Molecular and Cellular Pathobiology

Mutationally Activated BRAF\textsuperscript{V600E} Elicits Papillary Thyroid Cancer in the Adult Mouse

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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\textsuperscript{V600E}. One month following BRAF\textsuperscript{V600E} 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\textsuperscript{V600E}--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): 3863-71. ©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\textsuperscript{V600E} (4). Once mutationally activated, the BRAF\textsuperscript{V600E}→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\textsuperscript{V600E} is required for PTC maintenance because pharmacologic inhibition of BRAF\textsuperscript{V600E} by PLX-4032 in thyroid cancer patients led to tumor regression (5).

We have previously described the utility of Braf\textsuperscript{CA} 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\textsuperscript{T2}) under the control of the thyroglobulin promoter (Thyro:CreER\textsuperscript{T2}), we explored the consequences of induced BRAF\textsuperscript{V600E} expression in adult thyroid. Shortly after BRAF\textsuperscript{V600E} 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\textsuperscript{T2}, untreated Thyro:CreER\textsuperscript{T2}, Braf\textsuperscript{CA} 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\textsuperscript{V600E} induces a clearly defined stage of benign tumorigenesis, BRAF\textsuperscript{V600E} 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\textsuperscript{V600E}→MEK→ERK signaling.

Materials and Methods

Mouse breeding and manipulation

Braf\textsuperscript{CA} mice were described previously (6, 7). Thyroglobulin: CreER\textsuperscript{T2} (Thyro:CreER\textsuperscript{T2}) 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\textsuperscript{T2} 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-HMG12 (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 SDS, 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), α-pERK1/2, pAKT, and α-actin (1:5000; Santa Cruz). 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 BRaf CA allele or a Cre-activated KRas G12D allele, so that we could compare and contrast the effects of oncogenic BRAFV600E to KRAS G12D 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 KRAS G12D expression on thyroid size in Thyro::CreERT2; KRas G12D mice at any time, even after repeated tamoxifen administration (Data not shown; ref. 10). By contrast, tamoxifen-treated Thyro::CreERT2; BRAf CA 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; BRaf CA mice also displayed increased thyroid volume, which may be a consequence of stochastic BRAFV600E expression due to leaky CreERT activity.

Histologic analysis of untreated BRAf CA 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; BRAf CA mice displayed 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. 1B). 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 BRAf CA allele was rearranged to encode BRAFV600E in the majority of thyrocytes. Despite the

**Expression of BRAF V600E, but not KRAS G12D, induces PTC in adult mice**

To assess the consequences of BRAF V600E expression in adult mouse thyrocytes, we generated compound Thyroid Peroxidase (TPO):Cre; BRaf CA mice such that expression of BRAF V600E is initiated during thyroid development under the influence of the TPO:Cre transgene (8). Although TPO:Cre; BRAf CA 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.

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remarkably rapid onset of changes to thyroid architecture, it was unclear exactly when thyrocytes assumed malignant characteristics. At 3 months, occasional foci of hyperplastic tall cells were detected (Fig. 2D). However, the first clear indication of thyroid malignancy was detected 6 months after BRAFV600E expression (Fig. 2E) when nodules of tumor cells displaying a characteristic papillary structure were readily apparent. By 12 months, we detected evidence of extensive thyroid tumors, they were not overtly sick and did not require 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; BrafCA 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 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 and is a manifestation of the profound hypothyroidism associated with widespread BRAFV600E expression in thyrocytes. Consistent with the absence of an overt thyroid phenotype in Thyro::CreERT2; KrasLSL mice, we detected no perturbation in serum T4 or TSH levels 1 month after tamoxifen administration of these mice (Figs. 1C and D).
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 (19-24 months post-tamoxifen) were stained with antisera against CK19, Gal-3, HMGA2, TTF-1, and Ki67, the last being a general marker of cell proliferation (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...
prepared 2 weeks following tamoxifen administration to \( \text{B}^{\text{CA}+} \) mice that either did or did not carry the \( \text{Thyro::CreER}^{\text{T2}}; \text{B}^{\text{CA}+} \) 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 (þ Tamox, \( \text{T} = 9.5 \) mo) thyrocyte-specific expression (Cre+) of \( \text{BRAF}^{\text{V600E}} \) led to an elevation of pERK1/2 compared with similarly treated controls (Cre−, 9.5 mo). Moreover, both \( \text{BRAF}^{\text{V600E}} \)-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 \( \text{BRAF}^{\text{V600E}} \) leads to elevated ERK1/2 MAP kinase signaling.

**BRAF\(^{\text{V600E}}\)-induced elevation of serum TSH is not required for thyroid tumorigenesis**

Previous research indicated that \( \text{BRAF}^{\text{V600E}} \) 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 \( \text{T} \), \( \text{Cdkn2a} \), or \( \text{Pten} \) are deliberately silenced (6, 7). By contrast, \( \text{BRAF}^{\text{V600E}} \) 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 \( \text{BRAF}^{\text{V600E}} \) expressing thyrocytes, may prevent the senescent phenotype associated with \( \text{BRAF}^{\text{V600E}} \)-induced benign tumor cells (6, 7).

To test this, we took advantage of the fact that the \( \text{Thyro::CreER}^{\text{T2}}; \text{B}^{\text{CA}+} \) transgene displays 4-hydroxytamoxifen-independent activity in untreated mice. Consequently, these mice were predicted to have stochastic activation of \( \text{BRAF}^{\text{V600E}} \) expression in thyrocytes without the wholesale dysregulation of thyroid function that occurs in tamoxifen-treated \( \text{Thyro::CreER}^{\text{T2}}; \text{B}^{\text{CA}+} \) mice. Consequently, we aged a cohort of 9 \( \text{Thyro::CreER}^{\text{T2}}; \text{B}^{\text{CA}+} \) 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; BrfCA/ 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; BrfCA/ 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::CreERT<sup>2</sup>; BrfCA/ 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

**Figure 4.** Thyro::CreERT<sup>2</sup>; BrfCA/ mice develop PTC without tamoxifen administration. A, H&E staining of histologic sections of thyroid from a representative 13-month-old Thyro::CreERT<sup>2</sup>; BrfCA/ mouse that was not administered tamoxifen at low (40×, middle) and high power magnification (400×, left and right). Cells displaying abnormal nuclear cytology are indicated with arrows (right). B, immunofluorescence analysis of histologic sections of thyroid from Thyro::CreERT<sup>2</sup>; BrfCA/ without tamoxifen injection. DAPI in blue and CK19, Gal-3, HMGA2, TTF-1, and Ki67 in green as indicated. Magnification is 200×. C, analysis of serum concentration of TSH and T4 from approximately 1-year-old Thyro::CreERT<sup>2</sup>; BrfCA/ mice that were not administered tamoxifen.

n, number of mice in each group. N.S., not significant.
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 BrarfCA 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:CreER BrarfCA mice that display stochastic recombination of the BrarfCA 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 cyto logic 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 costimulatory 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 nontamoxifen-treated Thyro:CreER2; BrarfCA mice, in which no alterations in serum TSH or T4 were detected. Moreover, administration of exogenous T3 to tamoxifen-treated Thyro:CreER2; BrarfCA 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 elic tit 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
BRAFV600E-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 BRAFV600E-induced thyroid cancers with concomitant silencing of relevant tumor suppressor genes such as Trp53, Cdkn2a, or Pten (6, 7). Indeed, preliminary evidence suggests that BRAFV600E can cooperate with dominant-negative TPS3 and PTEN for thyroid cancer progression (data not shown). Finally, primary treatment for thyroid cancer often involves systemic radioiodide therapy. However, the effectiveness of such therapy is reported by the ability of BRAFV600E to inhibit sodium–iodide symporter (NIS) expression. Hence, agents that target BRAFV600E signaling might promote NIS reexpression, thereby sensitizing thyroid tumor cells to radioiodide 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 → MEK → ERK signaling. It will be interesting to test the effects of deliberate tumor suppressor gene silencing on the propensity of BRAFV600E-induced PTC to progress to more aggressive disease and on the response of thyroid cancer cells to pathway-targeted therapy.

References


Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all members of the McMahon lab for support and helpful discussions. In particular, we thank Takashi Hirano for technical support, Christy Trejo for assistance with tumor analysis, and Victoria Marsh for advice on the manuscript. We thank Allen Vlännuva for mouse husbandry support and the UCSF Mouse Pathology core for assistance with tissue preparation. We thank Dr. Shiko Kimura (NIH) for provision of TPO-Cre transgenic mice, Drs. Raymond Grogan and Orlo Clark (UCSF Department of Surgery) for interesting and informative discussions on the pathology of human thyroid cancer, Drs. James Fagin and Ronald Gosewin (Shano Kettering Institute) for advice on thyroid histology and for communicating unpublished results, and Dr. Byron Hann (UCSF Preeclinical Therapeutics core) for instruction and support for Vevo770 ultrasound imaging and drug administration. Finally, we thank Dr. A. Farlow (National Hormone and Peptide Program) for timely support for serum TSH and T4 measurements.

Grant Support

This research was supported by a grant from the National Cancer Institute (CA131261). R-P. Charles was supported by a fellowship from the Swiss National Science Fund.

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Received December 13, 2010; revised February 28, 2011; accepted March 24, 2011; published OnlineFirst April 21, 2011.
Mutationally Activated BRAF^{V600E} Elicits Papillary Thyroid Cancer in the Adult Mouse

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Cancer Res 2011;71:3863-3871. Published OnlineFirst April 21, 2011.

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