ERCC2 Mutation May Contribute to Cisplatin Response in Urothelial Carcinoma

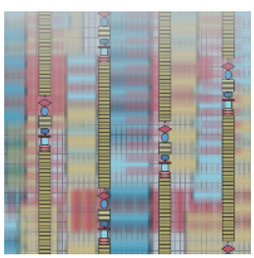
- Somatic *ERCC2* mutations are enriched in cisplatin-responsive urothelial carcinoma samples.
- ERCC2 mutants confer enhanced cisplatin and UV sensitivity in ERCC2-deficient cells.
- Defective nucleotide excision repair may promote cisplatin sensitivity in urothelial carcinoma.

Neoadjuvant platinum-based chemotherapy followed by surgical resection is the standard of care for patients with muscle-invasive urothelial carcinoma. Patients whose tumors exhibit a complete pathologic response to neoadjuvant cisplatin have a significant survival advantage; however, cisplatin is highly toxic, and the lack of a predictive biomarker for clinical benefit has limited its use in these patients. Van Allen, Mouw, and colleagues performed whole-exome sequencing of pretreatment tumor and germline DNA from 50 patients with muscle-invasive urothelial carcinoma who had received cisplatin-based chemotherapy, half of whom had an extreme response with no residual invasive disease following treatment. Excision repair cross-complementation group 2 (*ERCC2*), which encodes a nucleotide excision repair (NER) helicase involved in repair of cisplatin-induced DNA damage, was the only significantly mutated gene enriched in cisplatin responders as compared with nonresponders and unselected patients with bladder cancer. All identified somatic nonsynonymous *ERCC2* mutations occurred at conserved positions within or adjacent to the helicase domains. In contrast to wild-type ERCC2, stably expressed ERCC2 mutants failed to rescue cisplatin and UV sensitivity and were associated with increased genomic instability in ERCC2-deficient cells, suggesting that *ERCC2* mutations may contribute to enhanced cisplatin response by impairing NER. These results establish an approach to study exceptional responses to commonly used cytotoxic drugs and suggest that somatic *ERCC2* mutations may provide a means of selecting patients with urothelial carcinoma who are most likely to benefit from cisplatin-based chemotherapy. ■

See article, p. 1140.

Activated NOTCH1 and *HES4* Are Biomarkers of Response to NOTCH Inhibition

- Activating gene rearrangements leading to partial *NOTCH1* or *NOTCH2* deletion were identified in TNBC.
- High levels of activated NOTCH1 and *HES4* expression are correlated with GSI sensitivity in TNBC.
- NOTCH1 activation was associated with GSI response in *NOTCH1*-mutant adenoid cystic carcinoma.



Aberrant activation of NOTCH signaling via mutation or gene rearrangements has been implicated in several cancers, including T-cell acute lymphoblastic leukemia and breast cancer. However, biomarkers that are predictive of clinical response to NOTCH pathway suppression with gamma-secretase inhibitors (GSI) are lacking. Using whole-exome sequencing, Stoeck, Lejnine, and colleagues identified rearrangements in the *NOTCH1* and *NOTCH2* genes specifically in triple-negative breast cancer (TNBC) cell lines and primary tumors. In contrast to *NOTCH2*-rearranged cells, which were resistant to GSI treatment, mutations that disrupted the negative regulatory region (NRR) in NOTCH1 were correlated with increased GSI sensitivity in most *NOTCH1*-mutant TNBC cells. *NOTCH1*-rearranged cells exhibited high levels of activated NOTCH1, as measured by NOTCH intracellular domain (N1-ICD) protein levels, which were correlated with GSI sensitivity both *in vitro* and in TNBC xenograft models. In addition, increased expression of the NOTCH target gene *HES4* was associated with activating *NOTCH1* mutations and poor outcome in patients with TNBC, suggesting that this gene may represent a prognostic biomarker in this tumor type. NOTCH pathway inhibition resulted in the induction of senescence and apoptosis in *NOTCH1*-rearranged cells, and combined administration of paclitaxel enhanced the antitumor activity of GSI treatment. Furthermore, gain-of-function point mutations in the NOTCH1 NRR were also detected in adenoid cystic carcinomas and were associated with increased N1-ICD levels and enhanced GSI sensitivity. These results suggest that these biomarkers may identify patients most likely to respond to GSI-based therapies. ■

See article, p. 1154.

A Functional Screen Detects Tumor Suppressors that Inhibit FGFR Signaling

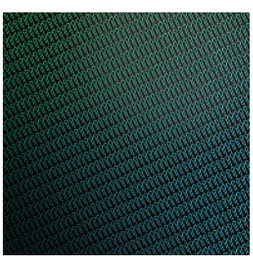
- An RNAi-based screen in mice identified tumor suppressors that are downregulated in hLSCC.
- Knockdown of many TSGs enhanced FGFR signaling and conferred FGFR inhibitor sensitivity.
- FGFR activation in the absence of *FGFR1* amplification or mutation promotes lung tumorigenesis.

Transcriptionally or epigenetically silenced tumor suppressor genes (TSG) critical for tumor initiation are obscured by traditional cancer genome sequencing. Therefore, Lin and colleagues employed a large-scale functional genomics screening approach in which nontransformed murine fibroblasts were transduced with shRNA pools and assessed for tumorigenic capacity in mice. Further analyses validated 24 candidate TSGs with human homologs that were downregulated in more than 70% of human lung squamous cell carcinoma (hLSCC) samples. For a subset of these TSGs, promoter hypermethylation was significantly increased in hLSCC samples compared with matched normal tissue, indicative of epigenetic silencing. Consistent with frequent genetic alterations of *FGFR1* that activate the FGFR pathway in hLSCC, many TSGs encoded repressors of FGFR signaling; knockdown of 17 TSGs transformed nontumorigenic human bronchial epithelial cells via increased FGFR1 signaling and conferred sensitivity to FGFR inhibitors. The mechanism of FGFR1 activation was varied and included FGFR1-dependent and FGFR1-independent activation of the downstream effector FGFR substrate 2. For example, loss of the candidate TSG serine/arginine–rich splicing factor 9 (*SRSF9*) altered the pre-mRNA splicing of SH3-domain binding protein 2 (*SH3BP2*), which encodes an FGFR1-interacting protein, resulting in *SH3BP2* missplicing, SH3BP2 protein degradation, and increased FGFR1 levels. Furthermore, low *SRSF9* levels correlated with aberrant *SH3BP2* splicing in hLSCC samples. Taken together, these results highlight the utility of unbiased functional genomics screens and identify candidate TSGs that promote FGFR-dependent lung tumorigenesis in the absence of *FGFR1* amplification or mutation. ■

See article, p. 1168.

Combinatorial RNAi Therapy Is Effective in *KRAS*-Mutant Colorectal Cancer

- An RNAi library of potent siRNAs targeting RAS pathway genes was generated using a Sensor assay.
- Low-dose siRNA treatment enables combination gene knockdown and reduces off-target effects.
- Nanoparticle-delivered siRNAs targeting KRAS and effector nodes inhibit *KRAS*-mutant tumors *in vivo*.

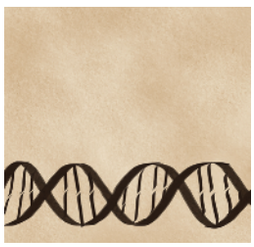


Efforts to pharmacologically inhibit KRAS either directly or by targeting the downstream RAF and MEK effector pathways have thus far been unsuccessful, emphasizing the need to develop alternative therapeutic strategies for *KRAS*-mutant cancers. To assess the potential for RNAi therapy in *KRAS*-mutant cancer, Yuan, Fellmann, Lee, and colleagues used a Sensor assay, in which siRNA efficiency was determined by measuring knockdown of a fluorescent reporter, to generate a library of potent siRNAs targeting RAS pathway genes. Low-dose siRNA treatment suppressed target gene expression and minimized off-target effects, and siRNA payloads were effectively delivered *in vivo* using nanoparticles. RNAi-mediated depletion of KRAS inhibited MAPK signaling, resulting in reduced viability of *KRAS*-mutant colorectal cancer cell lines *in vitro* and impaired growth of *KRAS*-mutant xenograft tumors. In addition, combined administration of potent siRNAs targeting various KRAS effectors, including RAF, MEK, and ERK isoforms, enabled simultaneous and robust depletion of multiple genes; codepletion of all three RAF isoforms suppressed ERK signaling, induced apoptosis in *KRAS*-mutant but not *KRAS*–wild-type colorectal cancer cells, and reduced tumor growth *in vivo*, similar to the effects of KRAS knockdown. Furthermore, combined depletion of KRAS and PI3K isoforms resulted in enhanced inhibition of *KRAS*-mutant tumor growth. These results highlight the utility of this approach to identify and validate therapeutic target combinations and provide evidence that targeted knockdown of driving oncogenes and effector nodes via RNAi may represent an effective therapeutic strategy in KRAS-driven cancers. ■

See article, p. 1182.

Phosphorylation of MPG by ATM Contributes to Temozolomide Resistance

- 3-methylpurine-DNA glycosylase (MPG) loss increases temozolomide (TMZ) sensitivity in pediatric GBM cells.
- Phosphorylation by ATM promotes MPG activity, providing a link between ATM and BER.
- Inhibition of ATM and MPG increases TMZ sensitivity and prolongs survival in a pediatric GBM model.

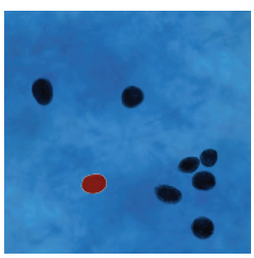


The alkylating agent temozolomide (TMZ) has shown activity in adults with glioblastoma (GBM) but is largely ineffective in pediatric GBM. Reduced expression of O^6-methylguanine–DNA methyltransferase (MGMT), which repairs alkylated O^6 lesions, correlates with TMZ sensitivity, but MGMT is often not expressed in pediatric GBM, suggesting that MGMT-independent mechanisms of DNA repair and TMZ resistance exist. Agnihotri and colleagues screened for genes whose knockdown increased TMZ sensitivity in pediatric GBM cells and identified multiple base excision repair (BER) genes as well as ATM, a master regulator of the cellular response to DNA double-strand breaks. Among BER components, expression and activity of 3-methylpurine-DNA glycosylase (MPG), which repairs cytotoxic N^3-methyladenine residues, was correlated with TMZ sensitivity, and *MPG* overexpression and copy-number gains were observed in pediatric GBM. Moreover, high MPG expression was associated with shorter overall survival and poor TMZ response. *In silico* analysis revealed a highly conserved ATM phosphorylation motif on MPG, and ATM-dependent phosphorylation of MPG at serine 172 was required for full MPG glycosylase activity, suggesting that ATM can induce MPG-dependent BER to promote TMZ resistance. Evidence of ATM-mediated MPG phosphorylation was identified in pediatric GBM samples, and knockdown or inhibition of MPG, ATM, or both sensitized pediatric GBM cells to TMZ and prolonged survival *in vivo*, suggesting that targeting the ATM–MPG axis may increase the efficacy of TMZ in the pediatric setting. ■

See article, p. 1198.

IκB Kinase Inhibitors Sensitize Melanoma to MAPK Pathway Blockade

- MAPK pathway inhibitors increase macrophage accumulation and TNFα levels in *BRAF*-mutant melanoma.
- TNFα promotes resistance to MEK inhibitors through IκBα phosphorylation and MITF upregulation.
- IKK inhibitors enhance the efficacy of MEK inhibitors by preventing TNFα-induced resistance.

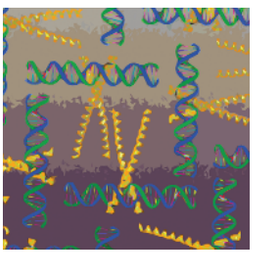


Inhibition of the MAPK pathway is a common therapeutic approach in melanoma, but resistance that can occur through a variety of mechanisms limits long-term efficacy. TNFα has been shown to regulate melanoma growth and progression, prompting Smith, Sanchez-Laorden, and colleagues to investigate its role in MAPK pathway inhibitor resistance. $Braf^{V600E}$-mutant tumor growth was significantly reduced in TNFα-deficient mice, confirming that TNFα is necessary for melanoma cell survival. Interestingly, stromal cell analyses revealed that M1- and M2-polarized macrophages expressed high levels of TNFα, and depletion of TNFα specifically in the myeloid lineage impaired tumor growth and reduced macrophage accumulation in $Braf^{V600E}$-positive tumors. Macrophage-derived TNFα protected melanoma cells from MEK inhibitor (MEKi)–induced cell death through stimulation of IκBα phosphorylation, NFκB/p65 nuclear translocation, and NFκB-dependent upregulation of microphthalmia-associated transcription factor (MITF), a lineage-specific melanoma survival factor previously implicated in MAPK inhibitor resistance. MEK/BRAF inhibitor treatment increased tumor-associated macrophage recruitment and TNFα and MITF expression in murine and human *BRAF*-mutant melanomas. Intriguingly, combined treatment with IκB kinase inhibitors (IKKi) and MEKi suppressed both macrophage-derived TNFα expression and MITF expression in melanoma cells and resulted in enhanced inhibition of tumor growth in mice, consistent with *in vitro* experiments showing that IKKi blocks NFκB-driven MITF expression and sensitizes melanoma cells to MEKi-induced cell death. These findings highlight the role of the immune microenvironment in MAPK inhibitor resistance and suggest that IKKi therapy may improve the efficacy of MAPK pathway inhibitors by preventing TNFα-mediated resistance. ■

See article, p. 1214.

CREB1 Drives Aberrant TGFβ Signaling in Glioblastoma

- TGFβ stimulates a CREB1- and SMAD3-mediated autocrine loop that induces *TGFβ2* in glioblastoma.
- Levels of active CREB1 correlate with TGFβ2 and poor prognosis in human glioblastoma samples.
- CREB1 may represent a biomarker to predict patient response to TGFβ inhibition in glioblastoma.



The TGFβ pathway has been implicated in multiple aspects of carcinogenesis, including immune suppression, proliferation, angiogenesis, and epithelial-to-mesenchymal transition. Despite the development of inhibitors targeting this pathway, biomarkers that predict patient response to TGFβ blockade are lacking. Aberrant TGFβ signaling has been observed in glioblastoma; however, no known genomic alterations in the TGFβ pathway have been detected, prompting Rodón and colleagues to examine the molecular mechanisms that underlie TGFβ hyperactivation in glioblastoma. Analysis of human glioblastoma samples compared with normal brain tissue revealed increased levels of *TGFB1* and *TGFB2* mRNA, which were correlated with poor prognosis. Stimulation with exogenous TGFβ ligands led to upregulation and increased secretion of TGFβ2 in glioblastoma cell lines, which was reversed by inhibition of TGFβ receptor 1, indicative of an autocrine loop. Characterization of the *TGFB2* promoter identified proximal binding elements for SMAD proteins and cAMP responsive element binding protein 1 (CREB1), which cooperate in driving gene transcription. In support of this idea, *TGFB2* induction was dependent on CREB1 activation by PI3K and p90 ribosomal S6 kinase and required CREB1 interaction with SMAD3 at the *TGFB2* promoter in response to TGFβ. TGFβ2 and active CREB1 levels were positively correlated and associated with poor prognosis in human glioblastoma tumors. Importantly, silencing of CREB1 in a patient-derived xenograft model reduced TGFβ2 levels and tumor burden and prolonged survival. Together, these results highlight CREB1 as a potential biomarker to predict clinical response to TGFβ pathway blockade. ■

See article, p. 1230.

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