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EDITORIAL

BRAF mutation assessment in papillary thyroid cancer: are we ready to use it in clinical practice?

Efisio Puxeddu · Sebastiano Filetti

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In 2002, large-scale genomic screens allowed uncovering the occurrence of *BRAF* mutations in melanomas (67 %), colon carcinomas (12 %), and ovarian tumors (14 %) [1]. In the subsequent years, constitutively activating mutations in the *BRAF* oncogene, encoding a serine/threonine protein kinase activating the MAP kinase/ERK-signaling pathway, have been reported in papillary thyroid carcinomas (PTC) (44 %) [2] and in hairy cell leukemias (100 %) [3]. Accordingly, the impact of *BRAF* mutation on clinical outcome has been extensively exploited in a variety of cancers, often resulting in controversial or unsettled findings. A T-to-A transversion at nucleotide 1799, which results in a valine to glutamate substitution at residue 600 ($BRAF^{V600E}$), is by far the most common mutation in differentiated thyroid cancer (DTC) [2]; however, it has been found as well in a fraction of poorly differentiated (PDTC) (10–15 %) and anaplastic thyroid carcinomas (ATC) (10–44 %) [4, 5]. The $BRAF^{V600E}$ mutation's high prevalence and detection easiness have facilitated the understanding of its pathogenetic, diagnostic, prognostic, and therapeutic roles in thyroid tumors.

Experimental studies with transgenic mice characterized by thyroid-targeted expression of the oncogene consistently indicated that $BRAF^{V600E}$ is a clonal-initiating event in thyroid follicular cell carcinogenesis [6]. Mutual exclusivity in PTCs of the most common genetic mutations, including *BRAF* and *RAS* mutations and *RET/PTC* rearrangements, indicate that each event is self-sufficient to constitutively activate the tumor-initiating MAPK pathway [6]. Conversely, more recently, a study that used pyrosequencing to determine the percentage of mutant *BRAF* alleles in conventional PTCs concluded that $BRAF^{V600E}$ is a rare clonal event and more often a nonclonal mutation, occurring late during PTC progression [7]. If confirmed, this finding would have important ramifications because it would undermine the rationale for using therapies targeted against this oncoprotein. An intense scientific debate is ongoing on this topic [8] and, hopefully, it will help in clarifying this controversy soon.

Another important question is if *BRAF* mutation might have a diagnostic role as malignant marker in a thyroid nodule aspirate, especially in the case of indeterminate fine-needle aspiration cytology (FNAC). The latter cytological picture occurs quite frequently (10–26 % of nodules) and harbors an intermediate risk of malignancy (14–48 %). Improvements of thyroid cytopathology by the introduction of thin core needle biopsy, with the assessment of the nodule capsule and the perinodular tissue, have appeared to ameliorate recognition of malignancy in cytologically indeterminate nodules [9]. Similarly, combination of nodule ultrasound features with measures of nodule stiffness, obtained by real-time elastography, has appeared to increase sensitivity for malignancy and to strengthen the selection of patients who do not need further diagnostic testing [10]. However, the analysis of diagnostic molecular markers on indeterminate FNAC specimens has caught the greatest interest. Search of DNA-based point mutations of *BRAF* has a sound biological rationale in view of its high frequency and driving role in PTCs and peculiar assay robustness due to DNA stability [6]. However, more than 50–60 % of DTCs do not harbor this molecular

E. Puxeddu
Department of Medicine, University of Perugia, Via Gambuli, 1, 06132 Perugia, Italy

S. Filetti (✉)
Department of Internal Medicine and Medical Specialties, University of Rome "Sapienza", V.le del Policlinico, 155, 00161 Rome, Italy
e-mail: sebastiano.filetti@uniroma1.it

mutation, including follicular carcinomas and follicular variants of PTC, cytological results of which frequently fall in the indeterminate subgroup, making the sensitivity of this marker unacceptably low [6]. Unfortunately, also, the simultaneous assay of a panel of other genetic markers of thyroid carcinoma, including *RET/PTC* and *PAX8-PPARγ* rearrangements and *RAS* mutations, does not improve significantly sensitivity, because 30–40 % of DTCs will still not harbor any known molecular mutation. Recently, a gene expression classifier composed by 142 genes [11] has displayed a higher sensitivity and NPV (>94 %), allowing an accurate separation of the patients in which surgery can be deferred from those with suspicious nodules. Conversely, use of genetic markers characterized by a high specificity and PPV, including *BRAF* mutations, might become useful in suspicious nodules to confirm malignancy as well in defining the extent of surgery.

In thyroid cancer, several genetic markers, including mutations in *RAS*, *PI3K*, *PTEN*, *P53*, *ALK*, and *BRAF* genes, have shown promise as prognostic molecular markers. Indeed, these mutations increasingly occur and coexist in thyroid tumors from low-to-high grade being involved in the stepwise activation of the crucial oncogenic MAPK and PI3K–AKT pathways. The best-defined prognostic marker is the *BRAF* mutation, which appears to be linked to recurrence and metastasis in PTC.

Indeed, several studies have shown a strong association of *BRAF* mutation with lymph node metastasis, extrathyroidal extension, advanced disease stages III and IV, and disease recurrence [12]. Interestingly, this association was also noted for conventionally low-risk cancers. More recently, a strong association of *BRAF* mutation with PTC mortality was also shown, although *BRAF* mutation only worsened the prognostic effect of negative clinical features and did not result independent of them [13]. These findings are at least in part related to the fact that *BRAF* mutation drives a loss in radioactive iodine avidity of recurrent PTCs, rendering the disease refractory to radioiodine treatment [14]. The high NPV for recurrence of a *BRAF* negative test shown by some studies has suggested the use of this test in clinical practice [15].

Accordingly, a positive *BRAF* mutation status has been considered a reliable factor to guide prophylactic central neck lymph node dissection, although routine application of this practice in the absence of prospective studies has been recently questioned [16]. Moreover, *BRAF* mutation status has been hypothesized to be useful in defining necessity to perform radioiodine ablation in low risk patients, targets of TSH suppression, intensity of recurrence surveillance, and threshold of FDG-PET execution.

Intriguingly, there are a few studies that contradict the prognostic value of *BRAF* mutation analysis [15]. One of those is the study by Barbaro et al. [17] published in this issue of Endocrine. Those authors enrolled prospectively 110 patients characterized by British Thyroid Association Thy4 and Thy5 FNAC results, who underwent total thyroidectomy and prophylactic central neck dissection and radioiodine ablation according to their management protocol. BRAF analysis was performed before surgery on FNAC samples. No differences could be detected between the 88 *BRAF* mutated PTCs and the 22 *BRAF* wild-type cancers in terms of initial pTNM staging and 8 months' follow-up. The results of that study and of the others reaching similar conclusions might be influenced by several factors: smallness of the examined casuistries; selection biases, such as inclusion only of patients with a presurgical Thy4 and Thy5 FNAC or patients at an early disease stage; and differences in the genetic background of the considered populations [6].

Finally, $BRAF^{V600E}$ is a promising target for novel, targeted therapies also in advanced RAI-refractory thyroid carcinomas [6]. Several small molecular inhibitors have been developed, and a few have already reached the stage of clinical trials [18]. In detail, sorafenib, a multikinase inhibitor, which targets also RAF family of kinases, has recently been tested in a phase III trial. A preliminary release of the obtained results showed that the drug significantly increased progression-free survival versus placebo [10.8 vs. 5.8 months; HR (95 % CI): 0.587 (0.454–0.758), $p < 0.0001$] [19]. Influence of *BRAF* mutation status on the outcome of the treatment still needs to be evaluated. Preliminary results in three metastatic PTC patients harboring *BRAF* mutation showed promising results [20] by treatment with vemurafenib, a selective $BRAF^{V600E}$ inhibitor, which has recently been approved for melanoma. An open-label, multicenter, phase II study with vemurafenib in advanced *BRAF*-mutated PTCs is ongoing.

In conclusion, many features of *BRAF* oncogene in papillary thyroid cancer have been elucidated. Many aspects still need to be clarified through prospective and randomized studies. However, *BRAF* mutation status in thyroid cancer appears as a promising marker to provide a basis for developing a personalized management.

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