Biological insights into BRAF\textsuperscript{V600} mutations in melanoma patient

Not mere therapeutic targets

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Some experimental evidence indicates that uncommon BRAF mutations consisting in the substitution of 2 adjacent nucleotides within codon 600 are in a cis configuration and associate with BRAF gene amplification. These findings suggest that BRAF\textsuperscript{V600} mutations are unlikely to occur as homozygous alterations in clinical melanoma samples, with gene amplification perhaps contributing to mask the heterozygous state.

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway is a highly conserved signal transduction cascade involved in the regulation of cell proliferation, differentiation, and survival in response to extracellular cues, and is frequently altered in human neoplasms. The most potent activator of MAPK/ERK kinases (MEKs) is the non-receptor kinase v-raf murine sarcoma viral oncogene homolog B1 (BRAF). The most predominant activating mutation of BRAF detected in human tumors involves a thymidine to adenosine transversion at nucleotide 1799 (exon 15), resulting in the substitution of valine at residue 600 with glutamic acid (V600E).\textsuperscript{1} BRAF\textsuperscript{V600E} exhibits a 500-fold increase in kinase activity, relentlessly stimulating the activation of MEK/ERK signaling in the absence of extracellular stimuli. This corresponds to the emancipation of malignant cells from the need of external growth signals. The MEK/ERK pathway is frequently mutated in melanoma, with BRAF mutations being found in up to 70% of cases and BRAF\textsuperscript{V600E} being the most frequent alteration of all (> 90%).\textsuperscript{1}

Because of its key role in the MEK/ERK signaling pathway, BRAF has been the subject of intense investigation, leading to the approval by regulatory agencies of 2 distinct BRAF inhibitors (BRAFis), vemurafenib and dabrafenib, for use in melanoma patients. Moreover, the assessment of BRAF mutations nowadays constitutes a fundamental diagnostic procedure.\textsuperscript{2} However, many melanoma patients harboring the BRAF\textsuperscript{V600E} mutation frequently become resistant to BRAFis. This corresponds to a median duration of clinical responses that is significantly shorter than 1 y, in many cases followed by rapid disease progression.\textsuperscript{2}

Novel therapeutic options for advanced melanoma rely on immune checkpoint inhibitors or the adoptive transfer of tumor-derived T cells (i.e., tumor-infiltrating lymphocytes, TILs) expanded and optionally activated in vitro. These approaches frequently result in the activation of robust antitumor T-cell responses and (at least in some cases) induce spectacular tumor regressions, reflecting the recognition of tumor-associated antigens (TAAs) presented on the surface of malignant cells in complex with MHC molecules.

Keywords: BRAF, homozygosis, immunotherapy, melanoma, MHC, vemurafenib

Abbreviations: BRAF, v-raf murine sarcoma viral oncogene homolog B1; BRAFi, BRAF inhibitor; ERK, extracellular signal-regulated kinase; FISH, fluorescence in situ hybridization; IFN, interferon; LOH, loss-of-heterozygosity; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; TAA, tumor-associated antigen; TIL, tumor-infiltrating lymphocyte

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mutation, as assessed by a conventional sequencing procedure, are homozygous. In further support of their findings, Sapkota et al. cite a study by Sigalotti et al. suggesting that the zygosity of \( \text{BRAF}^{\text{V600E}} \) can change over time from heterozygous to homozygous, as demonstrated using metachronous melanoma metastases from different anatomical locations, even though \( \text{BRAF}^{\text{V600E}} \) appears as a "stable" and early (present also in benign nevi) mutational event. Sapkota et al. concluded by speculating that the assessment of \( \text{BRAF}^{\text{V600E}} \) zygosity may warrant further examination as a biomarker in melanoma patients bearing this mutation.

At variance with these results, we believe that, perhaps with a few notable exceptions, somatic (oncogene-activating?) mutations such as those found in most melanoma are unlikely to be stably expressed by immune effector cells. As interferons (IFNs) are potent inducers of MHC class I and class II molecules, they are generally expressed in the tumor microenvironment and can be used therapeutically; Sapkota et al. have recently investigated whether the \( \text{BRAF}^{\text{V600E}} \) mutation may affect the expression of MHC molecules by melanoma cells, to optimally integrate strategies based on targeted kinase inhibitors and immunotherapeutic agents.

According to these authors, \( \text{BRAF}^{\text{V600E}} \) reduces the amount of MHC molecules on the cell surface and its inhibition significantly boost the ability of IFN\(\gamma\) and IFN\(\alpha\) to upregulate MHC expression levels. Thus, the inhibition of \( \text{BRAF}^{\text{V600E}} \), either as a stand-alone therapeutic intervention or combined with the administration IFN\(\alpha\), may be a valid approach to enhance the expression of MHC class I molecules on the surface of melanoma cells, thus promoting their recognition by cytotoxic T cells (be they naturally present and active in the tumor microenvironment or unleashed by immunotherapy).

One important aspect of this study is the impact of \( \text{BRAF}^{\text{V600E}} \) zygosity on the capacity of vemurafenib to boost the expression of MHC molecules induced by IFN. According to Sapkota et al., indeed, this effect occurred in melanoma cell lines harboring homozygous but not heterozygous \( \text{BRAF}^{\text{V600E}} \) mutations. These authors proposed that the percentage of homozygous \( \text{BRAF}^{\text{V600E}} \) mutations in melanoma is unclear but not a rare event. In this regard, a study from Rubinstein et al. was cited, showing that roughly 50% of melanoma patients harboring the \( \text{BRAF}^{\text{V600E}} \) mutation, as assessed by a conventional sequencing procedure, are homozygous.

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homzygous, as homozygosity is typical of germinal mutations and hereditary disorders. As a matter of fact, melanoma cells generally exhibit randomly acquired mutations that stochastically affect one of both alleles, as it normally occurs in cells replicating by mitosis (as opposed to the mitotic formation of gametes). In this setting, mutations are very unlikely to simultaneously affect the same genetic locus on both alleles, rather resulting in a heterozygous somatic mutational pattern. Events that can “mask” heterozygous mutations and make them appear as homozygous are essentially two: the amplification of the mutated allele and the loss-of-heterozygosity (LOH) of one of the 2 alleles.

While gene amplification is not seen in normal cells, it occurs quite often in tumor (melanoma) cells. In the course of oncogenesis, indeed, (pre)malignant cells are under strict selective pressure and obtain a survival/growth advantage by expressing an inherently active protein, such as BRAFV600E, that stimulates tumor growth, conferring a survival/growth advantage by expressing under strict selective pressure and obtaining LOH, indeed, (pre)malignant cells are (melanoma) cells. In the course of oncogenesis, it occurs quite often in tumor (melanoma) cells. In the course of oncogenesis, it occurs quite often in tumor (melanoma) cells.

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