A Germline Variant in *KDM4A* Enhances Sensitivity to mTOR Inhibition

- A coding SNP in *KDM4A* promotes its protein turnover and is associated with worse outcome in NSCLC.
- Lung cancer cells homozygous for SNP-A482 are more sensitive to drugs targeting the mTOR/PI3K pathway.
- Rapamycin reduces KDM4A SNP-A482 levels, and KDM4A depletion increases mTOR inhibitor sensitivity.

Single nucleotide polymorphisms (SNP) occur frequently in human DNA and are often associated with disease risk or outcome; however, little is known about how germline coding variants affect the function of chromatin-modifying enzymes in cancer. Van Rechem and colleagues characterized a nonsynonymous coding SNP (SNP-A482) in lysine (K)-specific demethylase 4A (*KDM4A*), which resulted in an E482A amino acid substitution. Different allelic frequencies of SNP-A482 were observed across various ethnic groups, and homozygosity for this polymorphism was associated with poorer outcomes in patients with non–small cell lung cancer (NSCLC). In comparison to the wild-type protein, KDM4A SNP-A482 exhibited increased interaction with the SKP–cullin–F-box containing (SCF) complex and enhanced ubiquitination, resulting in decreased protein stability. In an unbiased drug screen, lung cancer cells homozygous for SNP-A482 were significantly more sensitive to drugs targeting pathways related to mTOR/PI3K signaling; this increased sensitivity was not observed with two noncoding *KDM4A* SNPs, suggesting a specific relationship between SNP-A482 and the mTOR pathway. Consistent with this idea, KDM4A SNP-A482, but not wild-type KDM4A, protein levels were significantly reduced by rapamycin treatment, and depletion of KDM4A resulted in enhanced sensitivity to mTOR inhibition. These data document a germline coding variant within a chromatin modifier that influences NSCLC outcome and drug response. In addition, this SNP may represent a candidate biomarker for mTOR inhibitor therapy and may enable improved stratification and treatment of patients with NSCLC. ■

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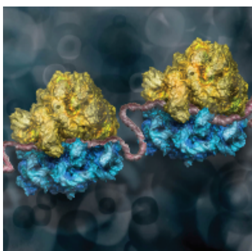
The Lysine Demethylase KDM4A Regulates Protein Synthesis

- KDM4A interacts with and modulates the distribution of translation initiation factors in polysomes.
- Depletion of KDM4A decreases protein synthesis and potentiates the effect of mTOR inhibitors.
- JmjC demethylase blockade impairs translation initiation and increases mTOR inhibitor sensitivity.



Lysine (K)-specific demethylases (KDM) are best characterized as chromatin-modifying enzymes that remove methylation from histone substrates in the nucleus. However, the function of KDMs in the cytoplasm remains poorly understood. Van Rechem and colleagues found that KDM4A interacted with components of the translation initiation complex and was enriched in the translation initiation fractions of polysome profiles. Overexpression of catalytically inactive KDM4A disrupted its enrichment within the initiating fractions of polysomes, suggesting that the enzymatic activity of KDM4A plays an important role in regulating its distribution. Consistent with a role for KDM4A in modulating translation, depletion or overexpression of KDM4A altered the distribution of translation initiation factors in polysome fractions, suggesting that optimal levels of KDM4A expression are critical for protein synthesis. Furthermore, KDM4A depletion resulted in decreased protein synthesis and enhanced the suppressive effects of the mTOR inhibitor rapamycin on translation initiation and cell viability. Similarly, treatment with the JmjC demethylase inhibitor JIB-04, which targets the KDM4A and KDM5A enzymes, impaired translation initiation, decreased protein synthesis, and increased the sensitivity of various cancer cell lines to mTOR inhibition. These findings define a role for cytoplasmic KDM4A in the regulation of protein synthesis, and suggest that inhibition of lysine demethylases may potentiate the antitumor effects of translation inhibitors. ■

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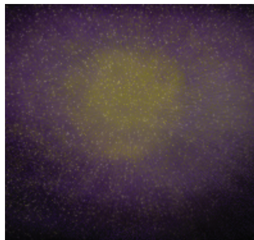
EPHA2-Driven Invasion Underlies Metastasis in Drug-Resistant Melanoma

- Ligand-independent EPHA2 signaling is upregulated upon chronic BRAF inhibitor treatment.
- EPHA2-driven cell invasion is dependent on AKT-mediated phosphorylation at S897.
- EPHA2 expression is induced in metastatic lesions that develop post–BRAF inhibitor treatment.



Acquired resistance to oncogenic BRAF inhibitors (BRAFi) results in tumor recurrence and metastatic outgrowth in patients with melanoma. Although reactivation of downstream MAPK signaling is a common mechanism of resistance to chronic BRAF inhibition or combined BRAF/MEK inhibition, increased receptor tyrosine kinase (RTK) signaling has also been suggested to contribute to targeted-therapy escape and the increased invasive potential of resistant cells. To dissect how long-term BRAF inhibition rewires signaling networks in *BRAF*-mutant melanoma, Paraiso and colleagues used a mass spectrometry–based phosphoproteomic approach and found that vemurafenib-resistant cells were enriched for phosphoproteins involved in cell adhesion and migration, including the ephrin type-A receptor 2 (EPHA2) RTK. In line with these results, BRAFi- or BRAFi/MEKi–resistant melanoma cell lines generated *in vitro* exhibited enhanced motility, increased EPHA2 phosphorylation at S897, and induction of ligand-independent EPHA2 signaling. Suppression of EPHA2 in BRAFi-resistant cells or drug removal abrogated this invasive phenotype, whereas introduction of EPHA2 in melanoma cells lacking EPHA2 expression increased cell invasion. Mechanistically, phosphorylation of EPHA2 at S897 was dependent on AKT signaling and was required to promote invasion of BRAFi-resistant cells. Importantly, increased levels of EPHA2 and phosphorylated EPHA2 S897 were detected in vemurafenib-resistant patient-derived xenografts, on-treatment and post-relapse primary tumor samples, and in newly formed metastatic lesions arising post-vemurafenib treatment. Together, these results suggest that EPHA2 upregulation occurs in response to chronic BRAF inhibition and may potentially be therapeutically exploited to limit metastatic disease in patients receiving BRAFi or BRAFi/MEKi treatment. ■

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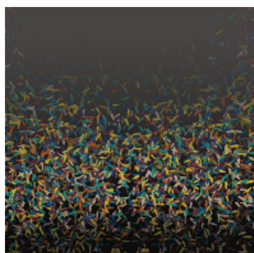
EPHA2 Induction Drives Vemurafenib Resistance in Melanoma

- EPHA2 upregulation mediates vemurafenib resistance in melanoma cell lines and primary tumors.
- First-in-class EPHA2 inhibitors suppress AKT and MAPK signaling to induce apoptosis.
- EPHA2 inhibition suppresses the growth of vemurafenib-sensitive and -resistant tumors *in vivo*.



Despite the clinical success of the BRAF inhibitor (BRAFi) vemurafenib, acquired resistance remains a clinical challenge. Given that activation of receptor tyrosine kinases (RTK) represent a mechanism of BRAFi resistance, Miao and colleagues hypothesized that ephrin type-A receptor 2 (EPHA2), an RTK that is upregulated in *BRAF*-mutant and *BRAF*–wild-type melanomas and that promotes melanoma cell survival, may play a role in mediating BRAFi resistance and melanoma progression. In support of this idea, EPHA2 expression was elevated in vemurafenib-resistant melanoma cell lines generated *in vitro*, negatively correlated with BRAFi sensitivity across 40 melanoma cell lines, and was upregulated in clinical samples of relapsed tumors post–BRAFi treatment. Overexpression of EPHA2 conferred BRAFi resistance, whereas suppression of EPHA2 heightened BRAFi sensitivity in both naïve and vemurafenib-resistant cell lines and reduced cell migration and colony formation in vemurafenib-resistant cells. Screening of an ATP-competitive inhibitor library highlighted two compounds, ALW-II-41-27 and HG-6-64-1, with strong EPHA2 inhibitory activity. Importantly, a positive correlation between EPHA2 levels and compound sensitivity was observed across cell lines, and pretreatment with EPHA2 inhibitors resensitized BRAFi-resistant cells to vemurafenib. Mechanistically, ALW-II-41-27 and HG-6-64-1 inhibited AKT and MAPK signaling downstream of EPHA2, resulting in cell-cycle arrest and induction of apoptosis in vemurafenib-sensitive and vemurafenib-resistant cells *in vitro* and suppression of xenograft tumor growth *in vivo*. Together, these findings highlight EPHA2 as a driver of BRAFi resistance and provide a rationale for the clinical development of EPHA2 inhibitors to overcome BRAFi resistance. ■

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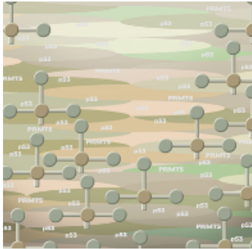
PRMT5 Drives Lymphomagenesis via Methylation-Dependent p53 Inhibition

- PRMT5 cooperates with oncogenic cyclin D1 to induce aggressive lymphomas in a mouse model.
- PRMT5-mediated arginine methylation of p53 inhibits proapoptotic p53 target gene expression.
- Elevated PRMT5 expression correlates with p53 methylation in cyclin D1–driven primary tumors.



Protein arginine methyltransferase 5 (PRMT5) is frequently upregulated in human lymphomas, but its contribution to tumorigenesis is unclear. Li and colleagues determined that coexpression of PRMT5 with a constitutively nuclear cyclin D1 mutant (D1T286A) in bone marrow hematopoietic stem/progenitor cells (HSPC) induced lymphomagenesis upon transplantation. In addition, inactivation of PRMT5 inhibited colony formation driven by other oncogenes such as NOTCH1 and c-MYC *in vitro*, supporting a critical role for PRMT5 in neoplastic transformation. In contrast to expression of D1T286A alone, which resulted in hematopoietic failure due to p53-dependent apoptosis, coexpression of PRMT5 antagonized p53-mediated cell death and promoted the survival of D1T286A-expressing HSPCs harboring wild-type p53. This protective function was dependent on the catalytic activity of PRMT5 and D1T286A-induced phosphorylation of methylosome protein 50 (MEP50, also known as WDR77), a component of the PRMT5 methyltransferase complex. Consistent with these findings, D1T286A triggered a significant increase in PRMT5-dependent p53 arginine methylation, leading to altered p53 chromatin binding and a reduction in proapoptotic p53 target gene expression. Notably, concurrent elevated PRMT5 expression and p53 arginine methylation were found in nuclear cyclin D1–driven primary human lymphoma and esophageal squamous cell carcinoma samples. These results indicate that PRMT5 can cooperate with oncogenes to induce lymphomagenesis via p53 methylation, defining an alternative mechanism for the bypass of p53-mediated tumor suppression in hematologic malignancies, and suggest PRMT5 as a potential therapeutic target. ■

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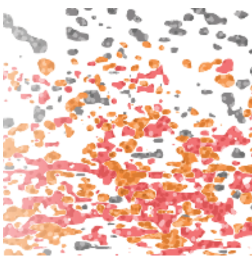
ETV1 Is a Therapeutic Target in Gastrointestinal Stromal Tumors

- The ETV1 transcription factor is essential for GIST tumor initiation and proliferation *in vivo*.
- ETV1, which is stabilized by MAPK signaling, directly induces *KIT* as part of a positive feedback loop.
- Dual KIT/MEK blockade targets ETV1 protein stability and synergistically inhibits GIST tumor growth.



Gastrointestinal stromal tumors (GIST) are frequently associated with activating mutations in the *KIT* oncogene and initially respond well to treatment with the tyrosine kinase inhibitor imatinib (Gleevec). However, the long-term clinical efficacy of imatinib is limited by the development of acquired resistance in most patients, underscoring the need for combinatorial therapeutic strategies. The transcription factor ETS variant 1 (ETV1) functions as a critical regulator of GIST precursor cells, the interstitial cells of Cajal, and is stabilized in GIST cells by MAPK signaling downstream of activated KIT, implicating ETV1 as a potential therapeutic target. Using genetically engineered mouse models of GIST driven by *Kit* mutation, Ran and colleagues found that *Etv1* ablation impaired GIST tumor formation and cell proliferation, indicative of an essential role for ETV1 in GIST tumor initiation *in vivo*. In addition, ETV1 depletion resulted in a decrease in *KIT* transcript levels; ETV1 directly bound the *KIT* enhancer regions to induce *KIT* expression, suggesting that ETV1 and KIT cooperate in a positive feedback loop to drive GIST tumorigenesis. Intriguingly, combined treatment with imatinib and the MEK inhibitor MEK162 destabilized the ETV1 protein and synergistically suppressed GIST tumor growth in imatinib-sensitive human xenograft models and a *Kit*-mutant GIST mouse model. These findings identify a therapeutic strategy to target ETV1 protein stability and suggest that this approach may be more effective than single-agent imatinib in patients with advanced GIST. ■

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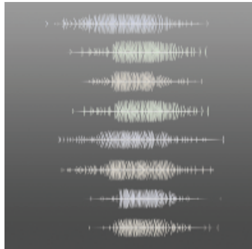
JAK Inhibitors Target Malignant and Nonmalignant Cells in MPN

- The output and heterogeneity of single-cell cytokine secretion in the bone marrow is increased in MPN.
- STAT3 signaling in both normal and malignant cells drives cytokine production and MPN severity.
- The JAK inhibitor ruxolitinib normalizes cytokine production by both malignant and nonmalignant cells.



Somatic mutations that activate the JAK–STAT pathway are prevalent in myeloproliferative neoplasms (MPN), bone marrow disorders characterized by hyperproliferation of myeloid lineages, and the JAK inhibitor ruxolitinib has shown clinical activity in patients with MPN. Levels of circulating proinflammatory cytokines are elevated in patients with MPN and decline in response to ruxolitinib, but it remains unclear whether this contributes to the clinical efficacy of JAK inhibition in MPN. Kleppe, Kwak, and colleagues performed microfluidic profiling of bone marrow cells from patients and mouse models to analyze secretory cytokine production at single-cell resolution and found that cytokine production per cell and cytokine heterogeneity significantly increased in MPN bone marrow compared with control bone marrow. Deletion of *Stat3* in the hematopoietic compartment of mice with MPN normalized cytokine production and reduced cytokine-mediated inflammation in association with reduced disease severity and longer overall survival, whereas MPN-specific deletion of *Stat3* had no effect on cytokine production or disease severity, suggesting that JAK–STAT signaling in both malignant and nonmalignant hematopoietic cells plays a role in MPN pathogenesis. Indeed, single-cell analysis revealed that the output and heterogeneity of cytokine production was elevated in malignant and nonmalignant cells in an MPN mouse model and that ruxolitinib treatment normalized cytokine secretion by both populations. These findings establish that JAK–STAT signaling drives abnormal cytokine production in MPN and suggest that inhibition of proinflammatory cytokine production by both malignant and nonmalignant cells underlies the activity of JAK inhibition in MPN. ■

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