The Melanocyte Lineage Factor miR-211 Promotes BRAF\textsuperscript{V600E} Inhibitor Resistance

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Resistance to targeted therapy and immunotherapy remains a major obstacle in improving care for patients with advanced melanoma. MicroRNAs play important roles in regulating gene networks involved in disease progression and resistance to therapy in cancers such as melanoma. MicroRNA miR-211 contributes to melanocyte and melanoma biology and has been implicated in targeted therapy resistance. Lee et al. (2020) report a novel mechanism by which miR-211 promotes resistance to BRAF\textsuperscript{V600E} inhibitor therapy via the upregulation of the extracellular signal–regulated kinase 5 signaling pathway.


Cutaneous melanoma is the third most common form of skin cancer. Its clinical importance is enhanced because of the high risk of metastatic progression and the poor prognosis of metastatic disease. Activating BRAF mutations (predominantly BRAF\textsuperscript{V600E}) are the oncogenic drivers in >50% of cutaneous melanomas. Whereas targeted inhibition with BRAF\textsuperscript{V600E} specific inhibitors (BRAFi), alone or in combination with MAPK/extracellular signal–regulated kinase (ERK) kinase (MEK) inhibitors, offers high response rates, intrinsic or acquired resistance leads to disease progression and death in almost all the cases. Immunotherapy with anti-CTLA4 and anti–PD-1 antibodies has transformed metastatic melanoma care, but this approach offers durable regressions in a minority of cases. Overall, a better understanding of therapeutic resistance mechanisms may profoundly impact the care of patients with melanoma.

It has been demonstrated that the dominant mechanisms through which BRAFi resistance occurs can be broadly classified as: (i) MAPK pathway reactivation through a variety of mechanisms such as BRAF alternative splicing or amplification and acquisition and/or selection for NRAS-activating mutation, (ii) upregulation of the MITF transcription factor, (iii) loss of PTEN or related pathway genes in miR-211 expressing melanocytes and melanoma, (iv) phenotype switching to a low-MITF–dederivedependent cell state. In particular, MITF upregulation has been shown to occur in up to 50% of tumors in patients treated with BRAFi (Boshuizen et al., 2018). MITF transcriptionally regulates numerous genes that are critical for melanocyte and melanoma function and survival, including cell cycle regulators and anti-apoptotic proteins such as BCL2, BCL2A1, and CDK2. It is believed that MITF upregulation may lead to therapy resistance through the upregulation of these genes and associated pathways, but other mechanisms have not been fully delineated.

Several microRNAs have been shown to be important for melanoma survival, with miR-211 serving as a notable and well-characterized example. MicroRNA miR-211 resides genomically within intron 6 of the TRPM1 gene. TRPM1 (also termed melanostatin 1) was originally identified as a possible tumor suppressor protein in melanoma. TRPM1 and miR-211 were previously observed to be downregulated during melanoma formation and metastasis; it has since been demonstrated that the loss of miR-211, but not TRPM1, promotes melanoma invasion and metastasis through the repression of genes that are centrally important to melanoma metastasis (Levy et al., 2010; Mazar et al., 2010). Levels of TRPM1 and miR-211 are directly regulated by MITF transcription factor, and miR-211 has been previously shown to be upregulated after BRAFi treatment through MITF upregulation and to play a role in BRAFi resistance (Díaz-Martínez et al., 2018; Lunavat et al., 2017). However, the downstream mechanism(s) by which miR-211 influences therapy resistance had not been elucidated.

Lee et al. (2020) report elegant studies that are designed to identify a novel mechanism by which miR-211 promotes tumor growth and regulates therapy resistance. They began their studies with a focus on the role of miR-211 in vivo using a tumor xenograft model. The overexpression of miR-211 strongly augmented the growth of xenografted A375 melanoma cells. In this setting, miR-211 did not lead to the strong metabolic switch previously identified in vitro, suggesting that other pathways in the tumor microenvironment may be involved in the protumorigenic miR-211 effect (Mazar et al., 2016). RNA sequencing of xenografted tumors showed a strong enrichment of ERK5 signaling pathway genes in miR-211 expressing tumors. RNA immunopurification and sequencing analysis from miR-211 expressing tumors showed enrichment for potential ERK5 regulators such as BIRC2 and DUSP family phosphatases in miR-211 overexpressing cells.

The authors then utilized in vitro cell culture assays to test the functional role of these putative ERK5 regulators and to show that the targeting of DUSP6 by the overexpression of miR-211 leads to...
ERK5 activation. Conversely, DUSP6 overexpression abrogates the effect of miR-211 overexpression on tumor promotion in the in vivo xenograft model. Lee et al. (2020) also show that ERK5 is upregulated in vemurafenib-resistant melanoma cells and that miR-211 expression (as quantified by qPCR) correlates with vemurafenib resistance in a large panel of cells. Overall, these data implicate the miR-211/DUSP6/ERK5 axis as a novel regulator of BRAFi sensitivity (Figure 1).

ERK5 (encoded by the MAPK7 gene) is a fascinating protein that consists of an N-terminal kinase domain with a high homology to ERK2 and a C-terminal domain that allows for nuclear localization and transcriptional coactivation of target genes (Gomez et al., 2016). Although some cytoplasmic substrates have been identified, ERK5 is believed to mediate most of its function in the nucleus, either through kinase activity or by acting as a transcriptional coregulator. The phosphorylation of ERK5 by MEK5 in the kinase activation domain not only allows catalytic activity but also allows for autophosphorylation and nuclear translocation. ERK5 nuclear translocation can also be regulated in MEK5-independent fashion. Further work can delineate the effect of the miR-211/DUSP6 axis on ERK5 nuclear localization and determine the precise ERK5 kinase and/or transcriptional targets involved in mediating BRAFi resistance. ERK5 can be activated by numerous stress and GF pathways, including oncogenic BRAF stimulation. Importantly, ERK5 has emerged as a possible therapeutic target in BRAF- and NRAS-mutated melanoma (Adam et al., 2020; Benito-Jardón et al., 2019). The findings of Lee et al. (2020) provide further evidence regarding the role of ERK5 in therapy resistance and demonstrate a novel mechanism by which miR-211 (through DUSP6) regulates ERK5 and that may have broad relevance to melanoma therapy.

Another interesting future direction would be to further clarify the role of miR-211 in responsiveness to therapy. The work by Lee et al. (2020) suggests that miR-211 is elevated after BRAFi treatment, and it is likely that in this setting, miR-211 upregulation occurs as a result of MITF induction. It is unclear whether miR-211 plays a role in resistance in the setting of the low-MITF—dedifferentiated phenotype that occurs after targeted therapy in some tumors. Further exploration of the role of miR-211 in different contexts of therapy resistance, and in particular in human patient samples, would help to characterize the precise clinical scenarios in which miR-211 may modulate therapeutic resistance. Finally, there is a subset of BRAF/MEK inhibitor—treated patients who exhibit impressive long-term clinical responses. It would be interesting to understand whether the miR-211/DUSP5/ERK5 axis exhibits a distinct behavior in this setting.

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CONFLICT OF INTEREST
DEF holds a financial interest in Soltego, a company that is developing salt-inducible kinase inhibitors for topical skin-darkening treatments that might be used for a broad set of human applications. DEF’s interests were reviewed and are managed by Massachusetts General Hospital and Partners Healthcare in accordance with their conflict of interest policies. SMO states no conflict of interest.

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Figure 1. MicroRNA miR-211 drives BRAFi resistance through DUSP6/ERK5 axis. Resistance to BRAFi treatment in many cases is driven by the upregulation of the MITF transcription factor. miR-211 is located genomically within intron 6 of the TRPM1 gene, and TRPM1/miR-211 are regulated transcriptionally by MITF. Lee et al. (2020) demonstrate that miR-211 targets DUSP6 to prevent its expression, leading to ERK5 activation, which in turn drives proliferation and BRAFi resistance of A375 melanoma cells. BRAFi, BRAF V600E specific inhibitor; ERK, extracellular signal—regulated kinase; P, phosphate.
Inhibition of the Epigenetic Reader BRD4 Reduces SIRPα-Mediated Phagocytosis and Melanoma Invasion

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BRD4 acts as an epigenetic reader to regulate gene transcription. It represents a valid therapeutic target in cancer, and several selective and potent small molecule inhibitors have been discovered. A study by Le et al. (2020) published in Journal of Investigative Dermatology (2020) demonstrates that BRD4 inhibition reduces the invasive behavior of melanoma cells associated with matrix metalloproteinase-2 downregulation and increases phagocytosis by myeloid cells through SIRPα downregulation.


BRD4 belongs to the bromodomain and extra terminal domain family of epigenetic reader proteins that bind to acetylated lysine residues in histones, recruiting chromatin-modulating enzymes and transcription factors to regulate gene expression. In melanoma, this protein is often expressed at high levels. This can be explained by genomic amplification of the chromosome 19p13 locus in a subset of cases (Segura et al., 2013). Through the regulation of transcriptional programs that promote cellular proliferation, BRD4 contributes to the development and progression of melanoma and other tumor types. In particular, the expression of the MYC oncogene is sustained by BRD4, and the secretion of proinflammatory cytokines is promoted (White et al., 2019; Zuber et al., 2011). From this, it follows that BRD4 is a valid therapeutic target in cancer, and several selective and potent small molecule BRD4 inhibitors have been discovered (Filippakopoulos et al., 2010). Treatment with BRD4 inhibitors has multiple effects on tumor cells as well as their microenvironments by affecting PD-L1 expression and VEGF secretion and by blocking the proliferation of tumor-associated macrophages (White et al., 2019; Yin et al., 2020).

In this issue of the Journal of Investigative Dermatology, Le et al. (2020) demonstrate that BRD4 inhibition reduces the invasive behavior of melanoma cells and increases phagocytosis by myeloid cells. Examining the consequences of BRD4 inhibition in melanoma cell lines, they found that both pharmacologic inhibition and small interfering RNA-mediated genetic suppression markedly reduced migration and invasion in vitro. In previous studies, different mechanisms have been proposed to account for the effects of BRD4 inhibitors on tumor metastasis. Le et al. (2020) examined the expression of several proteins that might confer metastatic capacity after BRD4 inhibition with JQ1 and demonstrated that matrix metalloproteinase-2 (MMP2) expression was diminished in melanoma cells (Figure 1). The metalloproteinase activity of MMP2 is involved in the degradation of extracellular matrix proteins, which allows tumor cells to invade and metastasize. Thus, JQ1-induced downregulation of MMP2 provides a potential mechanism for the observed reduction in invasive behavior. Confirming epigenetic regulation, treatment with the BRD4 inhibitor JQ1 depleted the acetylation of lysine 27 of histone 3 (H3K27Ac) at the MMP2 promoter. In accordance with their findings, in a very recent study on oral squamous cell carcinoma, it was found that BRD4 promoted metastatic behavior through the regulation of MMP2 (Yamamoto et al., 2020). It would

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Ogando A, Teixido J. Resistance to MAPK inhibitors on tumor metastasis. Le et al. (2020) demonstrated that BRD4 inhibition reduces the invasive behavior of melanoma cells and increases phagocytosis by myeloid cells. Examining the consequences of BRD4 inhibition in melanoma cell lines, they found that both pharmacologic inhibition and small interfering RNA-mediated genetic suppression markedly reduced migration and invasion in vitro. In previous studies, different mechanisms have been proposed to account for the effects of BRD4 inhibitors on tumor metastasis. Le et al. (2020) examined the expression of several proteins that might confer metastatic capacity after BRD4 inhibition with JQ1 and demonstrated that matrix metalloproteinase-2 (MMP2) expression was diminished in melanoma cells (Figure 1). The metalloproteinase activity of MMP2 is involved in the degradation of extracellular matrix proteins, which allows tumor cells to invade and metastasize. Thus, JQ1-induced downregulation of MMP2 provides a potential mechanism for the observed reduction in invasive behavior. Confirming epigenetic regulation, treatment with the BRD4 inhibitor JQ1 depleted the acetylation of lysine 27 of histone 3 (H3K27Ac) at the MMP2 promoter. In accordance with their findings, in a very recent study on oral squamous cell carcinoma, it was found that BRD4 promoted metastatic behavior through the regulation of MMP2 (Yamamoto et al., 2020). It would

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