**Concurrent HER2 or HER3 Mutations May Confer HER-Inhibitor Resistance**

- Patients with ERBB2-mutant metastatic breast cancer were treated with neratinib alone or plus fulvestrant.
- Some tumors exhibited selection for multiple ERBB2 (encoding HER2) mutations or additional ERBB3 mutations.
- These combinations of mutations may underlie some de novo and acquired resistance to HER inhibitors.

In breast cancer, some mutations in ERBB2 (encoding HER2) are associated with oncogenic addiction to HER2 signaling. Building on a phase II trial of single-agent treatment with the irreversible pan-HER inhibitor neratinib in patients with ERBB2-mutant solid tumors, Smyth and colleagues added a cohort of heavily pretreated patients with ERBB2-mutant metastatic breast cancer to evaluate the combination of neratinib with fulvestrant, a selective estrogen receptor (ER) degrader. Eighty-one patients were enrolled, including 47 (all HR+) treated with neratinib plus fulvestrant and 34 (23 HR+ and 11 HR-) treated with neratinib alone. Across both groups, unique ERBB2 mutations were identified in 22 patients, and the distribution of mutations was as expected for patients with breast cancer. The overall response rate (ORR) in the monotherapy group was 17.4% in patients with ER+ disease and 36.4% in patients with ER- disease, and the ORR was 29.8% in the group receiving combination therapy. Of note, the trial was not designed to compare efficacy between the monotherapy and combination-therapy groups. Deeper investigation revealed that a subset of tumors exhibited selection for multiple ERBB2 mutations and that mutations in ERBB3 (encoding HER3) were more prevalent in tumors with only one ERBB2 mutation—perhaps representing another mechanism to increase HER signaling—and that these tumors may be more recalcitrant to neratinib treatment. Together, the data presented in this study support the notion that some ERBB2-mutant tumors exhibit multiple mutations affecting ERBB2 or ERBB3, potentially underlying both de novo and acquired resistance to HER inhibitors.

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**RAS-Pathway Activation Drives Resistance to SYK Inhibition in AML**

- RAS-pathway activation drives spleen tyrosine kinase (SYK)-inhibitor resistance in acute myeloid leukemia (AML).
- Combining a SYK inhibitor with a MEK inhibitor was effective in *in vitro* and *in vivo* models of AML.
- This study elucidates mechanisms of resistance to SYK inhibition and suggests a method to overcome it.

SYK (encoding spleen tyrosine kinase) is not typically mutated in acute myeloid leukemia (AML), but SYK inhibitors have shown promise in treating patients with AML. Aiming to identify resistance mechanisms, Cremer and colleagues first validated that the selective SYK inhibitor entospletinib is effective in AML cell lines and patient-derived xenograft mouse models of AML. A genome-wide open-reading-frame (ORF) screen identified overexpression of wild-type RAS ORFs (HRAS and KRAS), RAS pathway-activator genes (e.g., CRKL), or RAS pathway downstream effector genes (e.g., RAF1, MAPK1, and MAPK3). Correspondingly, overexpression of wild-type NRAS and KRAS conferred resistance to entospletinib in AML cell lines, as did expression of NRAS with the hyperactivating G12D mutation, and AML cell lines naturally harboring RAS mutations exhibited greater entospletinib resistance. Experiments using AML cells chronically exposed to entospletinib showed that the development of drug resistance likely occurred via SYK upregulation or upregulation of pathways that bypass SYK, such as the JAK–STAT or RAS–MAPK–ERK pathways, with the latter predominating. Highlighting the potential clinical relevance of these findings, RAS–MAPK–ERK pathway activation was correlated with entospletinib resistance in patients with AML in a prior clinical trial. Additionally, in AML cell lines and a primary AML sample, SYK-inhibitor resistance driven by mutation of RAS could be overcome by adding a MEK inhibitor. This combination was also effective in mouse models of AML. This work provides new understanding of mechanisms of SYK-inhibitor resistance and suggests that combination SYK and MEK inhibition may be worth investigating further.

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The presence of desmoplasia caused by cancer-associated fibroblasts (CAF) is thought to be a major factor in the poor prognosis of pancreatic ductal adenocarcinoma (PDAC), and modulating CAF function has been shown to alter outcomes in some preclinical models of PDAC. In normal mouse pancreata as well as mouse PDACs, Dominguez, Müller, and colleagues found that cells positive for the mucin-type protein podoplanin (PDPN) were the predominant fibroblasts. These PDPN+ fibroblasts exhibited heterogeneity in their expression of CAF-associated markers, and single-cell RNA sequencing (RNA-seq) of mouse PDACs revealed the presence of several transcriptionally defined groups of CAFs. With tumor progression, there was an increase in frequency of one of these clusters, dubbed c2; in late-stage tumors, c2-cluster CAFs constituted approximately 60% of all CAFs. Bulk RNA-seq of PDPN+ CAFs from early- and late-stage tumors showed that Lrrc15, encoding a conserved transmembrane protein that exhibits increased expression in some human tumor types, was restricted to the c2 CAF population. Correspondingly, TGFβ-responsive LRRIC15+ CAFs were the most common fibroblasts in human PDAC samples. Data from The Cancer Genome Atlas indicated that LRRIC15+ CAFs were common not only in PDAC, but also in multiple other cancers. Notably, data from recent clinical trials involving patients with several cancer types revealed that an LRRIC15+ CAF signature was associated with poorer response to immunotherapy, demonstrating the translational relevance of these findings and indicating the need for further investigation of the transformation from normal fibroblasts to c2 CAFs. See article, p. 232.

Pyroptosis Mediates Melanoma Sensitivity to BRAF and MEK Inhibition

Combination treatment with inhibitors of BRAF and MEK are often effective in patients with BRAFV600E/K mutant melanoma; however, resistance to these targeted therapies can occur and is associated with a reduction in intratumoral CD8+ T cells and an increase in tumor-associated macrophages. In mouse models of BRAFV600E-mutant melanoma, Erkes, Cai, and colleagues found that an intact immune system is required for optimal response to the combination of BRAF and MEK inhibitors, and the amount of time required for treatment resistance to develop was dependent on immune-system status. Further, inhibition of tumor growth by combination treatment with BRAF and MEK inhibitors required T cells. Interestingly, multiple lines of evidence supported the notion that combination treatment with BRAF and MEK inhibitors caused the tumor cells to undergo pyroptosis, an inflammation-provoking, immunostimulatory form of programmed cell death that can be triggered by cleavage of gasdermin E (GSDME) by caspase 3. Supporting the importance of the role of pyroptosis in response to combination BRAF and MEK inhibition, tumors derived from cells with acquired resistance to the treatment did not undergo pyroptosis, and expression of the pyroptosis-inducing fragment of GSDME in resistant cells was sufficient to restore drug sensitivity. Importantly, pharmacologic treatment that rescued pyroptosis reduced tumor growth and improved survival in mice bearing tumors resistant to combination BRAF and MEK inhibition. In summary, this work provides a mechanistic basis for immune system–mediated resistance of BRAFV600E-mutant melanoma to BRAF and MEK inhibition and provides a potential method to circumvent treatment resistance. See article, p. 254.
**ASF1A Is a Potential Target in KRAS-Mutant Lung Adenocarcinoma**

- Deficiency of Asf1a enhanced anti–PD-1 response in mouse models of Kras-mutant lung adenocarcinoma.
- Asf1a deficiency with anti–PD-1 treatment enhanced M1-like macrophage polarization and T-cell proliferation.
- Investigation of ASF1A inhibition combined with anti–PD-1 or other immunotherapies is warranted.

KrAs mutations are common in lung adenocarcinoma, but KRAS has proven difficult to target, making the development of new therapies for patients with KRAS-mutant tumors crucial. Using an epigenome-wide CRISPR screen in a Kras-mutant mouse model of lung cancer, Li and colleagues found that Asf1a, encoding a histone chaperone, negatively regulated the response to immunotherapy with anti–PD-1. Corroborating the significance of this finding, in vivo experiments revealed that Asf1a deficiency enhanced response to anti–PD-1. Providing a possible explanation for this synergy, the combination of Asf1a deficiency and treatment with anti–PD-1 enhanced T-cell activation and increased M1-like macrophage polarization. Mechanistically, RNA sequencing (RNA-seq) and chromatin immunoprecipitation with sequencing experiments revealed that Asf1a deficiency activated TNFα signaling and upregulated the gene encoding GM-CSF, a cytokine that promotes granulocyte and macrophage proliferation. Further experiments revealed that the observed increase in M1-like macrophage polarization and T-cell proliferation associated with Asf1a deficiency was likely mediated by this upregulation of GM-CSF. Single-cell RNA-seq analysis of mouse lung adenocarcinomas confirmed this finding, showing that the mechanism by which the combination of anti–PD-1 treatment and Asf1a deficiency decreased tumor progression likely involved promotion of M1-like macrophage polarization and T-cell proliferation. Together, these results identify Asf1a as a potentially exploitable vulnerability in Kras-mutant lung adenocarcinoma, suggesting that the combination of ASF1A inhibition and immunotherapy with agents such as anti–PD-1 is worth investigating in further studies.

See article, p. 270.

**A RHoa Mutant Promotes Diffuse Gastric Cancer via PI3K and YAP–TAZ**

- RHoaY42A and inactivation of CDH1 (encoding E-cadherin) are commonly found in diffuse gastric cancers.
- RHoaY42A, a gain-of-function mutant, activates PI3K, promotes YAP–TAZ signaling, and promotes tumorigenesis.
- These functions of RHoa depended on focal adhesion kinase, providing a possible drug target.

The most common genomic abnormality in diffuse gastric cancers (DGC) is somatic inactivation of CDH1 (encoding E-cadherin). Missense mutations in RHoa (encoding RAS homolog family member A) are present in 15% to 26% of DGC cases, but the consequences of these mutations are unclear, with some work suggesting they are inactivating and other work suggesting the opposite. Mouse experiments by Zhang, Schaefer, and colleagues found that loss of Cdh1 along with expression of human RHoa bearing the hotspot mutation Y42C increased DGC development in vivo. In vitro experiments established that RHoaY42C is a gain-of-function mutant that promotes formation of actin stress fibers and assembly of focal adhesions. Mechanistically, the observed gain of function may be explained by the fact that RHoaY42C exhibits reduced intrinsic and GTPase-activating protein–stimulated GTP hydrolysis. In the absence of Cdh1, RHoaY42C activates PI3K, enhancing β-catenin activation, and promotes YAP–TAZ signaling. Deeper investigation into this pathway revealed that proper function of the YAP–TAZ and β-catenin pathways is essential for transformation mediated by RHoaY42C in the absence of Cdh1. The induction of PI3K-AKT and YAP–TAZ signaling in this context is mediated by activation of focal adhesion kinase (FAK), with FAK inhibition inhibiting cell proliferation and tumor growth in vitro and in vivo, respectively. Finally, experiments using human cell lines and patient DGC surgical samples implied that FAK inhibition may have antitumor effects. Together, this work elucidates a previously unknown pathway underlying DGC, a disease for which mechanistic understanding is limited.

See article, p. 288.
Amplification of oncogenes on genomic or extrachromosomal DNA is common in cancers. Clarke and colleagues found that histone 3 methylation at lysine residue 9 or 27 (H3K9Me or H3K27Me) decreased the likelihood of EGFR amplification. Further, overexpression of the H3K9/36 demethylase KDM4A increased EGFR amplification in normal and lung cancer cells, whereas treatment with a KDM4-family inhibitor reduced EGFR amplification and reduced response of the lung cancer cells to the EGFR inhibitor gefitinib. Mirroring the effect of overexpression of an H3K9 demethylase, depletion of multiple H3K9 methyltransferases tested increased the copy number of EGFR. Similarly, inhibition or depletion of the H3K27 methyltransferase EZH2 resulted in increased EGFR copy number and expression, as did overexpression of the H3K27 demethylases KDM6A or KDM6B. H3K4 methylation (generally associated with active transcription) also played a role, with increased H3K4Me promoting EGFR copy-number gain via KDM4A. Interestingly, exposure to hypoxic conditions for 24 hours transiently increased EGFR copy number, likely through stabilization of KDM4A. Additionally, 24-hour treatment with EGFR’s primary ligand, EGF, caused KDM4A-dependent EGFR amplification, and the combination of hypoxia and EGF treatment resulted in an additive increase in EGFR copy number. In summary, this study identifies epigenetic and physiologically driven mechanisms that control amplification of EGFR in normal and cancer cells, providing insight into why this specific oncogene is so commonly amplified and identifying potential targets to suppress EGFR amplification.

See article, p. 306.

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