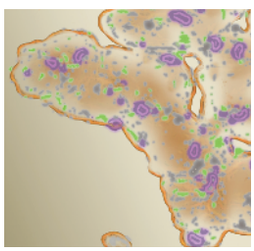
IN THIS ISSUE

Sustained MAPK Activity Confers Resistance to RAF Inhibitor Combinations

- MAPK reactivation drives resistance to RAF/MEK and RAF/EGFR inhibition in *BRAF*-mutant colorectal cancer.
- *KRAS*, *BRAF*, and *MEK1* alterations were identified in post-progression tumor biopsies.
- ERK inhibition suppresses MAPK signaling and overcomes resistance to RAF inhibitor combinations.



In contrast to *BRAF*-mutant melanoma, RAF inhibitor monotherapy is largely ineffective in *BRAF*-mutant colorectal cancer due to feedback reactivation of MAPK signaling, prompting the development of combinatorial therapeutic strategies that target RAF together with EGFR or MEK. However, although clinical trials suggest that these combinations have increased efficacy, resistance ultimately develops, underscoring the importance of identifying the mechanisms of clinical acquired resistance to RAF inhibitor combinations in *BRAF*-mutant colorectal cancer. Ahronian, Sennott, Van Allen, and colleagues found that *BRAF*-mutant colorectal cancer cells made resistant to RAF/EGFR or RAF/MEK inhibitor combinations *in vitro* developed *KRAS* mutations, which sustained MAPK pathway activation. Consistent with this finding, whole-exome sequencing of paired pretreatment and post-progression tumor biopsies from three patients with *BRAF*-mutant colorectal cancer treated with RAF inhibitor combinations identified genetic alterations in the MAPK pathway arising specifically in post-progression resistant lesions, including amplification of wild-type *KRAS* or mutant $BRAF^{V600E}$ and mutations in *MAP2K1* (which encodes MEK1). Overexpression of wild-type KRAS, $BRAF^{V600E}$, or $MEK1^{F53L}$ reactivated MAPK signaling and was sufficient to confer resistance to RAF/EGFR and RAF/MEK inhibitor combination therapy *in vitro*. Importantly, however, these cells retained sensitivity to treatment with an ERK inhibitor, which suppressed MAPK activity and overcame resistance driven by each of these alterations. These findings indicate that *BRAF*-mutant colorectal cancer cells are dependent on MAPK signaling and suggest that ERK inhibitors may be an effective therapeutic strategy. ■

See article, p. 358.

Genetic Variants in microRNA Binding Sites Influence Prostate Cancer Risk

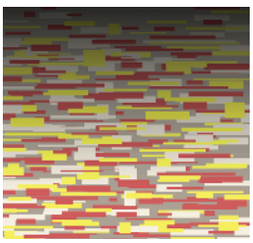
- Large-scale analysis identified 22 miRSNPs in 16 genes linked to prostate cancer risk.
- Interaction of *miR-3162-5p* with the *KLK3* rs1058205 SNP T-allele induces KLK3 downregulation.
- *miR-370-5p* targets the *VAMP8* rs1010 SNP A-allele with greater affinity.

Genome-wide association studies (GWAS) have linked 100 single-nucleotide polymorphisms (SNP) to prostate cancer risk. However, these SNPs account for only 33% of familial risk of prostate cancer, suggesting that additional genetic variants remain to be identified. Stegeman and colleagues carried out a comprehensive large-scale analysis of 22,301 prostate cancer cases and 22,320 controls of European ancestry from 23 studies and focused on genetic variants that are predicted to affect miRNA binding sites (miRSNP). Of 2,169 miRSNPs analyzed, 22 miRSNPs within 16 genes, including genes previously unmapped by GWASs, were associated with prostate cancer risk. The most significant miRSNP was rs1058205 within kallikrein-related peptidase 3 (*KLK3*, also known as *PSA*), and seven SNPs showed differences between aggressive and non-aggressive disease. The expression of seven of the 16 genes, including *KLK3* and vesicle-associated membrane protein 8 (*VAMP8*), was dysregulated in prostate cancer compared with matched normal prostate tissue. Functional validation studies revealed that *miR-3162-5p*, which was expressed in both cancerous and noncancerous prostate cell lines, had specific affinity for the *KLK3* rs1058205 SNP T-allele; overexpression of *miR-3162-5p* resulted in decreased expression of KLK3 mRNA and protein. In addition, *miR-370-5p*, which is known to be upregulated in prostate cancer, had higher affinity for the *VAMP8* rs1010 SNP A-allele, which was significantly linked to aggressive disease. These data suggest that functional evaluation of miRNAs and their interaction with miRSNPs may further illuminate the mechanisms of prostate cancer risk. ■

See article, p. 368.

Genomic Analysis Maps the Genetic Regulation of Splicing in Neuroblastoma

- Alternative splicing was characterized using integrative analysis of a neuroblastoma mouse model.
- Analysis of splicing quantitative trait loci identified genes that regulate alternative splicing.
- Intronic splicing motif mutations affect splicing in human cancers, including neuroblastoma.

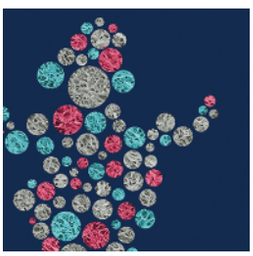


Alternative splicing of mRNA is a tightly regulated process that is controlled both in *cis* by mechanisms that act within the primary pre-mRNA transcript and in *trans* by recruitment of factors to splice sites. Deregulated splicing control has been implicated in many cancers, including neuroblastoma, in which differential splicing patterns have been observed between disease stages and are associated with high-risk disease alleles. To systematically assess the genetic regulation of alternative splicing in neuroblastoma, Chen and colleagues compared genome and transcriptome data from a genetically engineered mouse model of neuroblastoma and identified splicing quantitative trait loci (sQTL). *Trans*-sQTLs were scattered throughout the genome, occurred in a tissue-specific manner, and highlighted genes encoding known splicing factors, as well as two candidate splicing-related genes, *Rbmx2* and *Ddx26b*. Analysis of *cis*-sQTLs revealed mouse strain–specific alternative splicing of genes including *Fubp1*, which correlated with patient survival and resulted in altered MYC expression. Furthermore, comparison with whole-genome sequencing data defined unique intronic splicing motifs that were enriched for recurrent somatic mutations in several genes common to both human neuroblastoma and glioblastoma, including *DYNLRB1*, *AIMP1*, and *NPIPA1*. Mutation of these intronic splicing motifs resulted in functional changes in alternative splicing, and altered expression of the corresponding genes correlated with patient outcome in neuroblastoma. Together, this work demonstrates the use of an unbiased integrative genomic approach to identify splicing factors and intronic splicing motifs that modulate alternative splicing across cancers, and highlights candidate genes potentially involved in neuroblastoma. ■

See article, p. 380.

A Genetic Platform Enables Identification of Key Genes in Sarcomagenesis

- Hypoxic culture prevents BM-MSC transformation and provides a platform to model sarcomagenesis.
- *Lrf* deletion enhances *Trp53*-null BM-MSC transformation and formation of undifferentiated sarcomas.
- LRF-mediated inhibition of DLK1 and SOX9 is required for BM-MSC differentiation and tumor suppression.

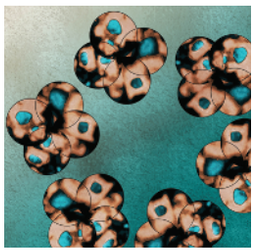


Undifferentiated sarcomas are aggressive soft-tissue tumors thought to arise from adult mesenchymal stem cells (MSC). However, the molecular mechanisms underlying sarcomagenesis remain poorly characterized, and effective therapeutic options for patients with these tumors are lacking. To address these issues, Guarnerio and colleagues developed an *ex vivo/in vivo* genetic platform that facilitates the identification of genetic drivers of undifferentiated sarcoma. Culture in hypoxic conditions prevented the spontaneous transformation of primary mouse and human adult bone marrow MSCs (BM-MSC), enabling the authors to screen for genes that combine with loss of *Trp53* to trigger BM-MSC transformation *in vitro* and generation of undifferentiated sarcomas *in vivo*. Depletion of zinc finger and BTB domain–containing 7A (*Zbtb7a*, also known as *Lrf*) favored the transformation of *Trp53*-null BM-MSCs and induced the formation of invasive undifferentiated sarcomas in transplanted mice, suggesting a role for LRF as a tumor suppressor in sarcomagenesis. Mechanistically, LRF was required for mesenchymal lineage commitment and promoted the differentiation of BM-MSCs into adipocytes, osteoblasts, and chondrocytes via transcriptional repression of delta-like 1 homolog (*Dlk1*). Furthermore, LRF suppressed the tumorigenic potential of *Trp53*-null BM-MSCs via negative regulation of *Dlk1* and inhibition of SRY (sex-determining region Y)-box 9 (SOX9) activity. These results establish the utility of this platform to model sarcomagenesis, highlight a role for LRF in BM-MSC differentiation and tumor suppression, and suggest that inhibition of DLK1 or SOX9 may be an effective therapeutic strategy. ■

See article, p. 396.

Autophagy Overcomes Senescence to Drive Melanoma Growth

- *Atg7* deletion or hydroxychloroquine treatment suppressed growth of *Braf*^{V600E}, *Pten*-null melanoma.
- Oxidative stress and senescence were increased in *Atg7*-deficient tumors compared with control tumors.
- *Atg7* loss potentiated dabrafenib-induced senescence and regression of *Braf*-mutant, *Pten*-null melanoma.

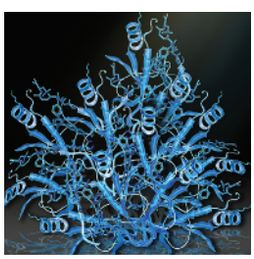


Cells degrade and recycle damaged proteins and organelles through a process known as autophagy, which can be oncogenic or tumor suppressive in different preclinical contexts. Some tumors harboring *BRAF* mutations have been shown to require autophagy for survival, but it remains unclear whether these findings will extend to other cancers in which *BRAF* mutations are common, such as melanoma. Using a genetically engineered mouse model of spontaneous melanoma in which both alleles of the essential autophagy gene *Atg7* could be inducibly deleted in melanocytes along with activation of *Braf*^{V600E} and deletion of *Pten*, Xie and colleagues showed that loss of *Atg7* significantly suppressed melanoma growth and extended survival. Treatment of established *Braf*^{V600E}, *Pten*-null melanomas with the autophagy inhibitor hydroxychloroquine also markedly suppressed tumor growth without toxicity, further indicating that autophagy supports *Braf*-mutant melanoma progression and that systemic autophagy inhibition may be a safe and effective treatment for this disease. In addition to the expected autophagy defects, *Atg7*-deficient tumors showed increased senescence in association with elevated oxidative stress, suggesting that ATG7 drives growth of *Braf*^{V600E}, *Pten*-null melanomas by opposing senescence. Moreover, *Atg7* deletion potentiated senescence induced by the BRAF inhibitor dabrafenib and led to more pronounced inhibition of tumor growth than dabrafenib alone, underscoring the role of autophagy in promoting survival in *BRAF*-mutant, *PTEN*-deficient melanoma and providing further evidence that inhibition of autophagy may be effective in this setting, alone or in combination with BRAF inhibitors. ■

See article, p. 410.

A Selective FGFR4 Inhibitor Is Effective Against FGF19-Driven HCC

- BLU9931 is a potent covalent inhibitor of FGFR4 with high FGFR paralog and kinome selectivity.
- Expression of an intact FGFR4 signaling complex is required for BLU9931-mediated growth inhibition.
- BLU9931 shows marked antitumor activity in HCC xenografts with *FGF19* amplification or overexpression.

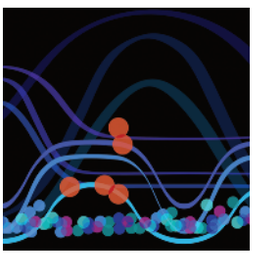


Overexpression of *FGF19* due to amplification or other as-yet-unidentified mechanisms results in autocrine activation of the FGFR4 signaling pathway in a subset of hepatocellular carcinomas (HCC). Using a structure-based design approach, Hagel, Miduturu, and colleagues developed a small-molecule inhibitor of FGFR4, BLU9931, which covalently binds a cysteine (Cys552) that is unique among FGFR paralogs and proximal to the ATP-binding pocket. BLU9931 potently and selectively inhibited FGFR4 while sparing FGFR1, 2, and 3, other kinases with a similar cysteine, and the vast majority of kinases in a large panel. Consistent with these findings, BLU9931 inhibited FGFR4 signaling and cell proliferation in a dose-dependent manner in HCC cells that express an intact FGFR4 receptor/ligand complex consisting of FGF19, FGFR4, and the coreceptor klotho β. In an HCC xenograft model that overexpresses *FGF19* due to amplification, BLU9931 treatment was well tolerated and resulted in induction of cytochrome P450 7A1 (also known as cholesterol 7-α-hydroxylase) mRNA, a potential biomarker, and dose-dependent tumor regression, including complete responses in two of nine mice. Furthermore, BLU9931 exhibited antiproliferative activity in cell lines derived from patient-derived HCC xenografts that overexpress *FGF19* without gene amplification and induced robust inhibition of tumor growth *in vivo*. Taken together, these results indicate that BLU9931 is a potent and selective inhibitor of FGFR4 that may be efficacious in the treatment of the subset of patients with HCC whose tumors harbor an intact FGFR4 signaling cascade. ■

See article, p. 424.

FGFR-Driven MAPK Reactivation Reduces Imatinib Sensitivity in GIST

- FGF2–FGFR1 signaling attenuates the antiproliferative effects of imatinib in *KIT*-mutant GIST cells.
- Imatinib relieves SPRY-mediated feedback inhibition of FGF signaling, resulting in ERK reactivation.
- Combined inhibition of FGFR enhances imatinib's antitumor effects *in vitro* and *in vivo*.



Activating mutations in the KIT and platelet-derived growth factor receptor α kinases are common in gastrointestinal stromal tumors (GIST) and have been successfully targeted using the tyrosine kinase inhibitor imatinib. However, despite improved patient prognosis, durable response to imatinib remains a challenge due to secondary mutations and acquired resistance. Recent work suggests that crosstalk between KIT and ligand-dependent receptor tyrosine kinases may also contribute to imatinib resistance. Using a secretome screen, Li and colleagues found that FGF ligands attenuated imatinib's antiproliferative effects in human GIST cell lines. In line with this result, FGF2 and FGFR1 were found to be highly expressed in primary GISTs. Combined treatment with imatinib and the FGFR1–3 inhibitor BGJ398 restored imatinib sensitivity in the presence of exogenous FGF2 and led to greater antiproliferative effects in GIST cells in the absence of FGF ligands. Mechanistically, long-term imatinib treatment induced FGF pathway activation and subsequent reactivation of ERK phosphorylation both *in vitro* and *in vivo*, in part by downregulating the expression of Sprouty 2 (SPRY2) and SPRY4, which repress FGF signaling as part of an ERK-dependent negative feedback loop. Importantly, cotreatment with imatinib and BGJ398 resulted in enhanced apoptosis and improved antitumor activity in patient-derived *KIT*-mutant GIST xenograft models. This work highlights how activation of parallel growth pathways via relief of negative feedback may contribute to imatinib resistance and provides a rationale for combined inhibition of KIT and FGFR in patients with GIST. ■

See article, p. 438.

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In This Issue

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