesis, BRAF<sup>V600E</sup> can promote thyroid cancer progression without deliberate manipulation of tumor suppressor genes. Moreover, this system shows utility in modeling the response of PTC to pharmacologic inhibition BRAF<sup>V600E</sup>→MEK→ERK signaling.

**Materials and Methods**

**Mouse breeding and manipulation**

*Braf<sup>CA</sup>* mice were described previously (6, 7). *Thyroglobulin::CreER<sup>T2</sup>* (*Thyroglobulin::CreER*) mice were generated by conventional transgenic technology and will be described in full elsewhere (Amendola and colleagues, manuscript in preparation). Thyrocyte-specific activation of CreER<sup>T2</sup> activity was achieved by
intraperitoneal injection of 100 µL of a 10 mg/mL stock of tamoxifen dissolved in peanut oil into adult (~30 days old) mice.

**Histology and immunofluorescence of mouse thyroid tissue sections**

Mice were euthanized by aortic dissection and thyroids removed, rinsed in ice cold PBS, and fixed for 4 hours in Z-Fix (Anatech). Four to 5 µm sections of formalin-fixed, paraffin-embedded tissues were stained with Hematoxilin & Eosin or processed for immunofluorescence with epitope unmasking carried out by boiling slides for 10 minutes (10 mmol/L Tris, 0.5 mmol/L EGTA, pH 9.0). Primary antibodies were obtained from the listed commercial sources: α-TTF-1 (thyroid transcription factor-1, 1:200; Santa Cruz Biototechnology, Inc.), α-Ki67 (1:300; Abcam), α-CK19 (cytokeratin-19, 1:300, TROMA-III; Hybridoma Bank, University of Iowa), and α-Galectin-3 (Gal-3, 1:200; Abcam), α-HMG2A (high mobility group AT-hook 2, 1:700; BioCheck). Primary antibody binding was detected by using either goat α-rabbit Alexa-488 (1:500) or goat α-rat Alexa-488 (1:500; Molecular Probes) and then counterstained with 4',6-diamidino-2-phenylindole (DAPI).

**Immunoblotting**

Snap-frozen thyroid specimens were extracted by using a TissueLyser (Qiagen) in 1% (v/v) Triton X-100, 20 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl, 1% (v/v) Glycerol, 1 mmol/L EDTA, and 1 mmol/L EGTA buffer supplemented with Halt protease/phosphatase inhibitor cocktail (Pierce). Western blots of cell extracts were probed with Halt protease/phosphatase inhibitor cocktail (Pierce).Primary antibody binding was detected by using either goat α-rabbit Alexa-488 (1:500) or goat α-rat Alexa-488 (1:500; Molecular Probes) and then counterstained with 4',6-diamidino-2-phenylindole (DAPI).

**Serum TSH and T4 measurements**

Mouse serum (0.5–1 mL) was collected from retro-orbital bleeds at the time of euthanasia. Serum thyroid stimulating hormone (TSH) and 3,5,3',5'-tetraiodothyronine (T4) were measured by using specific radioimmuno assays (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA).

**Drug administrations**

A suspension of the MEK1/2 inhibitor, PD0325901, was prepared by sonication in 0.5% (w/v) Hydroxy-Propyl-Methylcellulose (Sigma), 0.2% (v/v) Tween-80 that was prepared fresh every week. PD0325901 was administered to mice by oral gavage at 12.5 mg/kg for 4 weeks. Triiodothyronine (T3; Sigma) was supplemented into drinking water at 100 ng/mL with 1% (w/v) sucrose. Effective daily dose was estimated at 100 to 200 ng/mouse/day on the basis of mouse water consumption of 1 to 2 mL/day/mouse.

**Ultrasound imaging**

Mice were anesthetized by using 2% (v/v) isoflurane and fur around the neck was removed by using Veet depilatory cream. Ultrasound images were collected weekly by using the Vevo 770 system from VisualSonic. Thyroid size was assessed by counting pixels at the largest diameter of the thyroid by using ImageJ (NIH) and converting pixel count into area (mm²) by using scaling software internal to the device. Differences in thyroid size were plotted in relation to the area of the largest diameter of the thyroid at the start of the drug administration period (d0) compared with the end (d32).

**Results**

**Expression of BRAFV600E, but not KRASG12D, induces PTC in adult mice**

To assess the consequences of BRAFV600E expression in adult mouse thyrocytes, we generated compound Thyroid Peroxidase (TPO)::Cre; BrafC/A mice such that expression of BRAFV600E is initiated during thyroid development under the influence of the TPO::Cre transgene (8). Although TPO::Cre; BrafC/A mice were born at normal Mendelian frequency, they displayed low birth weight and a general failure to thrive, suggesting that BRAFV600E expression had deleterious developmental effects during mouse embryogenesis. Consequently, we discontinued these studies.

To elicit oncogene expression in the adult thyroid, we utilized Thyro::CreERT2 mice in which expression of CreERT2 is controlled by the thyroid-specific thyroglobulin promoter. Because mutationally activated RAS genes are also detected in 10% to 15% of PTC, we generated cohorts of Thyro::CreERT2 mice carrying either our Cre-activated BrafC/A allele or a Cre-activated KrasG12D allele, so that we could compare and contrast the effects of oncogenic BRAFV600E to KRASG12D on thyrocytes (9). One-month-old mice of each genotype were administered tamoxifen to induce oncogene expression and then dissected at different times from 1 week to 12 months thereafter. Consistent with previous data, we observed no effect of KRASG12D expression on thyroid size in Thyro::CreERT2; KrasG12D mice at any time, even after repeated tamoxifen administration (Data not shown; ref. 10). By contrast, tamoxifen-treated Thyro::CreERT2; BrafC/A mice developed a dramatically enlarged, goiterous, hypercellular thyroid that was up to 10 times larger than controls 1 month and up to 300 times larger 12 months after BRAFV600E expression (Figs. 1A and B). Interestingly, untreated Thyro::CreERT2; BrafC/A mice also displayed increased thyroid volume, which may be a consequence of stochastic BRAFV600E expression due to leaky CreERT activity.

Histologic analysis of untreated BrafC/A mice revealed normal thyroid architecture with spherical follicles full of eosinophilic colloid, similar to human thyroid histology (Fig. 2A). However, within 7 days after tamoxifen treatment, Thyro::CreERT2; BrafC/A mice displayed dramatically enlarged follicles throughout the entire gland (Fig. 2B). Fourteen days after BRAFV600E expression, thyrocytes displayed a squamous morphology accompanied by a large increase in follicle size and a loss of colloid (Fig. 2C). Consistent with the high frequency of LacZ expression in thyrocytes in tamoxifen-treated Thyro::CreERT2; Rosa26R mice (data not shown), it is likely that the BrafC/A allele was rearranged to encode BRAFV600E in the majority of thyrocytes. Despite the
without effect, expression of BRAFV600E in thyrocytes of adult mice initiated the process of thyroid carcinogenesis. Moreover, unlike the lung epithelium or melanocytes in which expression of BRAFV600E elicits benign tumors, expression of BRAFV600E in the thyroid epithelium resulted in malignancy with 100% penetrance but without requiring investigator-initiated silencing of tumor suppressor genes.

Thyrocyte-specific BRAFV600E expression leads to hypothyroidism

Given the rapid and dramatic increase in thyroid size combined with the observed loss of colloid, we assessed effects of oncogenic BRAFV600E on thyroid endocrine function. One-month-old Thyro::CreERT2; Braf<sup>+</sup> mice were treated with tamoxifen and were euthanized 1 or 3 months later, with serum samples collected for analysis of the concentration of TSH and the most abundant thyroid hormone thyroxine or T4 by radioimmune assay. Both 1 and 3 months following expression of BRAFV600E, we detected a profound decrease in serum T4 and TSH levels to the lower limit of detection of the assay (Fig. 1C), indicating a deficit in the synthesis and/or secretion of thyroid hormones. Concomitant with decreased T4, we also detected a 100-fold increase in serum TSH at the same time points (Fig. 1D). The robust elevation of TSH is an expected homeostatic response of the pituitary to the striking decrease in serum T4, indicating a deficit in the synthesis and/or secretion of thyroid hormones. Concomitant with decreased T4, we also detected a 100-fold increase in serum TSH at the same time points (Fig. 1D).

Figure 1. BRAF<sup>V600E</sup> expression induces goiter which progresses to PTC. A, representative images of the thyroid gland from control Braf<sup>+</sup>/Cre– (left) or Thyro::CreER<sup>T2</sup>; Braf<sup>CA/+</sup> mice (right) 9.5 months after tamoxifen administration. B, quantification of average thyroid volume of mice of the indicated genotype either untreated or treated with tamoxifen as indicated. n, number of mice in each group. C and D, serum concentration of T4 (C) or TSH (D) in Thyro::CreERT2 mice that also carry either a Cre-activated Braf<sup>CA</sup> or KRasLSL allele as indicated. Tamoxifen-treated (+Tamox) or control (–Tamox) mice were euthanized at 30 or 90 days as indicated and serum samples prepared. Serum T4 or TSH were measured by radioimmune assay as described in Materials and Methods. Dotted lines indicate the concentration of T4 (µg/dL) or TSH (ng/mL) in normal mice. n, number of mice in each group.
BRAFV600E-induced PTC in the mouse expresses markers of human PTC

In addition to characteristic cytologic features, human PTCs express a number of marker proteins including Gal-3 and CK19, which are used in the clinical setting to identify lesions, aid diagnosis and stage disease (11). In addition, expression of HMGA2 is useful for distinguishing benign thyroid lesions from malignant ones (12). Many thyroid cancers display expression of TTF-1/NKX2.1, which is a master regulator of thyroid (13) and lung development (14). To determine whether BRAFV600E-induced mouse PTC express these markers, tissue sections from control and PTC-bearing Thyro::CreERT2; BRafCA mice (Fig. 3A as indicated). Whereas normal thyroid (Cre-) was largely negative for CK19, Gal-3, and HMGA2 expression (Figs. 2A and C), these proteins were readily detected in BRAFV600E-induced PTC (Cre+). As expected, TTF-1 expression was readily detected both in normal thyrocytes and in PTC (Fig. 3A), which further serves to confirm thyrocytes as the cells of origin of papillary tumors in the Thyro::CreERT2; BRafCA mice. Finally, as expected, normal thyrocytes displayed low Ki67 expression, consistent with the fact that these cells proliferate slowly. By contrast, BRAFV600E-induced PTC displayed readily detectable Ki67 in a high percentage of cells indicating sustained cell proliferation. Similar results were observed by measuring DNA synthesis following injection of mice with bromodeoxyuridine (data not shown).

To determine whether PTC developing in tamoxifen-treated Thyro::CreERT2; BRafCA mice displayed evidence of ERK1/2 MAP kinase pathway activation, protein extracts were

Figure 2. Histologic analysis of thyroid tissue. Control (Cre-) or Thyro::CreER (Cre+) mice carrying either a Cre-activated BRafCA or KRasLSL alleles were either untreated or treated with tamoxifen as indicated. At different times thereafter (1 week–12 months), mice were euthanized for analysis of formaldehyde-fixed, paraffin-embedded thyroid tissue by Hematoxylin and Eosin (H&E) staining. A, control BRafCA mice; B-G, tamoxifen-treated Thyro::CreER; BRafCA mice analyzed: 1 week (B); 2 weeks (C); 3 months (D); 6 months (E), or 12 months (F and G) after tamoxifen treatment. H, control KRasLSL mice. I, Thyro::CreER; KRasLSL mice analyzed 6 months after tamoxifen treatment. Magnification is 200× with the exception of G, which is 400×.
prepared 2 weeks following tamoxifen administration to 
BrafCA/+ mice that either did or did not carry the 
Thyro::CreER2 transgene (Fig. 3B). Western blots were probed with antisera against either phospho-ERK1/2 (pERK1/2) or total ERK1/2 (tERK1/2). In parallel, mice were treated with PD325901, a specific and selective MEK1/2 inhibitor prior to euthanasia (Fig. 3B). As expected, tamoxifen-induced (+ Tamox) thyrocyte-specific expression (Cre+) of BrafV600E led to an elevation of pERK1/2 compared with similarly treated controls (Cre+, + Tamox, Fig. 3B). Moreover, both BrafV600E-mediated elevation and baseline pERK1/2 was strongly inhibited by PD325901. Similar results were obtained in a second cohort of mice analyzed 4 weeks after tamoxifen administration. These data indicate that thyroid-specific expression of BrafV600E leads to elevated ERK1/2 MAP kinase signaling.

BrafV600E-induced elevation of serum TSH is not required for thyroid tumorigenesis

Previous research indicated that BrafV600E expression in the lung epithelium or in melanocytes leads to a clearly defined state of benign tumorigenesis in which initiated benign tumor cells are restrained in their progression to malignancy, unless tumor suppressor genes such as Trp53, Cdkn2a, or Pten are deliberately silenced (6, 7). By contrast, BrafV600E expression in thyrocytes seems to promote malignant progression to PTC without deliberate manipulation of tumor suppressors. One possible reason for this may be due to the highly elevated serum TSH which, acting through the TSH receptor and its downstream signaling pathways in BrafV600E expressing thyrocytes, may prevent the senescent phenotype associated with BrafV600E-induced benign tumor cells (6, 7).

To test this, we took advantage of the fact that the Thyro::CreER2 transgene displays 4-hydroxytamoxifen-independent activity in untreated mice. Consequently, these mice were predicted to have stochastic activation of BrafV600E in thyrocytes without the wholesale dysregulation of thyroid function that occurs in tamoxifen-treated Thyro::CreER2; BrafCA/+ mice. Consequently, we aged a cohort of 9 Thyro::CreER2; BrafCA/+ mice to 12 months and assessed them for evidence of thyroid tumorigenesis (Figs. 4A and B). In addition, serum samples were collected for analysis of TSH and T4 levels (Fig. 4C). At euthanasia, the thyroid of all 9 animals presented regions of papillary thyroid cancer with adjacent histologically normal thyroid architecture (Fig. 4A). Such thyroid lesions were genotype dependent in that they were only detected in compound
Thyro::CreER; BRafCA mice. Consistent with a diagnosis of PTC, all of the tumor lesions stained positive for CK19, Gal-3, HMGA2, TTF-1, and Ki67 (Fig. 4B, as indicated). Importantly, analysis of serum revealed no statistically significant elevation of TSH or decrease in T4 compared with controls (Fig. 4C). Although these results do not rule out a possible role for normal levels of serum TSH in promoting thyroid tumorigenesis, they indicate that the logarithmic elevation of TSH observed in tamoxifen-treated Thyro::CreER; BRafCA mice is not required for PTC development.

**Pharmacologic blockade of MEK inhibits BRAFV600E-induced thyroid tumorigenesis**

Genetically engineered mouse models of human cancer provide useful preclinical platforms for testing novel anticancer therapies (15). However, for such models to be relevant and useful, they must recapitulate key features of the genetics and histopathology of the cognate human disease. We have previously shown that pharmacologic inhibition of MEK1/2 by using PD325901 has potent antitumor activity in mouse models of BRAFV600E-induced lung tumorigenesis and melanoma (6, 7). Given the frequency of BRAF mutation in human PTC, we sought to test the same approach in this new mouse model of BRAFV600E-induced PTC.

To do so, we administered tamoxifen to a cohort of 17 adult Thyro::CreERT2; BRafCA/+- mice and aged them for a further 5 months at which time, and as expected, all of the mice were shown to have an enlarged thyroid by using ultrasound imaging. Mice were randomly assigned into 4 treatment groups: group 1 (4 mice) received vehicle control; group 2 (5 mice) were administered with PD0325901 by oral gavage; group 3 (4 mice) were treated with synthetic thyroid hormone (T3) in drinking water in an attempt to normalize TSH levels, and; group 4 (4 mice) were treated with a combination of PD325901 and T3. Treatments were administered for 1 month with effects on thyroid size monitored weekly (Fig. 5A) by ultrasound imaging and enumerated by pixel counting as described in the Materials and Methods (Fig. 5B). At the end of the treatment period, mice were euthanized and their
thyroid subject to histologic analysis for the presence of PTC (Fig. 5C). Serum TSH and T4 were analyzed as previously described (Fig. 5D).

Both vehicle- and T3-treated mice displayed a progressive 15% to 20% or 20% to 30% increase, respectively, in thyroid size over the 32 days observation period (Figs. 5A and B). Mice treated with T3 displayed a greater increase in thyroid size compared with vehicle, but the difference was not statistically significant. By contrast, mice treated with PD325901, either alone or in combination with T3, displayed a progressive decrease in thyroid size that reached approximately 60% of the starting size by the end of the treatment period. The addition of T3 did not influence the reduction in thyroid size elicited by PD325901. Results inferred by ultrasonography were confirmed by postmortem analysis of thyroid size (data not shown). It should be noted that although the thyroid of PD325901-treated mice displayed a 60% decrease in relation to the starting size, they did not return to that of normal animals. Indeed, at the end of the treatment period, the thyroid of these animals remained approximately twice that of normal mice. Nevertheless, compared with vehicle- or T3-treated mice, MEK1/2 inhibition had a dramatic inhibitory effect on the ability of BRAFV600E to promote increased thyroid size.

Histologic analysis of the variously treated animals showed that vehicle- or T3-treated animals presented with PTC with characteristic cytologic features and altered follicular...
architecture (Fig. 5C) as described above (Fig. 1). By contrast, and consistent with the inhibitory effects on thyroid size, PD325901-treated mice displayed a more normal appearing follicular architecture with an increased prevalence of normal follicles lined by cuboidal thyrocytes with no evidence of nuclear atypia (Fig. 5C). Moreover, the follicles of PD325901-treated mice contained readily detectable eosinophilic colloid. Although aberrant cells were not entirely eradicated from the thyroid of PD325901-treated mice, the abundance of abnormal PTC cells was greatly diminished compared with vehicle-treated mice.

Consistent with effects of PD325901 on thyroid size and architecture, treatment with PD325901 led to a normalization of serum T4 levels and a 6-fold decrease in serum TSH levels (Fig. 5D), showing at least partial restoration of thyroid endocrine function. The effect of PD325901 on serum T4 and TSH was specific to mice with thyroid-specific BrafV600E expression (black bars) because similar treatment of control BrafCA mice lacking the Thyro::CreERT2 transgene had no significant effect on either serum T4 or TSH levels (white bars). Single-agent administration of T3 led to a decrease in serum TSH indicating that hormonal supplementation was at least partially effective. Furthermore, mice administered with T3 plus PD325901 displayed very low serum T4 levels because of inhibitory effects of exogenous T3 on T4 production. A cooperative effect of PD325901 plus T3 is evidenced because coadministration led to an even more striking decrease in serum TSH than either agent alone. T3 administration showed that hormonal supplementation alone did not provide any therapeutic benefit, unless coadministered with PD325901. Overall, these results show that the effects of BrafV600E on thyroid function and tumorigenesis are highly reliant on MEK—ERK signaling.

**Discussion**

Dysregulated RTK signaling, mediated by mutational activation of RET/PTC, K- or NRAS, or BRAF, seems to be a feature common to the majority of thyroid cancers (16, 17). Consequently, pharmacologic targeting of RTK-activated signaling pathways may be useful in the treatment of this disease. Here, we describe a new mouse model of BrafV600E-induced papillary thyroid tumorigenesis that recapitulates key features of the human disease. Despite the profound hypothyroidism and tumorigenesis induced by widespread expression of BrafV600E in thyrocytes, the mice displayed no overt signs of illness and none required euthanasia because of PTC. This stands in contrast to mice with constitutive, embryonic-onset expression of BrafV600E (18). This may reflect the timing of BrafV600E expression (embryonic vs. adult) in the 2 models and the fact that no obvious progression to more aggressive anaplastic thyroid cancer or metastasis was detected in our model. However, in both models, BrafV600E is likely expressed in the majority of thyrocytes resulting in profound hypothyroidism and a field cancerization that is not common in human PTC. To that end, untreated Thyro::CreERT2; BrafCA mice that display stochastic recombination of the BrafCA allele in thyrocytes to express BrafV600E may be a more accurate model of human PTC because, under these circumstances, there is no goiter or hypothyroidism associated with PTC initiation or progression.

In previous studies, we have documented that expression of BrafV600E in the lung epithelium or in melanocytes leads to a distinct phase of benign tumorigenesis that fails to progress to malignancy unless tumor suppressor genes are deliberately silenced (6, 7). Furthermore, it has been proposed that BrafV600E—induced benign tumorigenesis reflects the engagement of senescence as a cancer suppression mechanism (19, 20). However, in the thyroid, BrafV600E expression (whether induced by tamoxifen or not) invariably leads to development of PTC displaying characteristic cytologic features and protein marker expression of the cognate human disease. It remains unclear why thyrocytes expressing BrafV600E do not undergo senescence as a cancer suppression mechanism. It is possible that the 10- to 100-fold elevation of serum TSH might provide stimulatory signals through the heterotrimeric G-protein–coupled TSH receptor that might prevent engagement of senescence mechanisms. Indeed, TSH receptor couples to cAMP production through activation of Goα,GTP and to phosphoinositide 3-kinase and/or PLCβ through βγ subunits. However, progression to PTC was detected in non-tamoxifen-treated Thyro::CreERT2; BrafCA mice, in which no alterations in serum TSH or T4 were detected. Moreover, administration of exogenous T3 to tamoxifen-treated Thyro::CreERT2; BrafCA mice did not diminish the BrafV600E-induced increase in thyroid size and development of PTC. Although these results tend to rule out a role for elevated TSH in promoting PTC progression, they do not rule out a role for normal levels of serum TSH displayed by these mice. Consistent with this, while this article was under review, Franco and colleagues, described a similar mouse model in which embryonic-onset expression of BrafV600E led to PTC (21). In that model, concomitant silencing of either TSH receptor or Gsα expression delayed, but did not abrogate, BrafV600E-induced PTC. In addition, exogenous thyroid hormone had no effect on PTC maintenance. Notwithstanding differences in timing of the initiating genetic event, TSH receptor signaling plays a role in PTC initiation but not maintenance.

To our surprise, thyrocyte-specific expression of KrasG12D had no obvious effect on thyroid architecture and did not predispose to thyroid tumorigenesis. The failure of KrasG12D to elicit thyroid tumors might reflect a lack of Kras expression in mouse thyrocytes. However, it may also reflect an inability of KrasG12D to functionally engage downstream signaling effectors. Our results confirm those of others who showed that KrasG12D expression in the developing thyroid was largely without effect (10). However, in this model, KrasG12D cooperated with PTEN silencing to induce thyroid cancer indicating that Kras is expressed in mouse thyrocytes.

Considerable interest and excitement is focused on the effectiveness of pharmacologic agents that target Braf—MEK—ERK signaling in the treatment of cancers expressing mutationally activated Braf. Indeed, PLX-4032, a Braf inhibitor, has shown dramatic antitumor effects against metastatic melanoma and thyroid cancers expressing BrafV600E (5). Our data suggest that
BRAF<sup>V600E</sup>-induced PTC critically relies on MEK1/2 signaling because pharmacologic blockade of these enzymes had striking effects on thyroid size and function and clear antitumor activity. However, a more effective test of such agents might be in the context of BRAF<sup>V600E</sup>-induced thyroid cancers with concomitant silencing of relevant tumor suppressor genes such as <i>Trp53</i>, <i>Cdki2a</i>, or <i>Pten</i> (6, 7). Indeed, preliminary evidence suggests that BRAF<sup>V600E</sup> can cooperate with dominant-negative TP53<sup>ΔN/Δ3170R</sup> for thyroid cancer progression (data not shown). Finally, primary treatment for thyroid cancer often involves systemic radiiodide therapy. However, the effectiveness of such therapy is reported to be limited by the ability of BRAF<sup>V600E</sup> to inhibit sodium–iodide symporter (NIS) expression. Hence, agents that target BRAF<sup>V600E</sup> signaling might promote NIS reexpression, thereby sensitizing thyroid tumor cells to radiiodide therapy.

In conclusion, we describe here a new mouse model of adult-onset thyroid cancer that displays key features of the human disease, which will complement studies on human thyrocytes and thyroid cancer lines (22). In addition, we show the utility of this model system to test the antitumor effects of pharmacologic inhibitors of BRAF<sub>→</sub>MEK<sub>→</sub>ERK signaling. It will be interesting to test the effects of deliberate tumor suppressor gene silencing on the propensity of BRAF<sup>V600E</sup>-induced PTC to progress to more aggressive disease and on the response of thyroid cancer cells to pathway-targeted therapy.

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**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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