Molecular & Cellular Oncology 2:4, e1008291; October/November/December 2015; Published with license by Taylor & Francis Group, LLC

Inhibition of BRAF and BRAF+MEK drives a metastatic switch in melanoma

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Keywords: BRAF, ephrin, melanoma, metastasis, resistance

Recent analyses by our group and others showed that the majority of melanoma patients who fail BRAF inhibitor therapy do so at new disease sites. Using phosphoproteomics we showed that BRAF inhibition mediates a switch to an aggressive/metastatic melanoma phenotype that is driven by ligand-independent erythropoietin-producing hepatocellular receptor A2 (EphA2) signaling.

We recently demonstrated that chronic BRAF (RApidly growing Fibrosarcoma) inhibition was associated with increased metastatic dissemination in patients with *BRAF*-mutant melanoma, with 68% of those who failed therapy developing metastases at new sites compared to 35% of those who relapsed on the alkylating agent dacarbazine.[1] Our findings mirrored those of another study in which 50% of patients progressing on BRAF inhibitor therapy developed new metastasis.[2] *In vitro* investigations showed that melanoma cells with acquired resistance to BRAF inhibitors and the BRAF/MEK inhibitor combination were highly invasive compared to control drug-naïve cells. Using mass spectrometry-based phosphoproteomics we showed that acquired resistance to BRAF inhibitor enriched for pathways associated with the cytoskeleton, focal adhesion, and cell motility.[1] Serine 897-phosphorylated ephrin A2 (S897-EphA2) was identified as the central hub of the resistance-associated interactome network[1] (**Fig. 1A**).

Erythropoietin-producing hepatocellular (Eph) receptors form the largest family of receptor tyrosine kinases (RTKs). Activation of Eph receptors occurs following their ligation by both transmembrane and glycosylphosphatidylinositol (GPI)-linked Ephrin ligands.[3] This in turn initiates bidirectional signaling, in which "forward" signals are transduced in Eph receptor-expressing cells and "reverse" signals are transduced in the Ephrin ligand-receiving cells. In developmental settings, such as the establishment of organ boundaries and axon guidance, the Eph-Ephrin interaction generates repulsive signals that lead to inhibition of the mitogen activated protein kinase (Ras/Raf/MEK/ERK) and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways, an effect associated with reduced motile and invasive capabilities.[3] Recent work has shown that Eph receptors can also signal in a ligand-independent manner following their phosphorylation at S897 by AKT (**Fig. 1A**). This signaling is associated with a reversal of Eph function and leads to an invasive phenotype in many cancers.[4] The level of S897-EphA2 expression is a biomarker of tumor aggressiveness in some cancers and is correlated with tumor grade in astrocytoma. In other tumors, such as glioblastoma, S897-EphA2 is involved in stemness and self-renewal.[5]

The increased S897-EphA2 signaling that was observed following BRAF inhibitor treatment emerged rapidly and was associated with increased melanoma cell invasion *in vitro*. These results were recapitulated in *in vivo* melanoma mouse models of patient-derived xenografts (PDXs), in which chronic BRAF inhibition led to the emergence of new metastases in 50% of animals.[1] In all cases, high levels of S897-EphA2 were observed in the metastatic tumors that were lacking in the primary xenografts. Similar findings were also seen in specimens taken from patients on BRAF inhibitor therapy, with increased S897-EphA2 expression being seen from 14 days after the initiation of therapy. Increased S897-EphA2 expression was also observed in metastatic lesions that developed in patients on BRAF inhibitor therapy that were lacking in the original primary tumor.[1] Inhibition of S897-EphA2 signaling through small interfering RNA (siRNA) knockdown, PI3K inhibition, EphrinA1 ligand treatment, and following transfection of a kinase-dead (S897A) EphA2 plasmid all inhibited melanoma cell invasion[1] (**Fig. 1B**). There are already precedents from other cancers in which hostile microenvironments such as hypoxia, metabolic stress, and nutrient deprivation can cause tumor cells to detach and migrate to more favorable niches.[6]In some cancers, drug selection pressure also leads to phenotypic changes such as an epithelial-to-mesenchymal-transition (EMT), which

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Submitted: 01/07/2015; Revised: 01/08/2015; Accepted: 01/09/2015
http://dx.doi.org/10.1080/23723556.2015.1008291

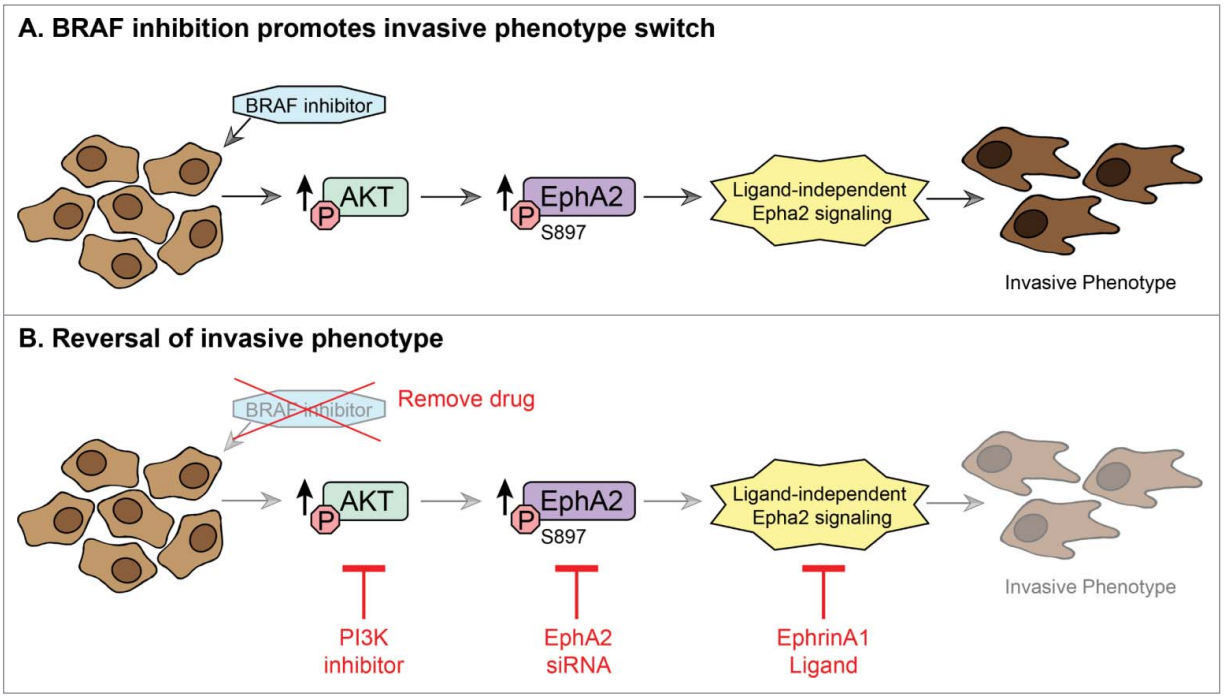

Figure 1. Mechanisms by which ligand-independent EphA2 signaling can promote the establishment of a metastatic phenotype following BRAF or BRAF+MEK inhibition. (**A**) AKT (protein kinase B, PKB) helps drive serine phosphorylation of erythropoietin-producing hepatocellular receptor A2 (EphA2) leading to increased invasion. (**B**) Possible sites of therapeutic intervention to prevent EphA2-mediated invasion.

has been associated with increased tumor invasiveness and metastatic spread. Although melanoma cells are not epithelial in origin, there is evidence from gastric cancer that EphA2 plays a role in EMT induction through activation of the wingless (WNT)/β-catenin signaling pathway. Previous studies have already hinted that BRAF inhibitor- and MEK inhibitor-resistant melanoma cells may have increased invasive potential through increased proto-oncogene tyrosine protein kinase Src-family signaling, with metastasis being inhibited through the combination of a BRAF inhibitor and the broad spectrum RTK inhibitor dasatanib.[7]

One of the more intriguing findings of our study was the observation that S897-EphA2 signaling and the increased melanoma cell invasion were dependent on continuous drug treatment.[1] The reversal of this phenotype upon drug removal suggested a role for epigenetic mechanisms in the BRAF and BRAF/MEK inhibitor-mediated regulation of S897-EphA2 signaling. There is already evidence that acquired resistance to BRAF inhibitor leads to epigenetic changes that impair the apoptotic response, and that these effects can be reversed through inhibition of histone deacetylase (HDAC).[8] In unpublished studies, we treated resistant melanoma cultures with the pan-HDAC inhibitor LBH-589 and observed decreases in EphA2 protein expression and its phosphorylation at S897 and inhibition of AKT phosphorylation. In agreement with the role of phosphorylated EphA2 in melanoma cell migration, treatment with the HDAC inhibitor LBH-589 significantly also reduced melanoma invasion.

Modeling of therapeutic responses in melanoma xenografts has already shown that BRAF inhibitor resistance can be dependent on continuous drug administration, and that tumor regression can occur following treatment withdrawal.[9] The reversibility of the S897-EphA2–mediated invasive phenotype following drug removal that we observed suggested that ligand-independent EphA2 signaling could be abrogated through discontinuous BRAF and MEK inhibitor dosing schedules. There is currently some debate in the field as to whether intermittent BRAF/MEK inhibitor dosing can forestall resistance better than continuous dosing, with evidence being provided for each scenario.[9,10] Our data support the notion that continuous BRAF and BRAF/MEK inhibitor dosing may increase the fitness and metastatic potential of melanoma cells. Whether this can be overcome through intermittent drug dosing remains to be determined and will be the subject of future studies.[1]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

Work in the Smalley lab is supported by R01 CA161107-01 and SPORE grant P50 CA168536-01A1 from the National Institutes of Health.

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