The overall aim of the work of this thesis was to progress in the understanding of the molecular alterations associated with thyroid cancer, particularly with papillary thyroid carcinoma (PTC).

Just before the starting point of the research project, some studies had reported that oncogenic mutations in BRAF, which encodes a downstream effector of RAS proteins through the RAF-MEK1/2-ERK1/2 pathway, were frequently detected in several neoplasias (e.g., cutaneous melanoma and colorectal carcinoma). Moreover, BRAF mutations virtually did not coexist in any lesion with RAS mutations, suggesting that the two alterations were alternative etiopathogenic events. As the most prominent genetic alterations known in thyroid tumors were RAS gene mutations, we hypothesized that BRAF mutations could also be frequent in thyroid carcinomas.

To test this hypothesis, we analyzed the BRAF mutational status in cases of sporadic nodular goiter, follicular thyroid adenoma, follicular thyroid carcinoma, and PTC. We found that BRAF mutations were only highly prevalent in PTC (46%), where they became the most frequent genetic alteration known. As in other neoplasias, BRAF mutations did not coexist with RAS mutations or RET rearrangements (RET/PTC). This finding suggested that the aforementioned genetic alterations were alternative events and that the RET/PTC(NTRK1)-RAS-BRAF-MEK1/2-ERK1/2 pathway underlies PTC tumorigenesis.1

PTC comprises several histological variants that also seemed distinct regarding the underlying molecular alterations: RET/PTC1 associated with conventional PTC, RET/PTC3 to the solid variant of PTC (SVPTC), and RAS mutations and PAX8-PPARγ rearrangements to the follicular variant of PTC (FVPTC) (Fig. 1). Therefore, we analyzed the mutational status of BRAF in a PTC series, including cases of several histotypes, and found that different mutation types were associated with distinct growth patterns: BRAFV600E was highly prevalent in cases of papillary or mixed papillary-follicular pattern, namely, in conventional PTC (46%), and absent in follicular-patterned cases; at variance, BRAFK601E was exclusively detected in FVPTC cases (7%). These results confirmed the existence of a close
FIGURE 1. PTC histotypes and related genetic alterations.
genotype–phenotype association and supported the singularity of PTC histotypes (namely, FVPTC). Interestingly, another mutation type—BRAFV600-1E—was detected in the only case displaying a solid pattern of growth. However, the rarity of SVPTC did not allow the elucidation of whether BRAFV600-1E is associated with the solid architecture or is an occasional event.

PTC usually has an excellent prognosis, but some cases manifest aggressive behavior. Despite some contradictory findings, most studies on record have reported the association of BRAF mutations with clinicopathological parameters of poor prognosis (older age at diagnosis, male gender, multicentricity, extrathyroid extension, lymph node or distant metastasis, and recurrence). Thus, some authors claimed that BRAF-mutant PTCs are more aggressive than PTC cases without the BRAF mutation. Moreover, it was also described that BRAF mutations are more frequent in lymph node metastases of PTC than in the respective primary lesions. In our series, the presence of BRAF mutations (V600E) was statistically associated with older age of the patients (46.5 years vs. 37.9 years; \( p = 0.0043 \)) but not to male gender, nor to any parameter of tumor aggressiveness (multicentricity, vascular invasion, extrathyroid extension, and lymph node metastasis). In another PTC series, in which the primary lesion and respective metastases were available for molecular studies, we found that both sets of lesions displayed a similar frequency of BRAF mutations (49%) and that the mutational status of the large majority of cases was maintained in the primary lesions and the respective metastases. Therefore, the results in the two series do not support the claim that BRAF mutations are a poor prognostic factor in PTC.

Given the occasional progression of PTC (and FTC) toward poorly differentiated thyroid carcinoma (PDTC) and undifferentiated thyroid carcinoma (UTC), BRAF mutations were also screened in a series of such aggressive tumors. BRAF mutations were frequent in UTC (35%) but absent in PDCT. These results indicate that BRAF-mutated PTC cases (namely, PTCs with a papillary pattern of growth) may progress to UTC.

**Comment by Giovanni Tallini, MD**
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Since 2003, the year of the first identification of BRAF-activating mutations in thyroid cancer, their role has emerged as one of the molecular alterations most important for thyroid tumorigenesis. They are now regarded as a reliable marker for papillary carcinoma or for tumors associated with it. BRAF mutations are identified in half of the carcinomas with papillary architecture, and in one third of the undifferentiated (anaplastic) carcinomas. They are uncommon in the follicular variant of papillary carcinoma—where alterations other than the canonical V600E mutation may occur—and rare in poorly differentiated carcinoma. Among papillary carcinomas, mutant BRAF is associated with older age at presentation, although the link with other clinico-
pathologic parameters of aggressive behavior is not entirely clear. Relevant to the molecular mechanisms of thyroid tumorigenesis is the finding that BRAF, RET/PTC, and Ras activation are alternative events, specifically selected in individual tumors because they all act on the common downstream MAPK signaling pathway.

Many of the seminal studies that have led to these conclusions have come from the Institute of Molecular Pathology and Immunology of the University of Porto, (IPATIMUP) headed by Professor Manuel Sobrinho-Simões. In many of these papers, Dr. Trovisco is the first author or one of the leading collaborators, and his work includes articles published as early as 2004. Of particular relevance is the definition of the genotype–phenotype correlation of BRAF with papillary thyroid carcinoma, the study showing that the BRAF mutation is a mutually exclusive event with respect to RET/PTC and Ras activation, and the identification of Braf mutations as different from the prevalent V600E form. Dr. Trovisco is also the author of several knowledgeable reviews on BRAF (see, e.g., Ref. 7).

It is not common to find a young investigator endowed with the competence and energy to generate a similar quantity of high-quality scientific works for his PhD thesis. Dr. Trovisco should be commended, together with his supervisors and with Professor Manuel Sobrinho-Simões. The summary of his thesis should be published and would be of much interest to the readers of Critical Reviews™ in Oncogenesis.

REFERENCES


