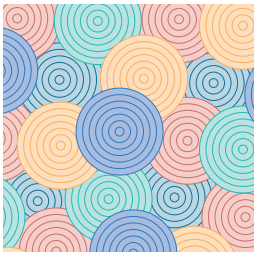
A Preexisting *PI3KCA*^{E545K} Mutation Confers Resistance to MEKi plus CDK4i

- A *PI3KCA*^{E545K} mutation is detected as a rare preexisting subclone in a patient after MEKi plus CDK4i.
- *PI3KCA*^{E545K} subclones are heterogeneously distributed, requiring multiregion sequencing to detect.
- S6K1 inhibitors may suppress *PI3KCA*^{E545K}-mediated resistance to MEKi plus CDK4i in NRAS-mutant melanoma.



Preclinical data indicate that CDK4 inhibition (CDK4i) in combination with MEK inhibition (MEKi) results in a more complete shutdown of NRAS signaling than single-agent MEKi, prompting evaluation of this combination in a phase Ib/II clinical trial of patients with NRAS-mutant melanoma, which achieved promising preliminary results. To identify mechanisms of resistance to this combination therapy, Romano and colleagues performed whole-exome sequencing of five longitudinal biopsies from a patient who initially responded to MEKi plus CDK4i, but eventually developed resistance. Comparison of pre- and post-resistance samples revealed an acquired oncogenic mutation: *PI3KCA*^{E545K}. To determine if this mutation may have eluded standard detection methods pretreatment, blocker displacement amplification was used to detect rare variants on multiple regions of the pretreatment tumor. This showed that the *PI3KCA*^{E545K} mutation was preexisting, found in only 3 of 7 pretreatment regions as a rare variant, and the *PI3KCA*^{E545K} population was rapidly expanded upon MEKi plus CDK4i treatment. *PI3KCA*^{E545K} expression conferred resistance to MEKi plus CDK4i in NRAS-mutant melanoma cell lines and increased phosphorylation of S6 and its upstream kinase S6K1. S6 is activated downstream of mTOR, and additional inhibition of mTOR or S6K1 reduced S6 phosphorylation and resensitized *PI3KCA*^{E545K} cells to MEKi plus CDK4i. *In vivo*, adding an S6K inhibitor to MEKi plus CDK4i treatment reverted *PI3KCA*^{E545K}-mediated resistance, inducing durable tumor regression in melanoma xenografts. Collectively, these findings indicate that thorough analyses are needed to uncover rare preexisting resistant cells and that unbiased molecular analyses can identify therapies to counter resistance. ■

See article, p. 556.

Targeting mTOR May Overcome Acquired Resistance to MEKi plus CDK4/6i

- Continuous MEKi plus intermittent CDK4/6i achieves maximal responses *in vivo* in a xenograft model.
- Acquired resistance to MEKi plus CDK4/6i is associated with increased S6 phosphorylation.
- Adding mTORC inhibitors may improve efficacy after patients have progressed on MEKi plus CDK4/6i.

MEK inhibitors (MEKi) have been approved for the treatment of advanced cutaneous melanoma, but more-effective treatment regimens are still needed. CDK4/6 inhibitors (CDK4/6i) are currently being investigated in clinical trials in patients with melanoma, but continuous dosing results in dose-limiting toxicities. Further, mechanisms of acquired resistance to CDK4/6 inhibition have not been determined. Teh and colleagues evaluated combined and intermittent dosing schedules for MEKi and/or CDK4/6i in melanoma models and investigated resistance mechanisms. In a BRAF-mutant melanoma mouse model expressing an E2F reporter to measure pathway activation downstream of CDK4/6, continuous MEKi plus intermittent CDK4/6i led to more complete responses than continuous CDK4/6i plus intermittent MEKi or intermittent treatment with both drugs. Further, continuous MEKi plus intermittent CDK4/6i was not associated with significant toxicities. However, two resistant tumors with E2F reactivation emerged. Acquired resistance was associated with an NRAS-mediated increase in phosphorylation of the S6 ribosomal protein. Consistent with these findings, elevated S6 phosphorylation was linked to relapse in tumors from patients with BRAF-mutant melanoma treated with combined BRAFi, MEKi, and CDK4/6i. S6 is downstream of mTOR, and treatment with an mTORC inhibitor reduced S6 phosphorylation and suppressed the growth of MEKi/CDK4/6i–resistant tumors *in vivo*. In addition to optimizing the dosing schedules for MEKi plus CDK4/6i, these findings suggest that the addition of an mTORC inhibitor may potentially overcome acquired resistance in patients with melanoma. ■

See article, p. 568.

A TEAD4–MYCN Feedback Loop Drives High-Risk *MYCN*-Amplified Neuroblastoma

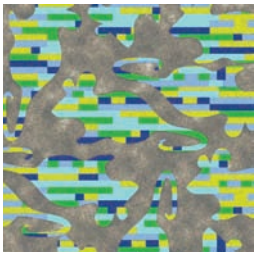
- The transcriptional state of *MYCN*-amplified neuroblastoma is induced by a 10-protein network.
- The *MYCN*-amplified master regulatory module is controlled by a TEAD4–MYCN positive-feedback loop.
- TEAD4 is a subtype-specific vulnerability and prognostic marker in *MYCN*-amplified neuroblastoma.

High-risk neuroblastomas are characterized by recurrent and complex chromosomal alterations, including frequent focal amplification of the *MYCN* oncogene; however, the molecular networks that are associated with these copy-number changes and drive these aggressive tumors remain unclear. Rajbhandari, Lopez, and colleagues used a systems biology approach to identify master regulator proteins that mechanistically control the transcriptional state of high-risk neuroblastoma subtypes and may represent therapeutic vulnerabilities. Gene expression analysis across two cohorts classified high-risk neuroblastomas into three molecular subtypes and inferred subtype-specific candidate master regulator proteins. Further validation in the *MYCN*-amplified subtype using RNAi screens defined a hierarchical 10-protein module of subtype-specific dependencies, which engaged in multiple autoregulatory loops and was controlled by the transcription factor TEAD4 together with MYCN. TEAD4 engaged in a positive-feedback loop with MYCN, enhancing MYCN expression via both transcriptional regulation and inhibition of MYCN protein degradation. In addition, TEAD4 activated transcription of cell cycle and DNA damage response genes in *MYCN*-amplified cells independent of YAP/TAZ activity; silencing of TEAD4 or MYCN reversed the subtype-specific transcriptional signature and diminished viability *in vitro* and *in vivo*. Consistent with this finding, TEAD4 expression was found to be an independent prognostic marker in patients with high-risk neuroblastoma. These results elucidate a protein network that drives the aggressive transcriptional phenotype of *MYCN*-amplified neuroblastoma and identify TEAD4 as a subtype-specific master regulator and potential therapeutic target in these tumors. ■

See article, p. 582.

Small Cell Lung Cancer PDXs Recapitulate the Human Disease

- SCLC PDXs from biopsies and circulating tumor cells maintain genetic alterations of the patient tumor.
- PDXs were successfully generated from 38% of circulating tumor cell samples and 89% of biopsies.
- PDXs derived from patients with SCLC accurately model clinical drug response and resistance.

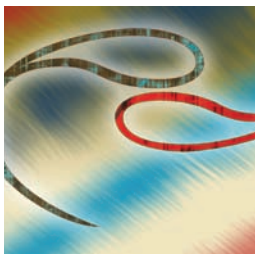


Patient-derived xenograft (PDX) models may recapitulate human tumors better than other preclinical models to facilitate translational research. However, the development of small cell lung cancer (SCLC) PDXs has been limited by the scarcity of tissue, as surgical resection and repeat tumor biopsies are not the standard of care in metastatic SCLC. Drapkin and colleagues developed an approach for efficient generation of SCLC PDX models from tumor biopsies and circulating tumor cells (CTC). PDXs were successfully generated from 16 of 42 (38%) CTC samples and 16 of 18 (89%) tumor biopsies. Both the biopsy- and CTC-derived PDXs recapitulated the histopathologic features of human disease, and whole-exome sequencing of 7 PDX models confirmed that somatic alterations present in the primary tumor are maintained in PDXs. Early-passage PDXs also maintained the genomic and transcriptional profiles of the founder PDX. Patients with SCLC often acquire resistance to chemotherapy with etoposide plus platinum (EP), and PDXs from EP-naïve patients exhibited a greater sensitivity to EP, suggesting that PDXs can recapitulate the chemosensitivity of SCLC in patients. A *MYC* expression signature emerged as a biomarker of EP resistance, as *MYC* target genes were upregulated in PDXs from EP-resistant tumors. Further, serial CTC-derived PDXs generated from a patient treated with olaparib plus temozolomide recapitulated the evolving drug sensitivity of the patients over time. In addition to developing an approach for efficient generation of PDXs, these data indicate that PDX models may recapitulate patient drug response and resistance and facilitate the identification of biomarkers. ■

See article, p. 600.

HOXA9 Cooperates with STAT5 to Drive T-ALL

- *JAK3* mutations activate STAT5, which co-localizes with HOXA9 at sites across the genome.
- HOXA9 upregulation is linked to *JAK3* mutations in T-ALL, and coexpression accelerates leukemogenesis.
- PIM1 may be a potential therapeutic target in patients with JAK/STAT/HOXA9-positive leukemia.

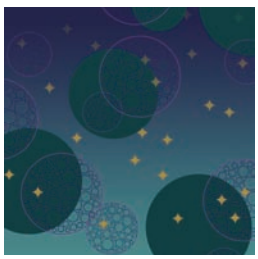


Mutations in *JAK3* occur frequently in patients with T-cell acute lymphoblastic leukemia (T-ALL) and drive leukemogenesis. However, patients frequently harbor additional oncogenic mutations that may cooperate to promote cellular transformation. De Bock and colleagues analyzed genetic data from JAK-mutant T-ALL and found that *JAK3* mutations are associated with HOXA upregulation. *In vivo*, HOXA9 expression cooperated with *JAK3* mutations to transform hematopoietic stem and progenitor cells and accelerate leukemia development. Retroviral coexpression of JAK3^{M511I} and HOXA9 in developing lymphoid cells induced oncogenic transformation, and transplantation *in vivo* resulted in T-ALL with a reduced latency. Mechanistically, JAK3^{M511I} activated downstream STAT5, and STAT5 and HOXA9 co-localized at sites across the genome in leukemia cells. HOXA9 enhanced the transcriptional activity of STAT5, thereby increasing expression of STAT5 target genes including the PIM1 kinase. PIM1 was also upregulated in patients with HOXA$^+$ or *JAK*-mutant T-ALL, and in patient-derived xenografts, dual inhibition of JAK1 and PIM1 reduced the leukemic burden. Assay for transposase-accessible chromatin using sequencing revealed changes in chromatin architecture when HOXA9 was expressed in JAK3^{MS511I} leukemia cells that resulted in upregulation of FOS and JUN. Taken together, these findings indicate that STAT5 and HOXA9 cooperate to drive T-ALL development and suggest the potential for therapeutic targeting of PIM1 and JAK1 in patients with HOXA$^+$ or *JAK*-mutant T-ALL. ■

See article, p. 616.

NUAK1 Drives the Antioxidant Response to Promote Colorectal Cancer

- NUAK1 is critical for colorectal cancer initiation and tumorigenesis.
- Oxidative stress activates NUAK1 to inactivate GSK3β and drive the nuclear translocation of NRF2.
- Targeting NUAK1 is a potential therapeutic strategy to enhance oxidative stress in colorectal tumors.

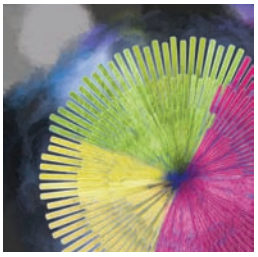


There is broad interest in understanding how to therapeutically exploit oxidative stress in cancer by manipulating antioxidant metabolic pathways, such as the cellular energy homeostasis pathway driven by the master energy sensor AMPK, or by inhibiting the transcription factor NRF2, which drives the expression of an antioxidant transcriptional program. Having recently shown that the AMPK-related kinase NUAK1 is critical for MYC-overexpressing cancers, Port and colleagues investigated the role of NUAK1 in colorectal cancer tumorigenesis. Elevated NUAK1 expression was correlated with worse outcome for patients with colorectal cancer, and pharmacologic NUAK1 inhibitors promoted MYC-dependent apoptosis in human colorectal cancer cell lines. *Nuak1* was essential for the formation of colonic polyps in the *Apc*$^{+/-}$;*Kras*^{G12D} mouse model of colorectal cancer and *ex vivo* spheroid formation of *Apc*$^{-/-}$;*Kras*^{G12D} murine colonic epithelium. *NUAK1* depletion resulted in decreased expression of the NRF2-regulated antioxidant transcriptional program, and oxidative stress induced apoptosis in *NUAK1*-depleted cells compared with control cells. By restraining PP1β phosphatase activity, NUAK1 enables inhibitory phosphorylation of GSK3β, which otherwise prevents the nuclear translocation of NRF2 during oxidative stress, and pharmacologic inhibition of GSK3b restored NRF2 nuclear accumulation in *NUAK1*-depleted colorectal cancer cells. *In vivo*, *Nuak1* ablation drove significant colon tumor regression, which was rescued by antioxidant treatment. These results characterize the role of NUAK1 in the antioxidant stress response in colorectal cancer initiation and maintenance, and identifies suppression of this kinase as a potentially therapeutically tractable approach to promote cancer-specific oxidative stress. ■

See article, p. 632.

MEK1 Mutations Activate ERK Signaling via Distinct Mechanisms

- *MEK1* mutations can be divided into 3 classes based on the mechanism by which they induce ERK signaling.
- All MEK1 mutants exhibit sensitivity to ATP-competitive MEK inhibitors.
- MEK1 mutants with constitutive kinase activity are resistant to allosteric MEK inhibitors.



Mutations in *MEK1* occur in a variety of tumor types and increase ERK activation to promote tumorigenesis. MEK1 mutations occur at different sites on the protein and are not clustered in a single hotspot, suggesting that *MEK1* mutations may have distinct allele-specific functions. Gao and colleagues investigated the mechanisms by which 17 different tumor-associated *MEK1* mutations activate downstream signaling. All 17 mutations activated ERK signaling to varying degrees, but the degree of MEK and ERK phosphorylation varied, separating the mutations into 3 different classes. The first class of mutants were completely RAF dependent, were hyperstimulated by phosphorylation, and could not autonomously drive ERK signaling. These mutants acted as amplifiers of ERK signaling driven by other coexisting mutations and were sensitive to ERK-dependent feedback inhibition of RAS/RAF signaling. The second class of mutants had baseline RAF-independent activity that could be further activated by RAF-mediated phosphorylation. These mutants were also sensitive to ERK-dependent feedback. The third class of mutants harbored deletions affecting amino acids 98–104 that produced constitutive kinase activity independent of RAF activity and phosphorylation, revealing an autoinhibitory MEK1 domain. Although the class 1 and 2 mutants were sensitive to allosteric MEK inhibitors, which bind to the inactive form of MEK1, the class 3 mutants were insensitive to allosteric MEK inhibition. However, all three classes of mutants were sensitive to an ATP-competitive MEK inhibitor. In addition to demonstrating allele-specific mechanisms of ERK activation, these findings suggest RAF-independent MEK1 mutants may be sensitive to ATP-competitive MEK inhibitors despite resistance to allosteric inhibitors. ■

See article, p. 648.

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