Concomitant BRAF V600E and NRAS Q61R mutations in the same thyroid nodule: a case report

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Background: Papillary thyroid cancer (PTC) is the most common type of well-differentiated endocrine malignancy. Generally, thyroid nodules with multiple oncogenic mutations are uncommon with an occurrence which may be related to more aggressive biological behavior of tumors. RET/PTC rearrangement, RAS, and BRAF mutations are considered to be mutually exclusive in PTC. Concomitant RET/PTC, RAS, or BRAF mutations have been documented, although the impact of these mutations for tumor growth and survival is debated.

Case Description: Here we present a rare case of woman 46 years old with a neck mass and thyroid nodule classified as TIR5 on cytological examination. We found contemporary BRAF p.(Val600Glu) [p.(V600E); c.1799T>A] and NRAS p.(Gln61Arg) [p.(Q61R); c.182A>G] mutations in morphologically different areas within the same lobe (the right one); The two lesions show different morphology. The mutated BRAF lesion showed morphological characteristics compatible with classic papillary carcinoma. The mutant NRAS lesion shows morphological features compatible with follicular variant papillary carcinoma. To the best of our knowledges, this is the first time that such mutations, which are normally mutually exclusive, have been detected at the same time.

Conclusions: The finding of synchronous mutations is a rare occurrence suggesting for intratumoral heterogeneity (ITH) even in PTC. Patients with multiple mutations have a clinical worse prognosis, generally characterized by an aggressive thyroid cancer, which may influence the surgical treatment, chemotherapy, and BRAF V600E mutation-targeting therapy.

Keywords: Papillary thyroid cancer (PTC); concomitant mutations; intratumoral heterogeneity (ITH); prognostic markers; case report

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* the pathologist is unfortunately dead.

Introduction

Thyroid cancer is the most common malignancy of the endocrine system (1,2). More than 95% of thyroid carcinomas originate from the follicular cells of the thyroid, while only a minority (~3%) originate from C-parafollicular cells, leading to the onset of medullary thyroid carcinomas (3,4).

Differentiated carcinomas have been identified in three histological subtypes: papillary thyroid cancer (PTC) (80–85%), follicular carcinoma (FTC) (10–15%), and oncocytic cells carcinoma (3–5%), whose development and prognosis is similar to that of FTC (5,6).

Despite most of PTCs and FTCs have a good prognosis with a 5-year survival rate of more than 90%, a low fraction might become more aggressive over time (7,8).

PTC is a solid tumor that normally occurs inside the thyroid; histologically it is characterized by the presence of papillae, epithelial cells arranged around a fibrovascular stem (9).

Despite most of PTCs are well-differentiated with a low rate of local invasion, recurrences, or metastases (regional or distant), there are several tumor variants, with distinct pathological, and molecular features. Because of their aggressive behavior, the latest American Thyroid Association (ATA) guidelines have classified these pathological subtypes as having an intermediate risk of recurrence (10).

The main variants of PTC with prognostical implications are the follicular variant PTC (FVPTC), diffuse sclerosing variant (DSV), tall cell variant (TCV), columnar cell variant (CCV), cribriform variant (CV), hurthle cell variant (HCV), and hobnail variant (HV). Their evaluation is usually histological due to the uncommon morphological features (11).

Highlight box

Key findings

- Improving knowledge of intratumoral heterogeneity in papillary thyroid cancer, would be beneficial in order to provide appropriate therapy advice and enhance survival of patients.

What is known and what is new?

- It is known that in papillary type thyroid cancer, carcinogenic alterations of the *BRAF* gene are frequent.
- It is new to have found the simultaneous presence of alterations which are generally mutually exclusive.

What is the implication, and what should change now?

- The result of report implies a patient-centered cancer therapy approach in order to get the correct drug(s) to the right patients at the right time and, as a result, overcome resistance mechanisms.

The main molecular alterations are the same as in traditional PTC, with the exception of the HV, which has changes in the *TERT* promoter (44.4%), *PIK3CA* (28.8%), *CTNNB1* (16.7%), *EGFR* (11.1%), *AKT1* (5.5%), and *NOTCH1* (5.5%) (12), and the HCV, whose oncocytic features are linked to mitochondrial DNA mutations (13). Alterations in *BRAF* gene have been referred as absent in the CV and uncommon in the DSV, where *ALK* gene rearrangement was recently identified (14).

The FVPTC, a well-circumscribed or encapsulated tumor with architecture that might be mistaken for follicular adenoma or FTC, has also been widely characterized it is known that mutations in the RAS oncogene have been linked to encapsulated/well-circumscribed tumors, whereas invasive FVPTC has been related to both BRAF and RAS mutations (15,16).

The gold standard approach for the diagnosis of PTC is fine needle aspiration and cytology (FNAC) which classifies thyroid biopsies as suspicious or malignant based on their cytological outcome. Although a FNAC can reveal papillary structures, preoperative diagnosis is primarily based on the detection of typical nuclear features such as "Orphan Annie" nuclei (clear intranuclear pseudoinclusions) and nuclear grooves (folds in the nuclear membrane) (17). PTC is confirmed by the presence of psammoma bodies, calcium salt deposits, in a cervical lymph node (18). The cytological examination allows for the diagnosis of cancer in most cases; however, due to limited sampling or a lack of well-expressed PTC markers, several nodules later confirmed as malignant are indeed classified as indeterminate by cytology (19). As a result, the use of molecular test is required in order to have a better understanding of the disease progression (20).

The most prevalent genetic mutation in PTC, BRAF V600E, as well as the less common RAS mutations, or RET (RET/PTC translocations) or NTRK rearrangements, play a key role in the PTC pathogenesis (18,20). Genetic changes in RET/PTC, RAS, and BRAF are thought to be mutually exclusive, and their overlapping has been debated for a long time. However, concurrent mutations in PTC have recently been reported and regarded as proof of existence of intratumoral heterogeneity (ITH) also in PTC (21). We present this article in accordance with the CARE reporting checklist (available at https://acr.amegroups.com/article/view/10.21037/acr-23-83/rc).

Case presentation

A 46-year-old, female patient was admitted to Pathology

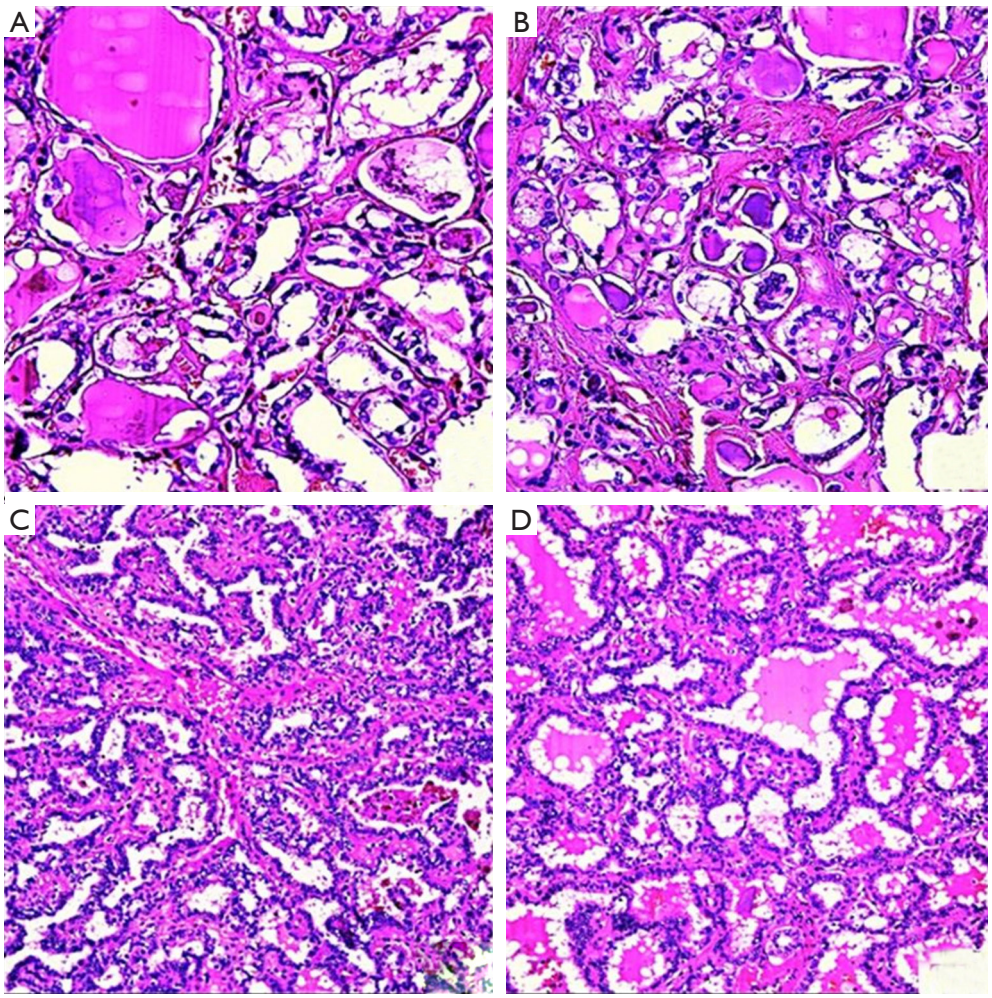

Figure 1 (A,B) High power magnification (20×) hematoxylin-eosin staining demonstrates the carcinoma cells BRAF V600E positive, with marked nuclear features and pseudoinclusions in the context of CPTC. (C,D) High power magnification (20×) hematoxylin-eosin staining demonstrates the carcinoma cells NRAS positive in the context of FVPTC. CPTC, classic PTC; PTC, papillary thyroid cancer; FVPTC, follicular variant PTC.

Unit, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale in May 2020 with a neck lump that grew in size over the year.

Previous history of thyroid disease in the family was not reported and laboratory test did not detect thyroid disorders such as hyperthyroidism and hypothyroidism.

The right lobe presented in the section, macroscopically, at the upper/middle III a nodule of 1.5 cm and a further nodule of 4 mm at the III medium.

Fine needle aspiration (FNA) from the right thyroid lobe was interpreted as TIR5, sign of aggressiveness and high level of tumor malignancy. Therefore, the patient underwent to total tiroidectomy with central neck dissection.

For the right lobe, the surgical pathology examination revealed a size of 5 cm × 3 cm × 2 cm; for the left lobe, 4 cm × 3 cm × 1 cm. In addition, two nodules, one 1.5 cm in diameter and the other 4 mm, have been identified in the right lobe.

Although both lesions were classified as PTC, morphological variances were detected (*Figure 1*).

The largest lump had a classic papillary histology with well-developed nuclear features of PTC and inflammatory cells mixed in typical nuclear features such as Orphan Annie nuclei (clear intranuclear pseudoinclusions) and nuclear grooves (folds in the nuclear membrane) suggestive of PTC, have been described. The diagnosis has been confirmed by

the presence of psammoma bodies (calcium salt deposits) in a cervical lymph node.

Instead, microscopic examination of the 4 mm nodule revealed morphological features of a variant follicular PTC, FVPTC, and extracapsular type.

Formalin-fixed paraffin-embedded (FFPE) tissue sample from surgical resection, were used for DNA extraction and each sample underwent molecular analysis to determine subclonality with regard to BRAF and RAS mutational status.

The slides were reviewed by two expert pathologists; areas tumor displaying distinct histological pattern were separately microdissected to ensure high tumor tissue content and a minimum of 10% tumor purity cells was required for sample processing.

DNA and RNA were extracted using respectively Qiagen QIAMP DNA FFPE Kit and RNeasy FFPE Kit according to manufacturer instructions and sample concentration was evaluated with nanodrop and Qubit.

BRAF and RAS mutational status was investigated by real-time polymerase chain reaction (PCR) using Thyroid Cancer Mutation Detection Kit (THDNA-RT64, EntroGen) intended for the detection of BRAF, KRAS, NRAS, and HRAS somatic mutation in human genomic DNA.

RET/PTC, RET/PTC2, RET/PTC3, and PAX8/PPARY translocation analysis on RNA were investigated by Easy Pgx Thyroid Fusion Kit and the one-step real-time PCR as amplification method. Neither area was confirmed rearranged in terms of gene fusions.

The results highlighted BRAF p.(Val600Glu) for the 1.5 cm nodule while NRAS p.(Gln61Arg) for the 4 mm nodule; we enforced our findings because of several clinical cases with concurrent mutations in PTC are referred in literature (7,8,22).

Genetic changes in the RAS gene, as well as RET/PTC fusion or TERT1 promoter alterations, were simultaneously observed in PTC BRAF V600E (7); however, to the best of our knowledges, this is the first time that the BRAF V600E and NRAS Q61R mutations, which are normally mutually exclusive, have been detected at the same time.

Ethical statement

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

PTC is an indolent tumor with a low death rate (22).

Several studies have identified two types of genetic alterations in thyroid cancer: point mutations in BRAF, KRAS, NRAS, or HRAS, and chromosomal translocations involving RET/PTC1, RET/PTC3, or PAX8/PPARY (10,17). The detection of these genetic markers allows for a definitive diagnosis of malignant tumor that is distinct from thyroid nodules, which are considered to be benign. Furthermore, these gene markers may provide important prognostic value for patients with various subtype.

All genetic alterations are assumed to be mutually exclusive and their overlapping has been debated for a long time. However, concurrent mutations in PTC have been discovered and regarded as a rare occurrence (23). RAS mutation and RET/PTC1 fusion co-expression were reported by Di Cristofaro *et al.* in 1/24 FVPTC and BRAF mutation RET/PTC3 fusion was found in 1/26 of classic PTC (CPTC) patients (18).

Henderson *et al.* reported 5/54 PTC patients with coexisting BRAF mutation RET/PTC fusion: the authors referred a correlation between mutation status and clinicopathological variables since patients with dual mutation were older and had more advanced tumor (80% in T4) than those with BRAF V600E mutation only (27% in T4) (11).

Xing *et al.* referred that BRAF V600E and TERT promoter mutations cooperatively identify an aggressive PTC with the worst clinicopathological outcome (10).

In 8/15 (53%) subclonal or nonclonal PTC, Zou *et al.* confirmed RET/PTC rearrangement and RAS or BRAF mutations, but none in clonal PTC. According to the authors concomitant mutations were considered to occur more frequently in advanced stages of disease, and long-term follow-up showed that patients with contemporary mutations had a poor response to treatment and a reduced disease-free survival rate (8).

Costa *et al.* observed concomitant BRAF V600E and KRASG13D+G12S mutations in 4/35 PTC (7) focusing on the assumption that BRAF alone isn't a predictor of poor outcome; nevertheless, when combined with other genetic alterations, it identifies a subset of PTC with higher risk of recurrence and decreased survival. In agreement with this, we have reported the first case of two distinct oncogenic

driver mutations, respectively BRAF V600E and NRAS Q61R within the same thyroid nodule; this could enforce the evidence of a wide variety of biological behaviors in PTCs, ranging from the most indolent (well-differentiated type) to the most aggressive malignancy. Only a few molecular markers linked to an increased risk of death are currently available, and their effectiveness in preoperative risk stratification and therapeutical planning remains unknown. As a result, a proper molecular characterization may be a useful tool for personalizing the initial surgical strategy, the follow-up, and ultimately to apply new therapies. Clinical trials using BRAF inhibitors for advanced thyroid cancer have revealed conflicting outcomes (22). In the context of a RAS mutation, recent investigations have shown that BRAF inhibitors might paradoxically boost MAPK activation (22,23). As a result, patients who have both a BRAF and a RAS mutation may not be candidates for BRAF inhibitors treatment since the reactivation of MAPK is involved in the resistance mechanism (23).

Moreover, the occurrence of concomitant mutations, enforced the evidence of ITH even in PTC. ITH refers to subclonal genetic variability within a tumor in contrast to the concept of a tumor as a clonal and homogeneous swarm (24). It is caused by genetic instability and the accumulation of genetic changes, both key factors in the growth of a tumor from an early stage to a more aggressive cancer.

The existence of ITH in PTC, its extension and biological impact are debated. However, several studies have shown that it is not a minor event in PTC, but a key factor for therapeutic failure and poor prognosis (25). Nowadays, in the era of personalized medicine, the discovery that some tumors are heterogeneous in terms of individual mutations has significant therapeutic implications as well as translational value (26,27). Tumors with a specific molecular alteration in only a minority of neoplastic cells are likely to have a low sensitivity to targeted therapies; as a consequence, the finding of this internal tumor-like complexity, was the starting point for the use of a drug combination to restrict tumor growth.

As a result, this report supports the assumption that synchronous mutations in PTC are linked to more aggressive tumor behavior, which could affect surgical procedure selection as well as post-surgery care. It also shows the potential impact of molecular testing/screening in selecting patients for more aggressive treatment, whatever the benefits of such an approach have yet to be confirmed. Anyway, it would be useful increase the understanding of ITH in order to properly advise therapy and improve survival of patients (28,29).

Conclusions

The identification of specific genomic alterations drivers for several malignancies has enabled the development of customized therapies with promising response rates. Unfortunately, most cancers are caused by a complex interaction of genetic, transcriptomic, and proteomic alterations, as well as anomalies in the tumor microenvironment and immune system.

The recent development of new technologies such as second-generation sequencing, next-generation sequencing (NGS), and the numerous advances in the field of genomics have allowed a continuous and further evolution of "precision oncology". While recognizing the value of morphological and histological data, the new paradigm of "mutational oncology", has opened the era of genomic profiling tests: this would improve the selection of the anticancer drug based on the "driver" mutation and agnostic approval, namely the therapeutic indication regardless of the tumor site.

Due to the high levels of tumor heterogeneity and individual genomic complexity, customized drug combination is a key factor in therapeutic management optimization. This implies a patient-centered cancer therapy approach in order to get the correct drug(s) to the right patients at the right time and, as a result, overcome resistance mechanisms.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://acr.amegroups.com/article/view/10.21037/acr-23-83/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://acr.amegroups.com/article/view/10.21037/acr-23-83/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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