

Somatic mutations in solid tumors: a spectrum at the service of diagnostic armamentarium or an indecipherable puzzle? The morphological eyes looking for *BRAF* and somatic molecular detections on cyto-histological samples

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ABSTRACT

This review article deals with the analysis and the detection of the morphological features associated with somatic mutations, mostly *BRAF*^{V600E} mutation, on both cytological and histological samples of carcinomas. Few authors demonstrated that some architectural and specific cellular findings (i.e. polygonal eosinophilic cells defined as "plump cells" and sickle-shaped nuclei) are able to predict *BRAF*^{V600E} mutation in both cytological and histological samples of papillary thyroid carcinoma (PTC) as well as in other carcinomas. In the current review article we evaluated the first comprehensive analysis of the morphological prediction of *BRAF*^{V600E} and other somatic mutations in different malignant lesions with the description of the possible mechanisms beneath these morphologic features. The detection of predictive morphological features, mostly on FNAC, may add helpful information to the stratification of the malignant risk and personalized management of cancers. Additionally, the knowledge of the molecular mechanism of different oncogenic drivers can lead to the organ-specific triaging selection of cases and can provide significant insight for targeted therapies in different malignant lesions.

INTRODUCTION

The recent findings of specific morphological parameters associated with *BRAF*^{V600E} neoplasms offer a promising and foreseeable insight into the morphological detection of somatic mutations supported also by the high specificity and positive predictive value of these parameters [1-6]. In this regard, this "morphological" evaluation seems to represent a valid and predictive support which may balance the difficulties in the use of

DNA-based molecular analysis. Conversely, it is mostly performed with molecular platforms including different types of sequencing [3-4,7-11]. Despite their invaluable advantages, little reluctance might be ascribed to the fact that they are more inquiring, and less cost-effective for their worldwide diffusion in any laboratory [7-11].

Several studies, referring to different malignant neoplasms such as melanomas, hairy-cell leukemia (HCL), colon carcinoma (CC), ovarian low-grade serous carcinoma (LGSC), Langerhans cell histiocytosis and

Erdheim-Chester disease (ECD) and glial neoplasms [12-21] have demonstrated the diagnostic and prognostic involvement of *BRAF*^{V600E} mutation in the molecular mechanism of cancers.

Additionally, in papillary thyroid carcinoma (PTC), *BRAF*^{V600E} mutation was found to be associated with some distinctive architectural and cellular morphological features (specifically plump cells defined by polygonal eosinophilic cytoplasm and sickle-shaped nuclei) with significant diagnostic accuracy [1-4].

Accordingly, these promising morphological insights were underscored in a recent study pointing to the detection in *BRAF*^{V600E} ovarian neoplastic lesions [5]. In this context, the authors identified a population of *BRAF*^{V600E} ovarian cells, characterized by the same abundant eosinophilic cytoplasm (EC) among the “ovarian atypical proliferative serous tumours” [5]. Furthermore, owing to the histological and molecular features, the authors suggested that *BRAF*^{V600E} mutation is highly likely to activate neoplastic initiation followed by a mechanism to restrain tumor progression and reach low-stage senescent disease as previously observed in vitro [5].

Peculiar morphological findings have been described in other *BRAF*-driven mutated cancers. In fact, hairy cell leukemia (HCL), characterized by specific morphological features (namely abundant eosinophilic cytoplasm and “hairy” membranous projections), demonstrate that *BRAF*^{V600E} mutation is the disease-defining genetic event inasmuch as this mutation is present in virtually all the HCL cases but rarely in other B-cell lymphomas [16-17]. Similarly, foamy and eosinophilic cytoplasm have been appreciated in several Langerhans cell histiocytosis and Erdheim-Chester disease (ECD) harbouring *BRAF*^{V600E} mutation [15].

Not surprisingly, some other neoplasms are likely to demonstrate a correlation between molecular alterations and distinctive morphological features. In fact, *PDGFRA* mutations in gastrointestinal stromal tumors (GISTs) have been found to correlate with several noteworthy morphological features (i.e. epithelioid morphology of tumor cells and tumor-infiltrating mast cells) allowing histological discrimination from GISTs with *c-kit* mutations. [19] Additionally, *ALK* translocations have been exclusively reported in lung carcinomas belonging to the acinar and solid predominant adenocarcinomas histotypes [20].

In this review article we emphasize that, investigating the genetic underpinnings of the detection of morphological features link to some somatic mutations on both cytological and histological samples of different malignant entities may pave the way for obtaining an accurate knowledge of the molecular mechanism of different oncogenic drivers. Hence, this knowledge may lead to the organ-specific triaging selection of cases in order to provide important insight for future tailored targeted therapies in different malignant lesions

***BRAF*^{V600E} AND THYROID**

Numerous studies have found a strict association between thyroid carcinoma and activating somatic mutations in the *BRAF* oncogene demonstrating a high prevalence of these mutations mostly in the classical papillary thyroid carcinoma (PTC) and/or its more aggressive variant (including for instance tall cell variant-TCV and sclerosing variant of PTC-SVPTC) [7-8, 21-30]. According to literature, the majority of *BRAF* mutations (more than 90%) involve the exon 15 with a final valine to glutamine substitution in the *Braf* protein (V600E) [21-26]. Nikiforov recognized that *BRAF*^{V600E}, *RAS*, *RET/PTC* and *PAX8/PPAR γ* were associated with malignancy in almost 100% of thyroid lesions demonstrating the role of somatic mutations and/or rearrangements in everyday practice [31-35]. Given that, in thyroid oncology, *BRAF*^{V600E} mutation is strongly associated with thyroid carcinoma mainly with the classical PTC histotype..

Besides the diagnostic role of *BRAF*^{V600E} mutation as a specific hallmark of thyroid tumorigenesis, this mutation has also provided significant insights into the prognostic unfavourable outcome of mutated carcinomas [24-27]. Despite contrasting evidence, *BRAF*^{V600E} has been found in cases with higher tumor aggressiveness demonstrated by more frequent local or distant metastases, multifocality and infiltration of the peri-thyroid tissue. [24-27, 36-39]. The recognition of this subset of patients with a specific genotype and aggressive phenotype induced Finkelstein [4] and Virk [3] to analyze the correlation between *BRAF*^{V600E} mutation and some specific architectural and cellular morphological parameters in the histological PTCs. Specifically this discovery included the evidence of foreseeable predictive features such as the presence of more infiltrative borders of the carcinomas, numerous psammoma bodies, well-developed features of PTC and peculiar polygonal cells with abundant and eosinophilic cytoplasm (defined as “plump cells”). In fact, Virk applied the term “plump cells” in order to define these large polygonal neoplastic cells, with nuclear features of PTCs but also with their height, less than twice the width, with squamous metaplasia and mostly with eosinophilic cytoplasm [3]. They demonstrated these peculiar cells in 72% of their *BRAF*^{V600E} PTCs but also in 28% of the wild type PTCs [3]. Their yields confirmed the data previously published by Finkelstein demonstrating plump in 65% of the *BRAF*^{V600E} PTCs [4].

Taking into account these findings, we were tempted to search for the same morphological features in our series of thyroid lesions diagnosed as positive for malignancy (favouring PTC) on cytology and processed with liquid based cytology (LBC). [1-2]. For the reason that we were searching these parameters on cytological specimens, we were likely to address our study only to the cellular features mostly directed to recognize these large polygonal eosinophilic tumor cells characterized by

nuclear features of PTC and homogeneous, moderate to abundant cytoplasm. The same morphological parameters were assessed on the corresponding histological samples. In contrast with the detection of “plump cells” in 72% of Virk’s *BRAF*^{V600E} cases; we recognized them in 100% of the mutated cases even if with a different intensity in the eosinophilic cytoplasm which may be attributed to the use of LBC [1-2; Figure 1]. Moreover,

the quantitative definition of these cells represents an additional critical parameter. Virk and Finkelstein appraised that even focal “plump cells” are correlated with *BRAF*^{V600E} mutation and this evidence was also found in our 12.7% of the *BRAF*^{V600E} mutated cases with focal “plump cells” [3-4]. Additionally we had 5 out of

25 wild type cases with focal “plump cells” [1]. In our opinion, in the interpretation of focal “plump cells”, there may be a problematic semi-quantitative evaluation of the percentage score which might not be predictive of the *BRAF*^{V600E} status. Importantly, for the first time to date, we reported the evidence of a particular and pathognomonic nuclear shape in all the *BRAF*^{V600E} cases. Specifically, these nuclei were smaller, in eccentric position and with a sickle shape which was absent in wild type *BRAF cases [1-2; Figure 1]. The sickle-shaped nuclei, characterized by nuclear grooves and pseudo inclusions, had also irregular nuclear membranes. The same morphological parameters were recognized on the corresponding histological samples (Figure 2).*

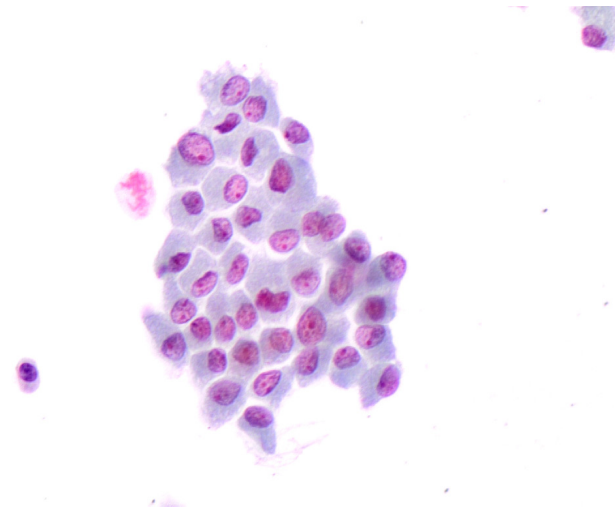


Figure 1: Details of the morphological features of plump cells and sickle shape nuclei in a cluster of “positive for malignancy” thyroid cells on liquid based cytology (LBC, 40X)

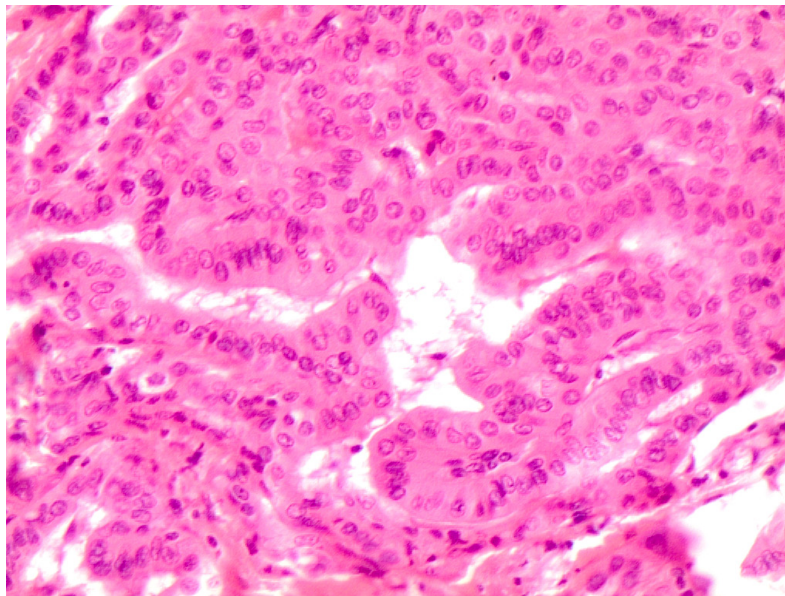


Figure 2: Specific histological details of polygonal cells with homogeneous eosinophilic cytoplasm (plump cells) in the bottom of the picture obtained from a Follicular variant of PTC (FVPC- H&E 60X)

In this perspective, the morphological evidence of altered nuclei/cytoplasm ratio (favoring a moderate/abundant cytoplasm in *BRAF*^{V600E} PTCs) cannot refute the argument that mutated cancer cells had a different metabolism due to the effect of mutated oncogenes on the pathway [7,12;Figure 3].

However, all these papers, including ours, limited their analysis to acknowledge the correlation of these “plump cells” with *BRAF*^{V600E} mutation in the PTCs without any description or study concerning the mechanisms beneath their nature [1-4].

Among authors, Hall suggested that these morphological findings are the consequence of the “Warburg effect” consistent in the ability of cancer cells of converting glucose to lactic acid [12; Figure 3]. According to their studies, Hall et al suggested that this effect may induce the activation of *RAS-BRAF-MEK-ERK* pathways which stimulate glycolysis also when there is presence of glucose [12-13, 40-41]. In this perspective, this up-regulation of glycolysis gene in *BRAF* mutated cells, may lead to an increased mitochondrial respiration justifying the abundant and eosinophilic cytoplasm of the

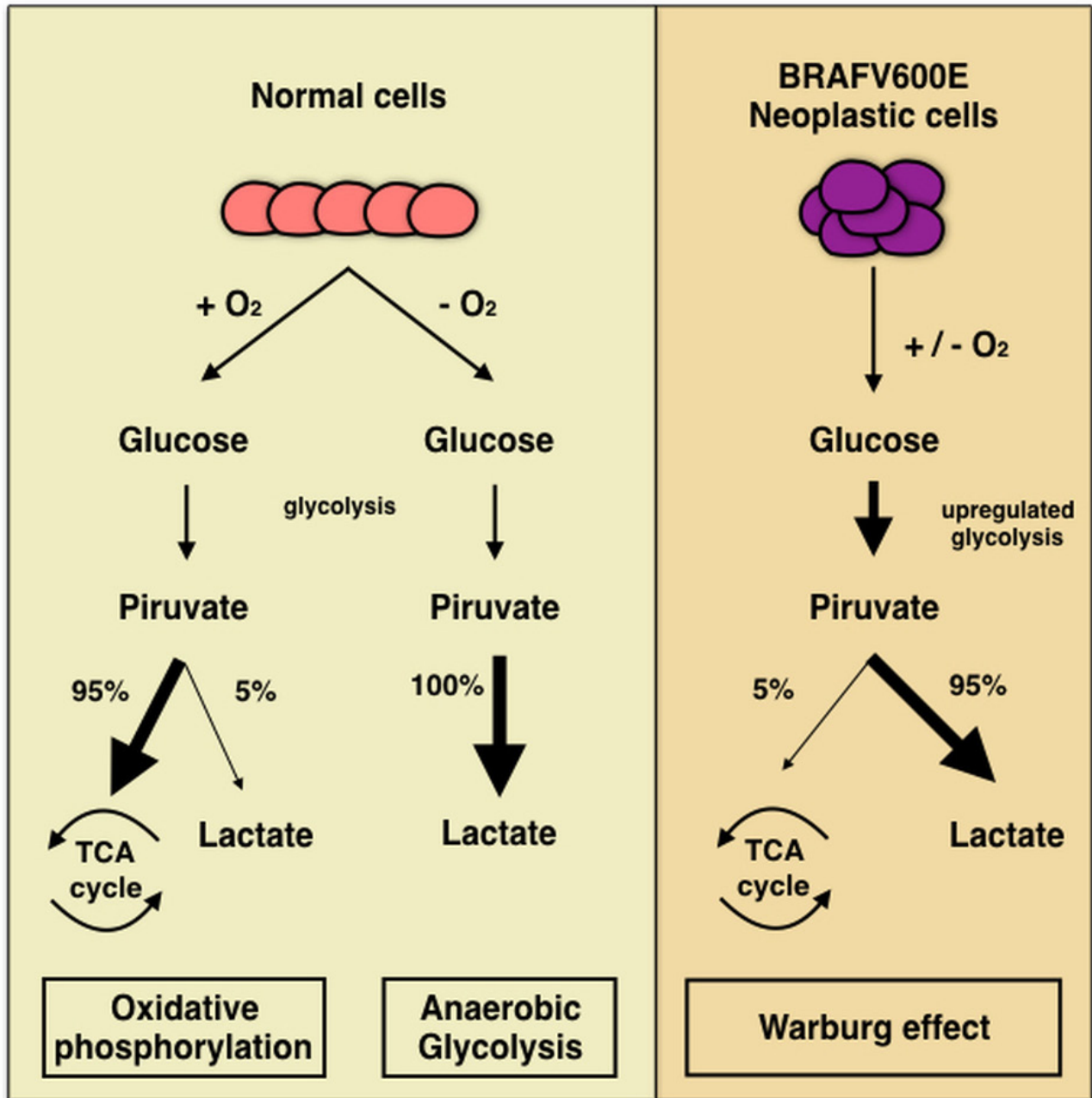


Figure 3: Comparative analysis of normal and neoplastic cells and “Warburg effect” pioneering the ability of cancer cells of converting glucose to lactic acid

cells [12-13, 42-43]. In this perspective, one explanation of this cytoplasm feature may be ascribed to the role of glycolysis through the accumulation of the lactate which was highlighted by the immunocytochemical analysis of the monocarboxylate (MCT) family. In fact, MCTs are involved in regulation of lactate and other monocarboxylates across cellular membranes.[44-50]. Inasmuch as glycolysis stimulates the accumulation of high amount of lactate inside cancers cells, MCTs regulate the maintenance of the intracellular PH of the neoplastic cells through the extrusion of lactate. According to literature, MCTs are transmembrane proteins including 14 members even though only the first 4 MCTs,(MCT1-4), are involved in the regulation of lactate and other MCTs across cellular membranes. The role of MCTs in thyroid *BRAF^{V600E}* carcinomas has not been completely defined so that their use as potential therapeutic targets needs to be demonstrated with practical application [51]. In our recent experience, we found that plump PTC cells yielded high positive predictive value (92%) and specificity (87%) for MCT1 whilst high predictive values and sensitivity for

MCT4 [52].). Their expression clearly depicts that the different metabolism of *BRAF^{V600E}* cells (involving the up-regulation of gene associated with glycolysis) may be responsible for the abundant eosinophilic cytoplasm of the plump cells [13-14]. Together, a recent study by Lee prospected the evidence of *BRAF^{V600E}* mitochondrial translocation involving both a metabolic remodeling and an anti-apoptotic effect through the caspases 3-9 [13].

In fact, it has been demonstrated that this localization confers apoptotic resistance and the characteristic metabolic phenotypes associated with suppressed oxidative phosphorylation due to the bind between *BRAF^{V600E}* and mitochondrial interacting molecules, which have not already been, identified [34]

In this regard, a possible molecular hypothesis, which might justify the higher aggressiveness encountered in mutated PTC, seems to involve the *BRAF^{V600E}* localization within the outer mitochondrial membrane. This evidence may imply a novel and potential therapeutic approach for the treatment of patients.

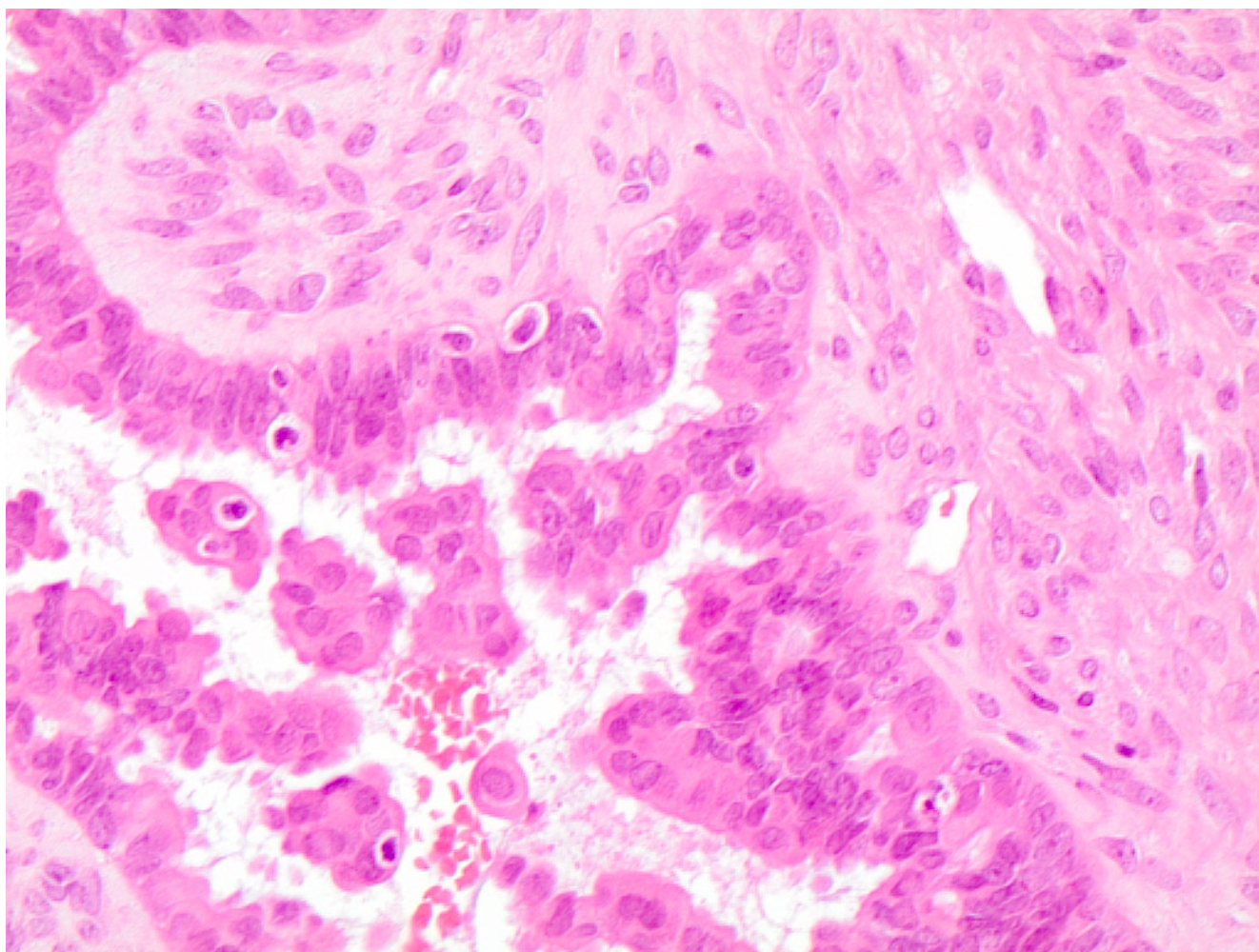


Figure 4: Histological details of the morphological features of plump cells on serous borderline mutated ovarian case (H&E 60X)

BRAF^{V600E} MUTATION IN OVARIAN NEOPLASMS

Among the broad range of human tumors found to harbor *BRAF* mutation, ovarian neoplasms, including atypical proliferative serous tumors (APSTs) and low grade serous carcinomas (LGSCs), account for two thirds mutated cases in different series by literature [5-6, 51-61]. However, Zeppernick examined its association with the clinicopathological characteristics in ovarian neoplasms [5-6].

More specifically, Zeppernick described some specific morphological features in 71 atypical proliferative serous tumors (APSTs) and 18 low grade serous carcinomas (LGSCs) and therefore their association with *KRAS* and *BRAF* mutations. They demonstrated that all the *BRAFV600E* APSTs were characterized by abundant eosinophilic cytoplasm (EC) whilst only 2 *BRAFV600E* LGSCs presented the same EC cells. Furthermore, the immunohistochemical studies revealed that these EC cells were characterized by low Ki-67 proliferation

index, significant decrease in steroid hormone receptors and strong p16 expression [5]. Owing to the histological and molecular features, the authors suggested that *BRAFV600E* mutation is highly likely to activate neoplastic initiation followed by a mechanism able to restrain tumor progression and to increase low-stage senescent disease, as previously observed in vitro [6]. Despite the same morphological evidence of *BRAFV600E* mutation in both ovarian (Figure 4) and thyroid neoplasms, we hypothesize that *BRAFV600E* activating mutation may produce a different or/and opposite effect on different body sites controlled by a specific metabolism and/or hormonal status. Hence, despite the similarities of cellular features associated with *BRAFV600E* mutation, it also activates organ-specific molecular mechanisms enabling different prognostic prediction in these cancers [5-6].

Given that, the association between *BRAFV600E* mutation and ovarian morphology demonstrates the pathogenesis of these specific lesions with an “oncogene-induced senescence” phenotype as described by Zeppernick, which may block the progression of

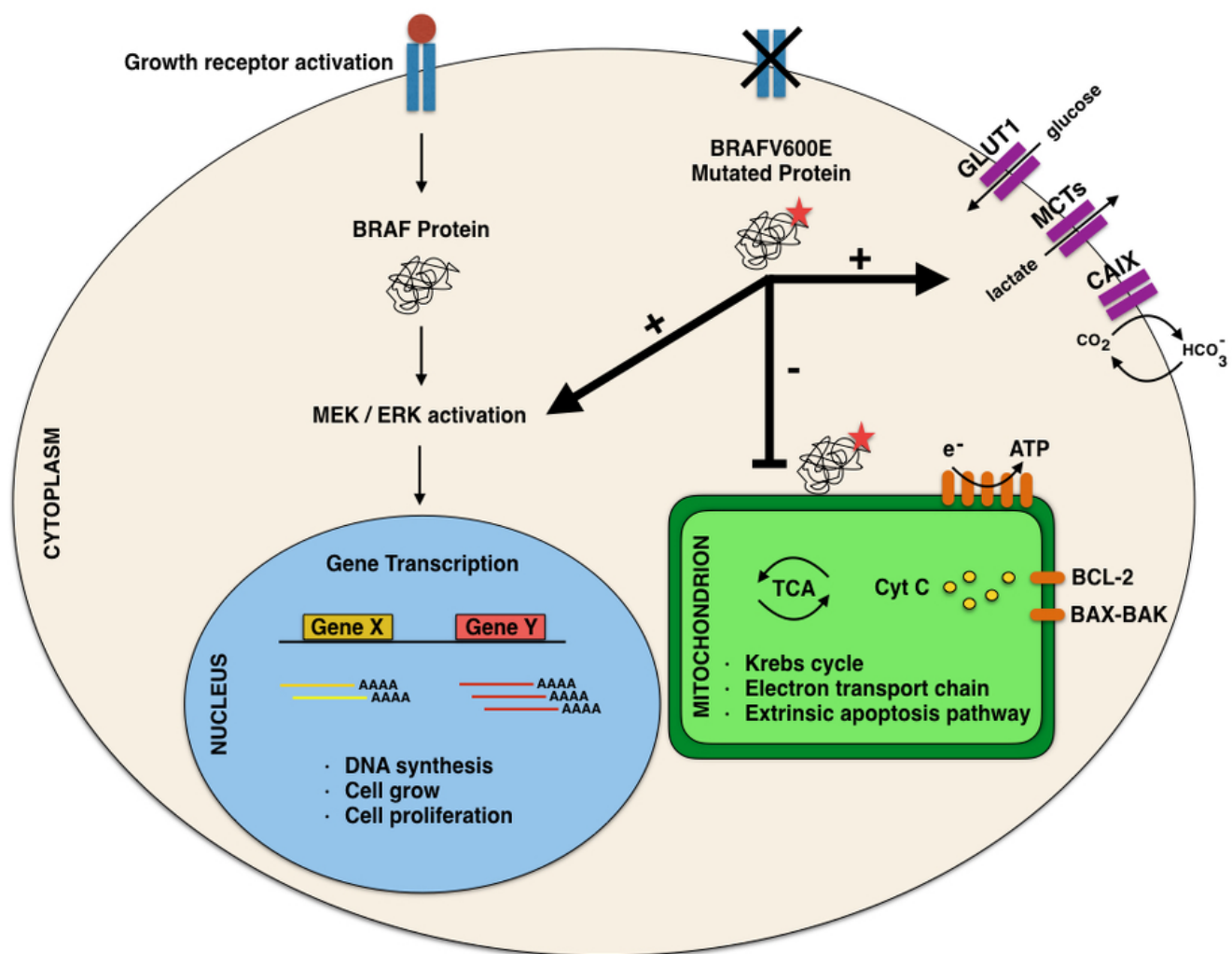


Figure 5: Details of the molecular mechanisms involving BRAF mutation in the different cell localizations

the precursor (APSTs) to cancer (LGSCs). Eventually the identification of EC cells may help in planning a personalized management especially because the majority of APSTs do not progress to LGSCs.

BRAF^{V600E} MUTATED MELANOMAS

As partially described in thyroid chapter, some of the mechanisms proposed as responsible for the

morphological features in mutated PTCs had been borrowed from the literature concerning *BRAFV600E* melanomas [12, 62-73]. Haq in his papers assessed that *BRAFV600E* mutation is the most frequent genetic mutation in melanomas and the activation of the BRAF/ MAPK decreases the oxidative metabolism [66-67]. As Salama and Flaherty summarized, *BRAF V600E* has garnered a great deal of attention in these years especially as the most frequent oncogenetic mechanism in up to

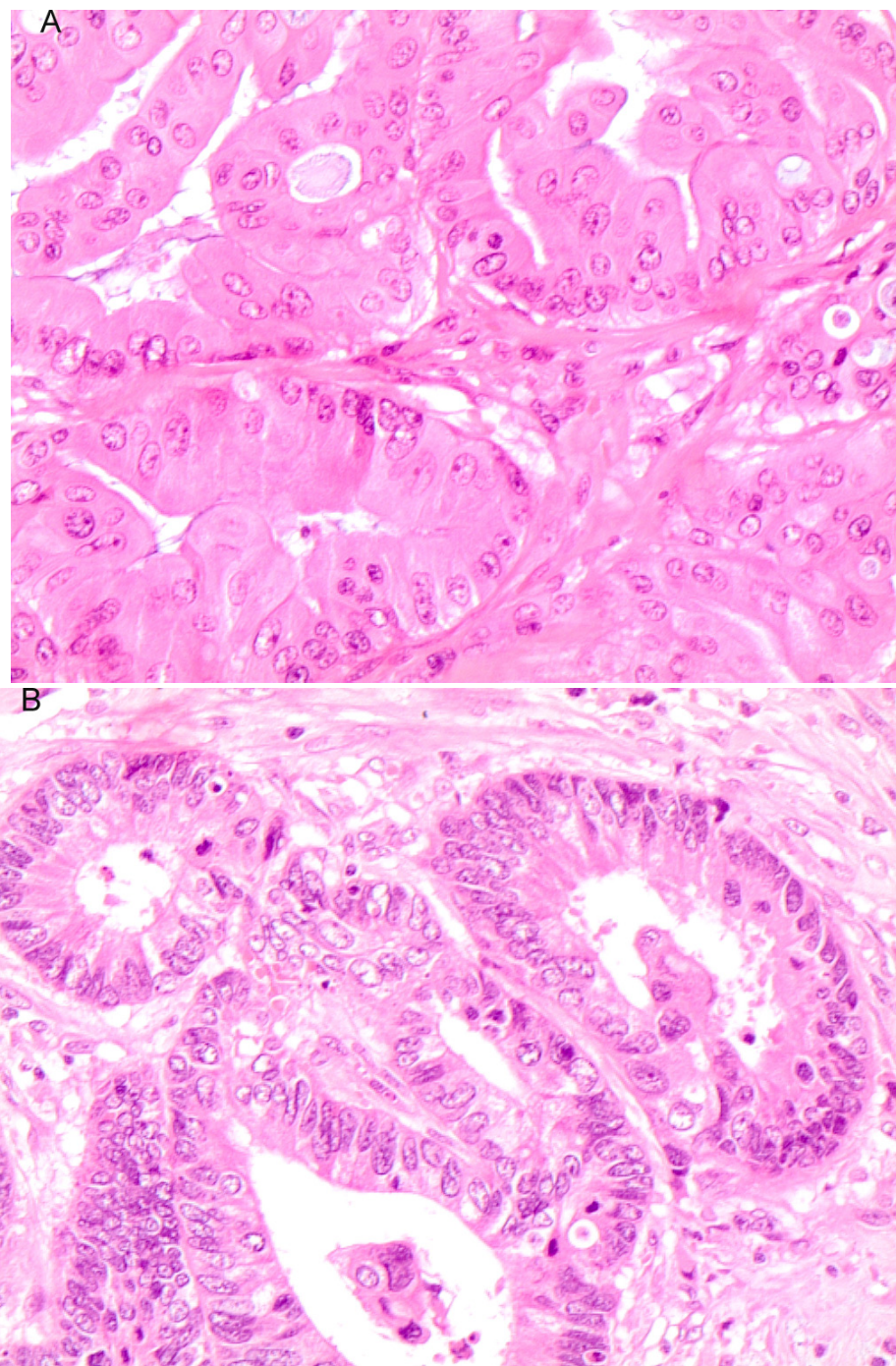


Figure 6: **A.** Histological details of the morphological features of cells on mutated intestinal adenocarcinoma case (H&E 60X); **B.** Histological details of the morphological features of cells on wild type intestinal adenocarcinoma case (H&E 60X).

60% of the cutaneous melanomas [62]. Nonetheless, these authors did not underline any correlation with specific phenotype of mutated melanoma cells. However, despite this lack of evidence, some authors found a higher incidence of *BRAF*V600E in melanomas developed on body sites intermittently exposed to the sun than those arisen in areas with chronic exposure [63]. Furthermore, Kim showed an association between *BRAF*V600E mutation and different types (acral lentiginous melanoma) and spreading of melanoma (superficial or nodular melanoma)[63]

On the other hand, Hall assessed that most cancer cells, including melanoma cells, display a strikingly different metabolism that is generated by intrinsic and extrinsic factors [12]. These authors proved the first evidence that *BRAF*V600E mutation stimulates the upregulation of gene involved in glycolysis in mutated melanoma cell lines [12]. Therefore, their results suggested that *BRAF* activation is associated with reduction of oxidative enzymes, mitochondrial number and alterations in the lactate (increasing amount). In contrast with the data from thyroid cancer cell, Hall did not find evidence of *BRAF* correlated suppression of oxidative phosphorylation in melanoma cells. This metabolic reprogramming triggered by *BRAF*V600E is linked with the suppression of melanocyte master regulator microphthalmia-associated transcription factor (MITF) and mitochondrial master regulator (PGC1 α) representing the major regulator of mitochondrial biogenesis and function [67-71; Figure 5].

Taking into account the results published in *BRAF* mutated melanomas, though unproved on thyroid PTCs, *BRAF* inhibition also stimulates some gene programs such as oxidative phosphorylation, mitochondrial mechanisms, and the levels of PGC1 α [67-71, 74]. Given that, the activation of the *BRAF*/MAPK pathway may suppress levels of MITF and PGC1 α and decrease oxidative metabolism [14, 72]. Data reported from melanomas cells, demonstrated that oncogenic *BRAF* promotes melanoma metastasis and aggressiveness through the down-regulation of the cyclic *GMP* (*cGMP*)-specific phosphodiesterase PDE5A with interaction between *RAS*/*RAF* and *cAMP* pathway [14,72]. Arozarena had previously demonstrated that *BRAF* V600E increases the levels of BRN2 (a transcriptor factor) in melanoma cells, which is responsible for invasiveness [64-65]. Additionally, it binds to the PDE5A promoter that is essential for the suppression of the PDE5A transcription by *BRAF* V600E. Despite the demonstration of these mechanisms in both in vivo and in vitro studies and cell lines, to date, they have not been correlated with peculiar morphological evidence on melanoma cells.

***BRAF*^{V600E} IN HEMATOLOGICAL NEOPLASMS**

As reported by Davies, *BRAF* oncogene was found

mutated also in some specific hematological neoplasms including hairy cell leukemia but also Langerhans cell histiocytosis and Erdheim-Chester disease [75-76]. In 2014, Machnicki discussed the role of *BRAF* in hematological entities even though it has not been clearly defined [77]. Although the oncogenic role of *BRAF*V600E has been reported in lymphoid and myeloid neoplasms, it seems to be mostly restricted to some specific subtypes. Conversely, the prevalence of *BRAF* V600E mutation in chronic and acute leukemias ranges between 0 and 10% with only Gustafsson demonstrated that 20.7% of acute lymphoblastic leukemia (ALL) had *BRAF* V600E mutations [78]. Despite the fact that these *BRAF*V600E mutated cases are devoid of any morphological finding linked to the mutation, the detection itself, restricted to specific histotypes is a relevant feature as target for *ERK* pathway inhibitors. Hence, other attempts were made to determine whether *BRAF* mutations are present in multiple myeloma (MM) and plasma cell leukemia (PCL) [79-90]. Despite the low incidence of *BRAF* mutations in these entities, Andrulis found 2.8% *BRAF* V600E cases out of the 379 patients with plasma cell disorders [79].

On the other hand, the selectivity of *BRAF*V600E mutation is also clearly stated in hairy cell leukemia (HCL), an uncommon B-cell neoplasm in which *BRAF*V600E mutation represents an almost 100% disease-defining genetic abnormality [17, 86-91]. The pathognomonic features of typical hairy cells with abundant cytoplasm and circumferential distribution of fine hair-like projections on the cellular surface have been worldwide accepted [86-91]. Conversely, the fact that HCLs show the peculiar hairy cytoplasmic projections is likely to represent the morphological evidence of *BRAF*V600E mutation recalling plump cells especially for the abundant cytoplasm. On the other hand, these hairy cells are not reported in other *BRAF* negative B-cell leukemias [86-91]. As recently reviewed by McCarthy, the *BRAF*V600E-MEK-ERK pathway might be promoted by the production of ketones [40]. In this regard, Kang demonstrated that HMG-CoA lyase (HMGCL), involved in the ketogenesis pathway, activates the interaction between *BRAF*V600E and MEK leading to the subsequent phosphorylation of MEK1 as shown in hairy-cell leukemia and melanoma cells [41].

According to literature, this mutational status has its major correlation with the diagnosis and treatment of HCL, so as to help in the choice of targeted therapies (namely interferon- α [IF- α] and purine nucleoside analogs [PAs]) allowing a complete remission in the majority of patients. However, additional studies may be addressed to understand how ketones stimulate the interaction between *BRAF*V600E and MEK and their role in inducing the morphological findings.

Additionally *BRAF*V600E mutation was also detected in other two rare histiocytoses: Langerhans-cell histiocytosis (LCH) and Erdheim-Chester disease

(ECD) characterized by a phenotype similar to CD1a⁺ S100⁺ Langerhans cells and foamy CD68⁺, CD1a⁻ S100⁻ histiocytes, respectively [15, 92-100]. Despite the controversy whether LCH and ECD are either flogistic entities or clonal neoplasms, the latter hypothesis is strongly supported by the detection of *BRAF* V600E mutation in up to half of the cases [92-98]. In the review article by Machnicki, the frequency of *BRAF* V600E ranged from 37.9% by Haroche to 68.8% by Satoh in LCH whilst it was at around 31.4% in ECD [77,100].

The high prevalence of *BRAF*V600E mutation restricted to these two histiocytoses is liable to encourage the management with vemurafenib or other ERK pathway inhibitors [100].

BRAF^{V600E} IN CENTRAL NERVOUS SYSTEM NEOPLASMS

Several authors estimated that *BRAF*V600E represents a frequent mutation in ganglioglioma (GG) [101-105]. On the other hand, the prevalence of *BRAF*V600E mutation in other low-grade gliomas has not been clearly recognized. In their paper, MacConaill reported a single case of pilocytic astrocytoma with *BRAF*V600E mutation, whilst others pointed out that some diffuse astrocytomas (WHO grade II) harboured *BRAF* V600E mutation [101, 102, 105].

Somatic mutations and specifically *BRAF*V600E through the MAPK signaling have been reported in 60% of the pleomorphic xanthoastrocytoma (PXA) representing a rare low-grade glial neoplasm of the central nervous system mostly occurring in children and young adults [103]. Specifically largepleomorphic giant cells with some xanthomatous change and showing dense deposition of intercellular reticulin and eosinophilic granular bodies are the peculiar features that are found in these tumors. It may be hypothesized that these cytoplasmic findings can be the effect of the altered cellular mechanisms activated by the somatic mutation. In this regard, the identification of *BRAF*V600E mutations, as commonly detected in PXAs, might help in discriminating them from classical glioblastoma multiforme and/or pilocytic astrocytoma [103,104]. The suggestion that *BRAF*V600E mutations is the hallmark of both PXA and GG is also demonstrated by the evidence that ganglioglioma (GG), shares significant clinicopathologic similarities with PXA, [102-104].

Moreover, Lee, in a series of 105 brain tumors (including 51 dysembryoplastic neuroepithelial tumors, 14 subependymal giant cell astrocytomas, 12 glioblastoma with neuronal marker expression, and 28 pleomorphic xanthoastrocytomas) assessed 51% and 42.9% mutated cases in the dysembryoplastic neuroepithelial tumor and subependymal giant cell astrocytomas. This data may support and encourage the aid of specific tailored therapies [104]

BRAF^{V600E} IN GASTRO-INTESTINAL NEOPLASMS

The evidence of morphological features associated with *BRAF*V600E mutations have been found in colon neoplasms [18, 106-110]. Specifically, recent data suggest that *BRAF*V600E mutation is one of the most frequent molecular abnormalities identified in hyperplastic polyps and sessile serrated adenomas which are early precursor lesions in the pathway of carcinogenesis [106-107]. In contrast, traditional serrated adenomas may be associated with either *BRAF*V600E or *KRAS* mutations [108]. According to Rosty, *BRAF*V600E mutation was assessed in several sessile serrated adenoma/polyps with or without dysplasia and microvesicular hyperplastic polyps with a percentage ranging from 95 to 76%. Hence, in their paper they attributed the morphological features of goblet cells tubulo-villous adenoma mainly to *KRAS* mutation [109].

The *BRAF* molecular correlation was also supported by the immunohistochemical positivity for VE1 suggesting a subclassification of polyps according to the mutation status. In this perspective, Mesteri proposed that the evaluation of serrated lesions with immunohistochemical or molecular *BRAF*V600E mutation may be the key to identify those lesions with higher potential to progression into *BRAF*V600E -mutated colorectal cancer [110; Figure 6A, 6B]. As assessed in the pictures 6a-6b the presence of *BRAF*V600E mutation induced the peculiar moderate-abundant cytoplasm, which were absent in wild type adenocarcinomas. These morphological features underline their unequivocal correlation with *BRAF*V600E mutation. Additionally, as reported by Rosenbaum et al, colon carcinomas harboring *BRAF*V600E mutation are also characterized by the expression of programmed cell death 1 (PD-1) and its ligand (PD-L1), microsatellite instability and a peculiar medullary morphology with frequent CD-8 positive tumor-infiltrating lymphocytes. Moreover, the PD-L1 expression in colorectal carcinomas is associated with a worse outcome as well as tumors with microsatellite instability.[111].

NOT ONLY BRAFBRAF^{V600E} MUTATION AND MORPHOLOGICAL FINDINGS

Not only did some authors report a correlation between *BRAF*V600E mutation and morphological features but also other papers documented some specific morphological clues linked with other mutations [19, 20, 112-115]. Although in 1998, the expression of c-kit protein associated with *c-kit* mutation has been considered a diagnostic hallmark of gastrointestinal stromal tumors (GISTs), a subset of them were *c-kit* wild type [114-115]. In fact, it has been well documented that a significant proportion of gastrointestinal stromal tumors (GISTs) harbour also activating mutations of platelet-derived

growth factor receptor a (*PDGFRA*). This latter mutation seems to be associated with peculiar morphological features allowing them to be discriminated from GISTs with *c-kit* mutations [19, 114-115]. Together, some authors, including Tajima, observed that GISTs with *PDGFRA* mutations are frequently characterized by epithelioid pattern, myxoid stroma, mast-cells infiltrating tumor, multi-nucleated neoplastic cells and some rhabdoid cells [19]. It stands to reason that, also among GISTs, the morphological finding may be a predictive factor of a sensitive mutation leading to personalized molecular-targeted therapy [19,114-115].

In the same perspective, mutations in the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue (*KRAS*), epidermal growth factor receptor (*EGFR*), v-Raf murine sarcoma viral oncogene homologue B1 (*BRAF*) and translocations of the anaplastic lymphoma kinase (*ALK*) gene locus have been found in several lung adenocarcinoma (ADC) with important implications for targeted therapies [20,112]. Warth highlighted that *KRAS* mutations were more frequently found in invasive mucinous ADCs whilst *EGFR* mutations were associated with lepidic growth and micropapillary ADCs [20]. On the other hand, *ALK* traslocations were recognized as oncogene drivers only in acinar and solid ADCs whilst *BRAF* mutation in micropapillary ADCs but not in papillary and lepidic ADCs [112]. This peculiar expression of mutations and/or translocations supports the association of morphology with predictive biomarkers impacting on the therapeutical management.

The glucose metabolism was also essential for RAS-driven cancers as demonstrated in inducible mouse model for oncogenic *KRASG12D* pancreatic duct adenocarcinoma (PDACs) [113]. In her review article, White described the results obtained by Ying demonstrating that RAS promotes glucose uptake and glycolysis even though tissue specific findings remain to be addressed and studied [42]. This determination represents a question mark especially because it is important to prove if this metabolic pathway may be attributed uniquely to PDACs or to other RAS-driven cancers [113]. It has been said that RAS transformation induces autophagy, which is required for tumor growth and mitochondrial function, whilst knockdown of autophagy genes causes impaired tumor progression [42].

Although *RET* proto-oncogene mutations are the most frequent mutation in medullary thyroid carcinoma (MTC), the presence of *RAS* mutations in sporadic MTC has been documented by the induction of MTCs in rasca1 transgenic mice with v-Ha-ras under the control of a specific promoter [116]. It has been reported that 0–41.2% and 0–40.9% of the cases had *HRAS* and *KRAS* mutations respectively, whilst and between 0–1.8% with *NRAS*. However to the best of our knowledge, the different type of *RET* and *RAS* mutation did not match with specific morphological features [117].

CONCLUSIONS

The insights into the involvement of metabolic mechanisms of mutated-driven cancers seem to contribute to the morphological detection of several somatic mutations. Targeting these findings may shed light on new metabolic management and inhibitors as well as metabolic flaws. It makes sense that the well-documented role of Warburg effect is a relevant characteristic of several tumors, which may justify the morphological effects of these mechanisms. Highlighting these morphological findings will represent a significant additional aid to prove a possible involvement of the metabolic processes. The knowledge of these metabolic finding of *BRAF*-driven cancers may raise additional researches and questions postulating that somatic mutations induce organ-specific molecular mechanisms enabling different prognostic prediction in different cancers.

CONFLICTS OF INTEREST

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

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