When Morphology Meets Somatic Mutations: The New Possible Scenario in Thyroid Fine-Needle Aspiration

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Key Words

BRAF mutation · Liquid-based cytology · Malignancies · Plump cells · Thyroid fine-needle aspiration

Abstract

This study points to the analysis of the morphological features suggestive of somatic mutations, mostly the *BRAF*^{V600E} mutation, on cytological samples of thyroid carcinomas. According to the literature, the application of ancillary techniques on cytology comes in handy as a challenging aid in ruling out a malignant outcome on both conventional and liquid-based cytological preparations. However, the evaluation of somatic mutations, including *BRAF*^{V600E}, usually performed by DNA techniques, may have some limitations in a worldwide diffusion. In this perspective, few authors emphasized the morphological search for *BRAF*^{V600E} mutations harbored in papillary thyroid carcinoma (PTC) and characterized by specific architectural and cellular findings (i.e. eosinophilic cells defined as ‘plump cells’ and sickle-shaped nuclei). Hence, the detection of eosinophilic cytoplasm of mutated PTC cells seems to suggest the possible involvement of the ‘Warburg effect’ pioneering the ability of cancer cells to convert glucose into lactic acid. The recent yields of immunohistochemical expression of monocarboxylate transporters in

mutated PTCs may suggest the accumulation of lactate in these plump cells. Equally importantly, the detection of these morphological findings using fine-needle aspiration cytology may be helpful in triaging thyroid lesions and limiting costs. Additionally, it may lead to the stratification of the malignant risk and personalized management in cases with multifocal lesions.

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The worldwide acceptance that somatic mutations represent a hallmark in the diagnosis and prognosis of cancer has been widely established [1–5]. Remarkable advances in the knowledge of the molecular mechanisms of cancer have been done in these last decades mostly due to the application of molecular platforms and next-generation sequencing able to identify the driver mutations of numerous different cancers [1–7]. Specifically, several scientific data have shown that molecular alterations in specific pathways play a pivotal role in different types of thyroid cancer and, equally importantly, arise early in the tumorigenic process so that they may be considered as strong markers of malignancy [1–11]. Especially papillary thyroid carcinoma (PTC), the most common thyroid malignancy, may carry *BRAF*, *RET/PTC* or *N-RAS* muta-

tions [12]. Among them, activating somatic mutations in the *BRAF* oncogene proved high prevalence (ranging between 45 and 70%) mainly in the classical variant of PTCs with more than 95% of *BRAF* mutations involving the hot spot identified on exon 15 of the B isoform of the *RAF kinase* gene [13].

Some Changes in The Molecular Approach

Although these innovative molecular approaches represent the gold standard for DNA and RNA evaluations, they do not fulfill a worldwide diffusion and application. In fact, their invaluable advantages are countered by several issues, including more laborious investigations, time-consuming, more expensive equipment, necessity of dedicated technical personnel or contamination by nonneoplastic components, which lead to an unpredictable rate of nondiagnostic molecular yields [14, 15]. Taking all these limitations into account, some authors cudged their brains in order to find some efficient and possible alternatives for a reliable detection of somatic mutations [15–21]. To overcome these drawbacks, some of them fostered the study and evaluation of antibodies against the mutated proteins as well as the possible identification of specific morphological features able to recognize mutated thyroid carcinomas.

The Role of VE1 Antibody

However, did these alternative approaches reach their goal? In 2012, Capper et al. [16] suggested that a new monoclonal antibody directed against the mutated *BRAF* V600E protein (clone VE1) was likely to be promoted as a valid option in several malignant neoplasms. Comparing the immunohistochemical yields with molecular assays, these authors showed that VE1 also had high sensitivity and specificity in a series including 47 melanoma metastases and 21 papillary thyroid carcinomas [16]. In a second paper, they yielded 97% correlation between VE1 and molecular *BRAF* with only 2 discordant cases [15]. Hence, Routhier et al. [19] found also high concordance between VE1 and molecular assays (97%) in a series of 152 malignant tumors including also 23 thyroid carcinomas. The application of VE1 on cytological samples resulted in 86% sensitivity and specificity as found in two different papers from Zimmerman et al. [21] and Rossi et al. [14]. Nevertheless, some criticism and controversial data have been raised mostly due to

the weak and focal positivity expressed in some thyroid lesions.

In fact, despite the fact that the literature exploited VE1 antibody as a feasible first-line approach for the evaluating *BRAF* mutations, Rossi et al. [14] highlighted that it was less accurate in identifying VE1-negative cases than molecular platforms.

The New Perspective of Morphological Findings of Mutation

Is there anything else helpful to predict mutations based on thyroid fine-needle aspiration cytology (FNAC)? The idea of the possible prediction of the mutational status of cancer cells was an alternative perspective suggested by recent papers on thyroid histological PTCs. Specifically, in 2012, Finkelstein et al. [22] proved that distinctive morphological features were harbored in histological samples of *BRAF*^{V600E}-mutated PTCs. Taking these data into account, the same results were pointed out by Virk et al. [23] in 2014 showing that some architectural (tumor-associated stromal reactions or infiltrative tumor borders) and cellular-specific parameters (nuclear features and polygonal eosinophils defined as ‘plump cells’) are linked with *BRAF*^{V600E}-mutated PTC. Simple fluke? Or may we exploit ‘mutational eyes’?

These recent papers shed light on the exciting, potential opportunity to implement the same search on thyroid cytology in order to offer a valid tool in selecting cases for molecular analysis and limiting costs. Consequently, the awareness of these histological findings has raised our interest in their evaluation in our cytological series of thyroid lesions diagnosed as positive for malignancy favoring PTC. Rossi et al. [24, 25] identified peculiar morphological features in all the 47 mutated cases, including also 6 *BRAF*^{V600E}-mutated cases with focal ‘plump cells’. Hence, an accurate morphological investigation suggested also the recognition of a peculiar nuclear shape in all the *BRAF*^{V600E} cases which had not been previously described in literature. In fact, these authors demonstrated the presence of mutated cancer cells characterized by nuclei which tended to be smaller, pleomorphic and eccentrically located with a particular sickle shape, which was absent in wild-type cases [24, 25]. However, the presence of these plump cells with sickle-shaped nuclei has recently been confirmed by Kwon et al. [26] in a series of 142 thyroid lesions diagnosed as suspicious for malignancy and performed with conventional cytology.

Moreover, a recent study of ours (data under publication) assessed the detection of morphological parameters associated with the *BRAF*^{V600E} mutation in a series of 119 pediatric cytological lesions emphasizing that they may be easily recognized regardless of the thyroid category and helped in guiding the correct management in pediatric thyroid lesions.

Nevertheless, these findings have recently also been documented in other *BRAF*^{V600E}-mutated cancers [27, 28]. For example, the morphological features of *BRAF*^{V600E} mutations were underscored in a recent study even in ovarian neoplastic lesions. Consistently, in 2014, Zeppernick et al. [27] described some morphological features that may predict *BRAF*^{V600E} mutations in ovarian serous borderline tumors which are highly likely to induce neoplastic initiation followed by a mechanism to restrain tumor progression and reach a low-stage senescent disease. Given that, are these new insights regarding thyroid and ovarian *BRAF*^{V600E} neoplasms driven and supported by the same metabolic mechanism?

How are *BRAF*^{V600E} and morphology linked? In this perspective, we emphasized that a *BRAF*^{V600E}-activating mutation is likely to exert a different effect on the nucleus/cytoplasm ratio in favor of abundant cytoplasm in mutated cases.

The Molecular Mechanism

In this regard, the detection of the abundant eosinophilic cytoplasm of mutated thyroid cancer cells seems to stand a chance of involving the 'Warburg effect' inducing a modified metabolism of cancer cells able to convert glucose into lactic acid due to the up-regulation of genes involved in glycolysis [29–31]. As recently published, it has been proven that the metabolic use of glucose in glycolysis, with production of ketones, promotes the *BRAF*^{V600E}-*MEK*.*ERK* oncogenic pathway [32]. Taking into account the glycolytic mechanisms beneath, these *BRAF*^{V600E}-mutated cells may hesitate in an increased mitochondrial respiration justifying the abundant and eosinophilic cytoplasm of the cells through both *MEK*/*ERK* activation and mitochondrial translocation with an anti-apoptotic effect [31, 33]. The anti-apoptotic effect and the enhanced glyco-phenotypes may be the final effect of the localization within the outer mitochondrial membrane inducing the cleavage effect of inhibition of the caspase pathway and lack of resistance to intrinsic and extrinsic apoptotic stimuli [31].

However, are we able to target this suggestion in our everyday practice even on FNAC?

The Expression of MCTs

Thus, the altered metabolic profile and the hypothetical glycolytic metabolisms seem to be supported by the immunocytochemical analysis of the monocarboxylate transporter (MCT) family which may give rise to the accumulation of lactate in these thyroid mutated cancer cells [34–37]. However, the exact and specific role of MCTs in thyroid *BRAF*^{V600E}-mutated carcinomas is still far from being fully characterized and thus their role as an additional therapeutic target can still not be translated into the clinical context [34–37]. Our preliminary data (not yet published) showed that the expression of both MCT1 and MCT4 in plump mutated thyroid cancers is likely to justify that lactate can be involved in these morphological features. In fact, MCT1 yielded 76% positivity in the mutated cases, especially in both plump cells and sickle-shaped nuclei, whereas MCT4 resulted in 100% positivity in mutated cases.

In conclusion, these morphological features may be a reliable foreseeable parameter of *BRAF*^{V600E} mutations especially in mutated PTCs suggesting plausible additional oncogenic *BRAF* mechanisms. Their morphological detection in cytological samples may lead to a selection of cases for molecular *BRAF* analysis limiting costs especially in cases of multifocal cancers. Hence, their recognition may predict a malignant cytological diagnosis regardless of the cytological diagnostic categorization. Furthermore, investigating the genetic underpinnings of the detection of plump cells and sickle-shaped nuclei may pave the way for obtaining an accurate knowledge of the molecular mechanism of other different oncogenic drivers so that this knowledge may lead to the organ-specific triaging of cases in order to provide important insight for future tailored targeted therapies.

Disclosure Statement

None of the authors listed above has a potential conflict of interest. None of the authors received any funding sources for the paper.

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