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Significance of *BRAF* mutations in papillary thyroid carcinoma: prognostic and therapeutic implications

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Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy, representing ~80% of all cases. In PTC, mutations of genes encoding effectors of the MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) PATHWAY are central to malignant transformation. In 70% of all cases rearrangements of the genes encoding the receptor tyrosine kinases RET or NTRK leading to expression of constitutively active fusion proteins, as well as activating-point mutations of *RAS* or *BRAF,* are found. In any given PTC, mutation only occurs in a single component of the MAPK pathway, supporting the idea that constitutive functional activation of any of these effectors alone is sufficient to foster development of PTC.[1]

The *BRAF* (T1799A) somatic mutation encodes the constitutively active kinase B-Raf (Val600Glu). Among differentiated thyroid neoplasms this mutation is found almost exclusively in PTC, accounting for approximately 44% of all cases.[2] A recently reported intrachromosomal rearrangement between *BRAF* and *AKAP9* also leads to expression of a constitutively active form of B-Raf, but is a rare event found mainly in a subset of radiation-induced PTC.[3] *BRAF* mutations are probably involved in tumor initiation, as they are found in microscopic PTC. Targeted expression of B-Raf (Val600Glu) in thyroid cells of transgenic mice results in PTC, indicating that inappropriate expression of this oncogenic form of the kinase can recapitulate the disease phenotype.[4] Prior exposure to ionizing radiation during childhood predisposes to development of PTC with *RET* rearrangements and, to a lesser extent, with *NTRK* or *BRAF* intrachromosomal inversions. These mutations are also found in children with PTC without known exposure to radiation. By contrast, point mutations of *BRAF* are exceedingly rare in this population, with an overall prevalence of 4–6%.[2] Thus, somatic mutation of *BRAF* is the most common early genetic event causally associated with development of PTC in adult patients without a history of radiation exposure.

There is a general consensus that PTCs with different mutations have distinct histopathologic appearance and biologic properties. Gene expression profiling of PTCs with *RET/PTC*, *RAS* and *BRAF* mutations accurately classified the mutational status of the cancers, further supporting the concept that each of these oncogenes induces specific phenotypic features.[5] Tumors associated with *RET/PTC1* rearrangements usually have the classical or conventional papillary histotype, whereas those with *RET/PTC3* are associated with solid-variant PTC. Follicular-variant PTCs frequently harbor *RAS* mutations or *PAX8/PPARG* rearrangements or, less commonly, distinct B-Raf mutations (Lys601Glu, Gly474Arg) and have a comparatively low frequency of lymph-node metastases.[6] Although most PTCs with *BRAF* mutations have a classical histology, almost 80% of tall-cell variant PTCs have the *BRAF* (T1799A) mutation; this histotype is believed to present more often at an advanced disease stage.

Although the majority of PTCs, regardless of genotype, are surgically removed at an early stage of the disease, most studies indicate that PTCs with *BRAF* mutations are overrepresented among those cases presenting at an advanced stage. These tumors have a higher frequency of extrathyroidal invasion and a predisposition to neck-lymph-node and distant metastasis.[7] PTCs with *BRAF* mutations also have a higher recurrence rate, and the metastatic recurrences have diminished radioiodine avidity.[7] A recent study describes a *de novo BRAF* mutation in lymphatic metastases in which the primary PTC did not seem to have this mutation.[8] This finding suggests that late acquisition of *BRAF* mutations could confer predisposition to lymphatic spread; however, it is also conceivable that the lymph-node metastases arose from a discrete focus of the primary PTC harboring a *BRAF* mutation. Regardless, these findings support the concept that *BRAF* predisposes to lymphatic involvement. Although most reports confirm the association of *BRAF* mutation with unfavorable clinicopathologic features, it should be noted that two studies from Italy and two

from Asia did not find a statistically significant association between *BRAF* mutation and these negative prognostic markers.

About 25% of undifferentiated thyroid cancers have *BRAF* mutations, and this proportion is probably higher in tumors with documented evidence of progression from a pre-existing well-differentiated PTC. In the latter case, the *BRAF* mutation is present in both components of the tumor—well-differentiated and undifferentiated—suggesting a role for oncogenic *BRAF* in disease progression.[9] By contrast, *RET/PTC* rearrangements are rarely, if ever, found in poorly differentiated or anaplastic thyroid carcinomas. Data from mouse models of thyroid cancer are consistent with these observations. Thus, PTCs arising in B-Raf (Val600Glu) transgenic mice are invasive and undergo transition to poorly differentiated carcinomas, whereas those that develop in *RET/PTC* transgenic mice do not progress to this form. Taken together, multiple lines of evidence indicate that PTCs with *BRAF* mutations have a worse prognosis. It remains to be seen whether this information will be of practical value and alter diagnostic or therapeutic strategies.

Detection of somatic mutations in fine-needle aspiration specimens of PTC with the *BRAF* (T1799A) mutation can be performed easily with good sensitivity and specificity.[2] Identification of a *BRAF* mutation in thyroid aspirates could help distinguish between benign and malignant tumors when the cytologic examination is inconclusive. A positive genotyping result is diagnostic for PTC, and has been shown in one small study to rectify the diagnosis after false-negative cytology. It seems unlikely, however, that this test alone will have much impact in resolving the clinical dilemma posed by the majority of aspirates considered 'suspicious' or 'indeterminate'. Most of these cases involve follicular neoplasms, which do not have *BRAF* mutations, or to the follicular variant of PTC, which has a low prevalence of this mutation or other B-Raf mutations (Lys601Glu, Gly474Arg). In the future, it is conceivable that preoperative genotyping of thyroid aspirates could alter the surgical approach, perhaps by mandating more aggressive lymph-node dissection; however, in view of the favorable outcome of most patients with PTC, even those with a *BRAF* mutation, this approach seems unlikely. Indeed, despite the poor prognosis of patients with *BRAF* (T1799A)-associated PTC, the majority of these cases still present at an early stage.

The fact that PTC is associated with mutually exclusive alterations to MAPK pathway effectors offers intriguing therapeutic opportunities. Ras, RET and B-Raf are part of a linear signaling pathway, RAF being required for the effects of RET and Ras on thyroid cell transformation and dedifferentiation. Thus, inhibitors of RAF kinase activity or the direct downstream effector, MAPK kinase, are logical candidates for treating patients with advanced PTC.[10] Targeted therapies for cancer tend to be most effective when the therapeutic compound inhibits a pathway that is constitutively activated during the early stages of tumor development, and this is certainly the case for RET and B-Raf. At least two clinical trials of multikinase inhibitors with strong activity against RAF (BAY 43-9006, Bayer Health Care, West Haven, CT and AMG706, Amgen, Thousand Oaks, CA) are ongoing in differentiated thyroid cancer. The results of these studies should pave the way for a new era in thyroid cancer therapy, in which the genetic basis of tumor development is used to tailor the best treatment for each patient.

GLOSSARY

MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) PATHWAY
Transduces mitogenic signals via activation of receptor tyrosine kinases, leading to the successive recruitment and activation of Ras and members of the RAF family of serine/threonine kinases

Competing interests
The authors declared they have no competing interests.

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