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BRAF inhibitors in colorectal cancer: Toward a differentiation therapy?

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BRAF inhibitor monotherapy appears to be ineffective in *BRAF*^{V600E}-positive colorectal cancer (CRC) as a result of inherent EGFR-mediated resistance mechanisms. This concept initiated combinatorial treatment approaches. Nevertheless, BRAF inhibition in isogenic CRC cell lines induced enhanced cell-cell adhesion and differentiation, underlining a potential benefit of BRAF inhibitors in CRC.

Colorectal cancer (CRC) is a heterogeneous disease characterized by a diverse set of genetic aberrations. The serine/threonine kinase BRAF, which is part of the mitogen-activated protein kinase (MAPK) pathway, is constitutively activated by the V600E mutation in 11% of CRCs.[1] This pathway controls a variety of tumor-promoting processes, including proliferation, survival, differentiation, migration, and invasion.

The frequency of *BRAF* mutations differs between microsatellite instable (MSI) versus microsatellite stable (MSS) CRCs, with higher frequencies in MSI tumors.[1,2] However, *BRAF*-mutant MSS CRCs are associated with aggressive behavior and a distinct pattern of metastatic spread, and predict a poor prognosis.[1,3] Recently, it has become clear that serrated polyps harbor *BRAF* or *KRAS* mutations and represent a histological subtype that progresses to serrated adenocarcinomas. Moreover, *BRAF*^{V600E} was identified as an early-stage event in the serrated pathway of carcinogenesis.[4] The clinical benefit of BRAF inhibitors such as vemurafenib and dabrafenib in *BRAF*-mutant melanoma also led to phase I extension trials in CRC. Although clinical data on single-agent vemurafenib treatment in CRC patients are limited, they indicate unresponsiveness to RAF inhibition.[5] Two studies attribute this finding to rapid feedback activation of the epidermal growth factor receptor (EGFR) in BRAF inhibitor-treated cells.[6,7] Corcoran et al. showed that vemurafenib treatment leads to reactivation of extracellular-signal regulated kinase (ERK) signaling by EGFR-mediated activation of Ras, CRAF, and AKT.[7] Prahallad and colleagues described feedback activation of the EGFR via inhibition of the ERK-induced phosphatase CDC25C, which dephosphorylates the EGFR.[6] These studies have initiated a series of ongoing combination trials involving BRAF or MAPK/ERK kinase (MEK) inhibitors in combination with therapeutic antibodies targeting the EGFR (www.clinicaltrials.gov).

Recently, we analyzed the effects of BRAF and MEK inhibitors, as well as allele-specific knockdown of BRAF^{V600E}, on the behavior of the *BRAF*-mutant CRC cell lines HT29 and Colo-205 in 3-dimensional (3D) tissue culture, an experimental system that more closely mimics tissue organization.[8] Our results complement the findings of Corcoran and Prahallad in conventional tissue culture by showing a similar rebound of AKT phosphorylation. Interestingly, the transcriptomic profile of PLX4720-treated 3D cultures revealed additional potential resistance mechanisms that could interfere with RAF inhibitor sensitivity. First, we observed that CDC25C was regulated not only at the protein level by ERK-mediated feedback as described by Prahallad et al.,[6] but also at the transcriptional level.[8] Secondly, and in addition to the rapid phosphorylation-driven CDC25C-mediated feedback, we observed the loss of expression of ERBB receptor feedback inhibitor 1 (ERRFI1, also known as MIG-6) protein, a well-established negative regulator of EGFR signaling. Moreover, we observed that oncogenic BRAF signaling induces the expression of cell migration inducing protein (CEMIP, also known as KIAA1199), a protein of ill-defined function that is overexpressed in a variety of solid tumors and is associated with migratory and invasive traits.[9] Recently, Shostak and colleagues showed that CEMIP can directly bind to the EGFR and promote EGFR stability and signaling by interfering with lysosomal degradation in cervical and breast cancer cells.[9] Although we have not addressed the role of CEMIP in EGFR signaling in our CRC models, the data of Shostak et al. and our study suggest that the interplay between MAPK-regulated proteins and feedback-regulated receptor tyrosine kinases (RTKs) is complex, multilayered in a spatiotemporal sense, and probably cell type- and context-dependent. These findings further highlight the need for a detailed

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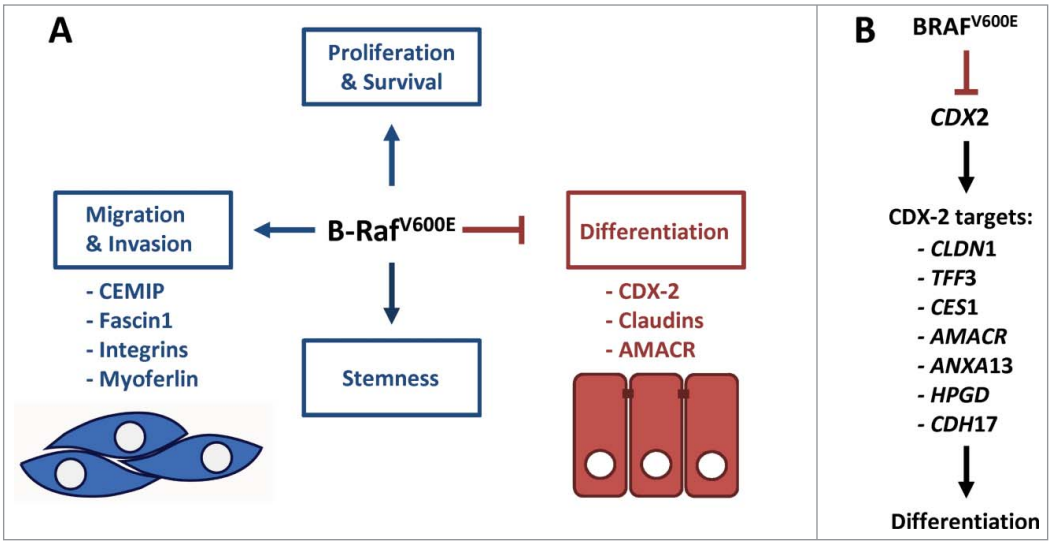

Figure 1. BRAF^{V600E}-mediated effects associated with tumor progression. (**A**) Besides its well-known effects on proliferation and survival, BRAF^{V600E} signaling is also involved in the regulation of migration and invasion, stemness, and differentiation. The listed gene products were identified as differentially regulated in PLX4720-treated or BRAF^{V600E}-depleted 3D Matrigel cultures of colorectal cancer (CRC) cell lines.[8] (**B**) Model linking BRAF^{V600E} signaling to an undifferentiated phenotype via repression of *CDX*2. For details see Herr et al.[8] AMACR, α-methylacyl-CoA racemase; *ANXA*13, annexin A13; *CDH*17, cadherin 17 (also known as liver-intestine "LI" cadherin); CDX-2, caudal type homeobox 2; CEMIP, cell migration inducing protein; *CES*1, carboxylesterase 1; *CLDN*1, claudin 1; *HPGD*, hydroxyprostaglandin dehydrogenase 15-(NAD); *TFF*3, trefoil factor 3.

understanding of the signaling networks in CRC for the rational design of effective combination therapies.

Although BRAF inhibitors will almost certainly be used in CRC therapy as part of a combinatorial regimen, we reasoned that it would be important to analyze the effects of BRAF loss or inhibition as a single perturbation on cellular processes such as migration, invasion, and adhesion. Indeed, BRAF^{V600E} depletion or inhibitor treatment reduced the migratory and invasive behavior of the CRC cell line models.[8] Surprisingly, global gene expression analysis revealed induction of a differentiation signature and downregulation of several invasion-associated genes in PLX4720-treated 3D cultures (**Fig. 1A**). Most strikingly, caudal type homeobox 2 (CDX-2), a tumor suppressor and master transcription factor of intestinal differentiation, was upregulated in response to BRAF depletion or inhibition in a set of *BRAF*-mutant CRC cell lines. This was confirmed by experiments *in vivo* in which HT29 xenografts presented with CDX-2-positive glandular structures. As loss of CDX-2 is associated with tumor stage and is frequently observed at the invasive front of colorectal tumors,[2,10] our finding that the BRAF/MEK/ERK axis suppresses *CDX*2 expression is of particular interest as it links loss of this homeobox transcription factor to endogenously expressed BRAF^{V600E} for the first time. To date, loss of *CDX*2 expression has been attributed to epigenetic mechanisms, signaling pathways including the Wnt, Notch, c-Jun N-terminal kinase (JNK), and ERK pathways, or epithelial-mesenchymal transition (EMT) regulators.[2,10] Although the negative correlation between *BRAF*^{V600E} mutation status, tumor location, and CDX-2 loss has increasingly been established in recent histopathological studies,[2] the mechanisms underlying these correlations have so far remained elusive. Furthermore, many of the differentiation-associated genes that we found to be upregulated following BRAF^{V600E} inhibition are well-known or potential CDX-2 target genes, such as claudin 1 and α-methylacyl-CoA racemase (AMACR) (**Fig. 1B**). This finding is consistent with the role of CDX-2 as a master regulator of intestinal morphogenesis and suggests that an entire suite of genes controlling epithelial differentiation and effector functions is suppressed by BRAF^{V600E} via repression of this transcription factor.

Our findings have several implications. Firstly, as differentiation indicates a more benign behavior of CRCs, its induction by inhibition of the BRAF/MEK-axis could reduce the risk of metastasis. On one hand, this could be a potential benefit in combination therapies, as the migration of disseminated cells that are already in the circulation to presumptive metastatic niches is slowed down. On the other hand, differentiation might have adverse effects if it favors the colonization of already disseminated cancer cells by promoting cell-cell adhesion. However, as a more differentiated phenotype often confers increased sensitivity toward chemotherapy, such micrometastases might become better targets for conventional strategies. Thus, it remains to be clarified whether BRAF inhibitors do indeed counteract metastasis and/or sensitize tumor cells to standard chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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