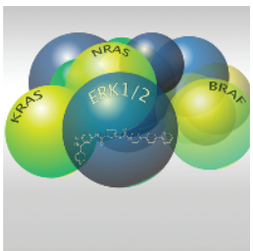
ERK Inhibition Overcomes Resistance to BRAF and MEK Inhibitors

- SCH772984 is an ERK-selective inhibitor that inhibits ERK1 and ERK2 with nanomolar potency.
- SCH772984 has features of both type I and type II kinase inhibitors.
- BRAF and/or MEK inhibitor-resistant cancer cells are sensitive to SCH772984.

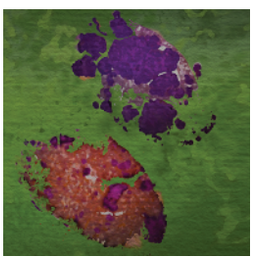


BRAF and MEK inhibitors have shown activity in *BRAF*- or *RAS*-mutant cancers, but acquired resistance ultimately develops, frequently through reactivation of downstream ERK signaling. Morris and colleagues developed SCH772984, a highly selective ERK inhibitor with nanomolar potency against ERK1 and ERK2. Like other type I kinase inhibitors, SCH772984 bound the ATP binding site of the active, phosphorylated form of ERK and inhibited ERK substrate phosphorylation in a dose-dependent manner. However, SCH772984 also inhibited phosphorylation of the ERK activation loop without inhibiting upstream ERK-activating kinases, suggesting that SCH772984 also acts as a type II kinase inhibitor that stabilizes the inactive form of ERK. SCH772984 selectively inhibited the proliferation of *BRAF*- or *RAS*-mutant cell lines and induced tumor regression *in vivo* in association with reduced ERK phosphorylation. Notably, SCH772984 also blocked ERK signaling and proliferation in BRAF or MEK inhibitor-resistant cell lines, regardless of the mechanism of ERK reactivation. Furthermore, SCH772984 was active in models of resistance to combination BRAF/MEK inhibitor therapy, which has recently been shown to have improved clinical benefit in *BRAF*-mutant melanoma compared with monotherapy. Acquired resistance to combination therapy was also due to ERK reactivation, either through secondary MAPK pathway mutations or upregulation of compensatory pathways. Targeted inhibition of ERK may therefore be an effective method to delay or overcome resistance to BRAF and MEK inhibitors used alone or in combination for treatment of *BRAF*- and *RAS*-mutant cancers. ■

See article, p. 742.

Inhibition of MSP/RON Signaling Prevents Metastatic Outgrowth

- Host expression of RON promotes conversion of micrometastases to overt metastatic colonies.
- RON deletion induces a CD8$^+$ T-cell antitumor immune response that suppresses metastasis.
- Pharmacologic RON blockade impairs the outgrowth of established pulmonary micrometastases.

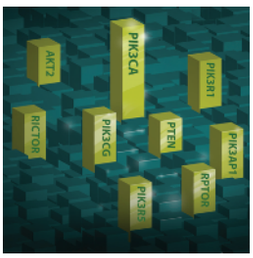


Antitumor immune responses mediated by CD8$^+$ cytotoxic T lymphocytes (CTL) promote tumor dormancy and prevent metastatic outgrowth, but the mechanisms by which micrometastatic tumor cells suppress immunosurveillance and transition to overt metastases are not well characterized. Recent studies have shown that upregulation of macrophage stimulating 1 (MST1, also known as MSP) promotes breast cancer metastasis in mice and is correlated with poor outcome in human breast cancer. Eyob and colleagues found that genetic deletion of the MSP receptor MST1R (also known as RON) from the host microenvironment impaired metastasis in mouse models of breast and lung cancer. Loss of RON did not affect the early steps of metastasis but specifically reduced the conversion of micrometastatic cells to metastatic colonies. This effect was mediated by increased proinflammatory cytokine secretion by macrophages in tumor-bearing RON-deficient mice, resulting in expansion of peripheral CD8$^+$ T cells and pulmonary infiltration of TNFα-expressing CTLs with enhanced tumor-specific cytotoxic activity. Expansion of CD8$^+$ T cells was both necessary and sufficient to inhibit metastasis in the absence of RON, suggesting that inhibition of RON reactivates antitumor immunity to block metastatic outgrowth. Consistent with this idea, treatment with a small-molecule RON inhibitor decreased pulmonary metastases in a CD8$^+$ T-cell–dependent manner and attenuated the outgrowth of pre-established micrometastases. These findings define a role for MSP/RON signaling in suppressing antitumor immunity and suggest that adjuvant therapy with RON inhibitors may limit metastatic spread. ■

See article, p. 751.

The PI3K Pathway Is Frequently Mutated in HNSCC

- Whole-exome sequencing analysis shows that the PI3K pathway is mutated in 30.5% of HNSCCs.
- Concurrent PI3K pathway mutations were more likely to occur in advanced-stage HNSCCs.
- *PIK3CA*-mutant HNSCC patient tumorgrafts are highly sensitive to PI3K pathway inhibition.

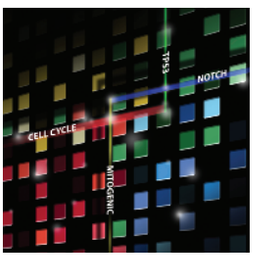


The high degree of genomic heterogeneity found in head and neck squamous cell carcinomas (HNSCC) has hindered the identification of targeted therapies likely to benefit a significant proportion of patients with this frequently lethal cancer. Given the wide mutational spectrum in HNSCC, Lui and colleagues took a pathway-based approach and assessed the mutation frequency of components of targetable mitogenic pathways in whole-exome sequencing data from 151 HNSCCs. Among the MAPK, JAK/STAT, and PI3K pathways, the PI3K pathway was the most frequently affected, with 30.5% of HNSCCs harboring PI3K pathway mutations. PI3K pathway-mutated tumors had a significantly higher mutation rate and incidence of cancer-related gene mutations than those without PI3K pathway mutations, suggesting that PI3K pathway mutations may promote genomic instability. Additionally, all HNSCC tumors with concurrent mutations in multiple PI3K pathway components were advanced (stage IV), indicating that concerted genetic alterations in this pathway may contribute to HNSCC progression. The most frequently mutated PI3K pathway gene in HNSCC was *PIK3CA*, which encodes the PI3Kα catalytic subunit. Several noncanonical *PIK3CA* mutations were identified in addition to known hotspot mutations, and each stimulated cell growth and PI3K pathway activation in HNSCC cells. Importantly, both noncanonical and hotspot *PIK3CA*-mutant patient-derived tumorgrafts were highly sensitive to pharmacologic inhibition of the PI3K pathway. These findings indicate that the presence of PI3K pathway mutations in a subgroup of patients with HNSCC may be an indicator of sensitivity to PI3K-targeted therapy. ■

See article, p. 761.

Potential Drivers Are Identified in Oral Squamous Cell Carcinoma

- Notch pathway defects are found in 66% of patients with oral squamous cell carcinoma.
- *NOTCH1* acts as a tumor suppressor gene in oral squamous cell carcinoma.
- Oral squamous cell carcinomas commonly harbor inactivating mutations of *FAT1* and *CASP8*.

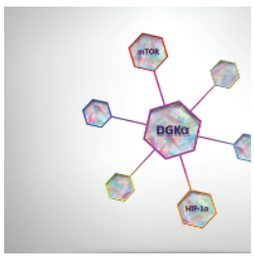


Improved therapies are needed for oral squamous cell carcinoma (OSCC), a subset of head and neck squamous cell carcinoma with a poor survival rate. The few genome-wide analyses of OSCCs to date have identified mutations in *TP53* and *NOTCH1*, but have not uncovered many clinically actionable genetic alterations. To identify potential drivers of OSCC, Pickering and colleagues performed integrated genomic analyses of 38 OSCCs. Recurrent copy number alterations and mutations most frequently affected cell cycle components, *TP53*, mitogenic signaling, and Notch signaling. Genomic alterations to Notch pathway components were found in 66% of OSCCs, and included frame-shift and nonsense mutations in *NOTCH1* in 9% of tumors, suggesting that NOTCH1 acts as a tumor suppressor in OSCC. Indeed, expression of wild-type NOTCH1 in NOTCH1-deficient OSCC cell lines led to cell cycle arrest and inhibited tumor growth *in vivo*. Other potential driving events in OSCC included loss of *FAT1*, a cadherin gene with roles in differentiation, in 46% of tumors, and inactivation of the proapoptotic gene caspase 8 (*CASP8*) in 10% of tumors. Encouragingly, despite the prevalence of inactivating mutations in OSCC, 80% of the tumors harbored at least one targetable genomic alteration, many of which have inhibitors already in clinical development. Together, these findings provide insight into the etiology of OSCC and suggest that use of targeted therapies may be possible for this disease. ■

See article, p. 770.

The Signaling Node DGKα Is a Potential Therapeutic Target

- Suppression of DGKα selectively induces apoptosis in glioblastoma and other cancer cells.
- DGKα promotes cancer cell viability via regulation of *MTOR* transcription and HIF-1α signaling.
- Small-molecule DGKα inhibitors impair tumor growth and angiogenesis and improve survival.



Diacylglycerol kinase α (DGKα) is a member of a family of metabolic enzymes that convert the lipid second messenger diacylglycerol to the phospholipid phosphatidic acid. Recent studies have implicated DGKα in the regulation of oncogenic signaling pathways, including NF-κB, hypoxia-inducible factor-1α (HIF-1α), mTOR, and VEGF, suggesting DGKα as a potential therapeutic target in cancer. In support of this idea, Dominguez and colleagues found that DGKα suppression via knockdown or treatment with small-molecule inhibitors selectively induced caspase-mediated apoptosis in glioblastoma and other cancer cell lines without reducing the viability of normal cells. DGKα overexpression enhanced glioblastoma cell proliferation despite only moderately increased expression or amplification in a subset of human tumors, suggestive of cancer-specific nononcogene addiction. The cytotoxic effect of DGKα inhibition was dependent on attenuation of DGKα-driven phosphatidic acid production and was mediated largely via downregulation of mTOR and HIF-1α, as expression of either mTOR or HIF-1α partially rescued the toxic effects of DGKα blockade. DGKα suppression specifically enhanced cyclic AMP levels downstream of phosphatidic acid and phosphodiesterases in cancer cells but not nontransformed cells, resulting in decreased *MTOR* transcription. Importantly, DGKα inhibition diminished glioblastoma and melanoma xenograft growth and vascularity and prolonged the survival of tumor-bearing mice without toxicity. These results suggest that inhibition of the DGKα signaling node may be an effective strategy to target multiple oncogenic pathways in glioblastoma and other cancers. ■

See article, p. 782.

ZNF365 Prevents Telomere and Fragile Site Instability

- *ZNF365* is induced in cells with telomere dysfunction in a p53-dependent manner.
- ZNF365 suppresses telomere defects and common fragile site expression.
- Unresolved replication at telomeres and fragile sites caused by ZNF365 loss leads to aneuploidy.

Telomeres are regions of repetitive nucleotide sequences that prevent loss of genetic material and inappropriate DNA repair at chromosome ends. When telomeres become too short, a p53-dependent checkpoint induces cellular senescence or apoptosis, but this checkpoint is often subverted in cancer cells. To identify components of the p53-mediated telomere checkpoint, Zhang and colleagues analyzed transcriptional changes after p53 reactivation in cells with defective telomeres and identified zinc finger protein 365 (*ZNF365*) as a direct p53 target gene in the context of telomere dysfunction. Upon replication stress, ZNF365 partially colocalized with telomeres, and ZNF365 knockdown led to increased DNA damage foci at telomeres in association with increased telomere defects, including heterogeneity, loss, amplification, and fusion. ZNF365 loss also led to alterations at a subset of common fragile sites, indicating that ZNF365 also contributes to the stability of other nontelomeric genomic regions that are sensitive to replication challenge. In mitotic cells, ZNF365 deficiency led to the formation of ultrafine DNA bridges arising from unresolved DNA replication of fragile sites and telomeres, which ultimately resulted in cytokinesis failure and aneuploidy. As germline polymorphisms of *ZNF365* have been associated with an increased risk of breast cancer in *BRCA2* mutation carriers, and decreased *ZNF365* expression was found to be associated with shorter relapse-free survival in breast cancer patients, these findings suggest that maintenance of telomere and fragile site integrity by ZNF365 may have a tumor-suppressive effect. ■

See article, p. 798.

SNPs Modulate SERM Therapy Benefit via Regulation of BRCA1 Expression

- SNPs in *ZNF423* and near *CTSO* are associated with breast cancer risk during SERM therapy.
- Estrogen- and SNP-dependent induction of ZNF423 and CTSO promotes *BRCA1* transcription.
- SERMs reverse the SNP-dependent expression pattern of ZNF423 variants and BRCA1.

Selective estrogen receptor modulators (SERM) such as tamoxifen and raloxifene decrease breast cancer occurrence in women at high risk for developing this disease. However, the potential for adverse effects has limited their broad use, underscoring the need for genetic factors that enable better selection of patients. Ingle and colleagues performed a genome-wide association study of patient samples from the two largest SERM breast cancer prevention trials and identified single-nucleotide polymorphisms (SNP) in genes encoding a zinc finger protein (*ZNF423*) and near the cathepsin O (*CTSO*) gene that were associated with risk of developing breast cancer. In the absence of a SERM, the expression of wild-type but not variant SNP-related *ZNF423* and *CTSO* was estrogen-inducible and was correlated with elevated expression of the breast cancer susceptibility gene *BRCA1*; ZNF423 directly interacted with the *BRCA1* 5′-flanking region to activate *BRCA1* transcription and promote double-strand break repair. One of the *ZNF423* SNPs was located near estrogen response elements and decreased ERα binding when the variant SNP sequence was present, but showed a reversal in the presence of 4-hydroxytamoxifen, an active tamoxifen metabolite. SERMs also reversed the SNP-dependent expression pattern of ZNF423 in response to estrogen, resulting in increased levels of ZNF423 when the variant SNP was present along with enhanced BRCA1 induction in these cells, consistent with decreased risk during SERM therapy. These results provide insights into the estrogen-dependent induction of BRCA1 and suggest strategies for individualized breast cancer prevention. ■

See article, p. 812.

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