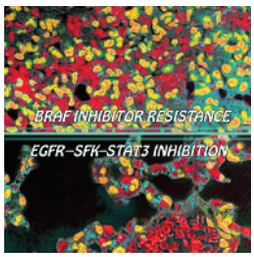
IN THIS ISSUE

EGFR and SFK Signaling Underlies BRAF Inhibitor Resistance in Melanoma

- EGFR is expressed in melanoma cells and activated in vemurafenib-resistant melanomas.
- SRC family kinase (SFK) signaling is elevated in cells with acquired vemurafenib resistance.
- Inhibitors of EGFR and SFK block growth and invasion of vemurafenib-resistant melanomas.



Small-molecule BRAF inhibitors such as vemurafenib significantly improve progression-free and overall survival in patients with *BRAF*-mutant melanoma, but most patients are intrinsically resistant to treatment or quickly develop acquired resistance. Because hyperactivation of receptor tyrosine kinases (RTK) contributes to BRAF inhibitor resistance in colorectal cancer, Girotti and colleagues evaluated RTK activation in vemurafenib-resistant *BRAF*-mutant melanoma cells. Surprisingly, phosphorylation of the EGF receptor (EGFR), which was not thought to be expressed in melanoma, was elevated in vemurafenib-resistant cells and the majority of vemurafenib-resistant patient samples, and the *in vivo* growth and dissemination of vemurafenib-resistant cells were sensitive to combined use of the EGFR inhibitor gefitinib and vemurafenib. To determine which pathways downstream of EGFR mediate vemurafenib resistance in melanoma, intracellular kinases and transcription factors were interrogated. Phosphorylation of SRC family kinases (SFK) was increased in resistant cells, and the pan-SFK inhibitor dasatinib blocked vemurafenib-resistant melanoma growth and metastasis. Importantly, analysis of paired pre- and posttreatment samples from one vemurafenib-resistant patient showed increased EGFR and SFK phosphorylation following vemurafenib treatment, and growth and dissemination of the posttreatment sample in mice could be suppressed with dasatinib. Together, these findings are consistent with a role for EGFR and SFK signaling in BRAF inhibitor resistance in melanoma and suggest that combined use of EGFR and BRAF inhibitors or use of broad-specificity kinase inhibitors that inhibit SFKs may be effective strategies to overcome resistance to BRAF inhibition in melanoma. ■

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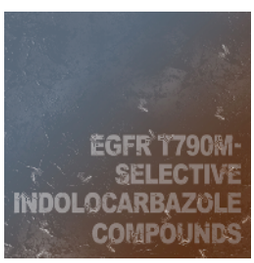
Indolocarbazoles Target EGFR T790M but Not Wild-type EGFR

- NSCLC cells with the EGFR T790M resistance mutation are highly sensitive to indolocarbazoles.
- Indolocarbazoles potently inhibit T790M-containing mutants without affecting wild-type EGFR.
- Dosing of EGFR inhibitors that spare wild-type EGFR may not be limited by on-target toxicity.



EGF receptor (EGFR) inhibition elicits responses in many patients with *EGFR*-mutant non–small cell lung cancer (NSCLC), but resistance frequently arises through a secondary T790M mutation that prevents drug binding. Second-generation covalent EGFR inhibitors suppress EGFR T790M but also inhibit wild-type EGFR, leading to toxic side effects that have limited dosing and clinical efficacy. In a kinase inhibitor sensitivity screen, Lee and colleagues noted that *EGFR*-mutant NSCLC cell lines were among the most sensitive to the staurosporine-related indolocarbazole compound Gö6976, a known inhibitor of protein kinase C (PKC). However, Gö6976 activity in *EGFR*-mutant NSCLC cells was PKC independent. Instead, this compound had significantly higher binding affinity for mutant EGFR variants compared with wild-type EGFR. Gö6976 inhibited the growth of *EGFR*-mutant cell lines at nanomolar concentrations, including an erlotinib-resistant cell line with the T790M mutation, and inhibited autophosphorylation of EGFR mutants while sparing wild-type EGFR. Because Gö6976 is a relatively nonspecific kinase inhibitor, the authors evaluated related indolocarbazole derivatives with greater kinase specificity, such as PKC412, a well-tolerated FLT3 inhibitor in clinical development for acute myeloid leukemia. Notably, compared with irreversible EGFR inhibitors, PKC412 had reversible yet more potent and selective activity against T790M-containing *EGFR* mutants than wild-type EGFR while similarly suppressing *EGFR*-mutant NSCLC growth *in vitro* and *in vivo*. Use of clinically available indolocarbazole compounds may therefore represent a feasible strategy for treatment of drug-resistant *EGFR*-mutant NSCLC without adverse effects caused by wild-type EGFR inhibition. ■

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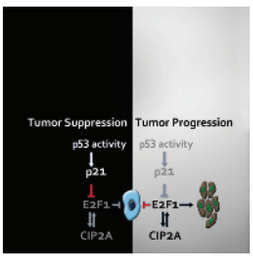
Inhibition of CIP2A Is Necessary for p53-Mediated Tumor Suppression

- p53 inactivation suppresses therapy-induced senescence via an E2F1–CIP2A feedback loop.
- CIP2A inhibition impairs tumorigenesis and is required for p53- and p21-induced senescence.
- High CIP2A levels are associated with poor outcome in a subset of patients with breast cancer.



The antitumor activity of chemotherapeutic agents is mediated in part by induction of senescence via p53. Inactivation of p53 in tumors leads to chemotherapy resistance; however, it is unclear whether p53 inhibition, in addition to causing defective checkpoint activity, actively contributes to senescence resistance. Laine and colleagues found that expression of cancerous inhibitor of protein phosphatase 2A (CIP2A, also known as KIAA1524), an oncoprotein that is upregulated in many cancers and is associated with p53 mutation in breast cancer, was inhibited by wild-type p53. CIP2A downregulation was dependent on p21-driven suppression of E2F1 downstream of p53 and was required for p53- and p21-activated senescence; CIP2A depletion was sufficient to induce senescence to a similar extent as p53, whereas CIP2A overexpression prevented senescence induction by doxorubicin or p53 reactivation with Nutlin-3. *Cip2a* deficiency also impaired tumor formation and promoted senescence in a mouse model of p53-mutant mammary tumorigenesis, supporting a role for CIP2A inactivation in p53-dependent tumor suppression. Furthermore, high CIP2A expression was associated with poor prognosis in patients with HER2-negative breast cancer, particularly those treated with senescence-inducing chemotherapy. Mechanistically, E2F1 directly stimulated *CIP2A* transcription, initiating a positive feedback loop in which CIP2A enhanced E2F1 protein stability by inhibiting protein phosphatase 2A-mediated dephosphorylation of E2F1 at serine 364. These results identify E2F1–CIP2A activity as a critical determinant of senescence sensitivity in breast cancer and suggest that inactivation of this pathway may activate senescence and provoke therapeutic response in p53-mutant tumors. ■

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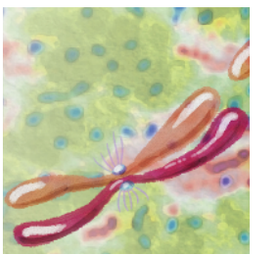
Transformed Cells Are Dependent on BUB1B

- BUB1B is essential for expansion of glioblastoma-initiating cells but not neural stem cells.
- Transformed cells have shorter interkinetochore distances (IKD) compared with normal cells.
- The BUB1B GLEBS domain suppresses kinetochore attachment defects in cells with short IKDs.



Because glioblastomas are thought to arise from tumor-initiating cells with stem-like properties, genes selectively required for the growth of these cells might represent potential therapeutic targets for glioblastoma. Through a combination of a short hairpin RNA–based kinome screen in patient-derived tumor-initiating cells with analysis of a glioblastoma-specific regulatory network derived from tumor samples in The Cancer Genome Atlas, Ding and colleagues determined that *BUB1B* was selectively required for expansion of tumor-initiating cells, a surprising finding given that BUB1B is an essential protein that regulates the mitotic spindle checkpoint. Glioblastoma cells that were sensitive to decreased levels of BUB1B had significantly shorter interkinetochore distances (IKD) than insensitive cells, and oncogenic transformation of normal cells reduced IKD length and conferred sensitivity to BUB1B loss, suggesting that transformed cells may have an added requirement for BUB1B due to altered kinetochore dynamics. Indeed, the BUB1B Gle2-binding sequence (GLEBS) domain, which regulates mitotic kinetochore localization and attachment but is not required for spindle checkpoint activation, was selectively required for the viability of genetically transformed and glioblastoma-initiating cells, and BUB1B loss specifically led to kinetochore–microtubule attachment defects and chromosome misalignment in cells with short IKDs in association with reduced tumorigenicity *in vivo*. These findings suggest that there may be a therapeutic window for targeting BUB1B in glioblastoma and suggest that short IKDs may predict for sensitivity to BUB1B inhibitors or agents that disrupt kinetochore stability. ■

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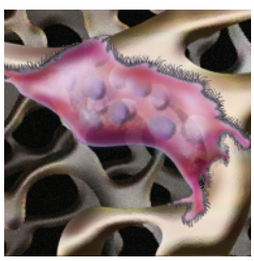
IAP Antagonists Augment Bone Metastasis via Osteoclast Activation

- Treatment with IAP inhibitors specifically drives tumor growth in the bone microenvironment.
- NIK stabilization and alternative NF-κB pathway activity promote osteoporosis and osteoclastogenesis.
- Osteoclast inhibitors such as bisphosphonates block IAP antagonist–induced bone metastasis.

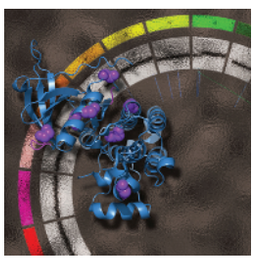


Inhibitor of apoptosis (IAP) proteins promote cancer cell survival via classical NF-κB signaling, and IAP antagonists have shown efficacy as antitumor agents. However, IAPs also modulate the tumor microenvironment via suppression of the alternative NF-κB pathway, which controls osteoclast-mediated bone turnover. To assess the effect of IAP inhibitors on bone metastasis, Yang and colleagues treated mice bearing breast cancer tumors with the IAP antagonist BV6. In contrast to its inhibition of soft tissue tumor growth, BV6 only partially decreased bone metastasis of drug-sensitive tumors and specifically augmented the growth and metastasis of drug-resistant tumors in bone. Treatment with BV6 or other IAP antagonists stimulated bone turnover, primarily due to increased osteoclast activity, resulting in reduced bone mass. This osteoporotic phenotype was mediated by enhanced osteoclast differentiation and was dependent on stabilization of the alternative NF-κB pathway kinase NIK (encoded by *mitogen-activated protein kinase kinase kinase 14*) in response to inhibition of IAPs, which ubiquitinate NIK and promote its degradation. NIK-induced activation of RelB/p52 but not p65 was required for osteoclastogenesis, and expression of constitutively active NIK in osteoclasts was sufficient to enhance tumor growth and osteolysis. Intriguingly, administration of the bisphosphonate zoledronic acid, an osteoclast blocking agent, reversed BV6-driven bone loss and diminished its prometastatic effects in bone. These results demonstrate that the bone microenvironment limits IAP inhibitor efficacy and suggest a combinatorial therapeutic strategy that may reduce bone metastasis. ■

See article, p. 212.

HER2 Mutants Are Therapeutic Targets in Breast Cancer

- HER2 mutations found in patients lacking *HER2* amplification were functionally characterized.
- Many mutations activate HER2 kinase activity, and all mutations are sensitive to neratinib.
- Patients with HER2 mutations may benefit from treatment with irreversible HER2 inhibitors.



Therapeutic agents such as lapatinib that target the HER2 kinase show clinical efficacy in patients with breast cancer harboring *HER2* gene amplification. Genome sequencing has also recently identified a subset of patients (1.6%) with somatic HER2 mutations in the absence of gene amplification, but the effect of these mutations on HER2 activity are unknown. Bose and colleagues functionally characterized a panel of HER2 mutations, including several recurrent mutations, which largely clustered in either the HER2 tyrosine kinase (68%) or extracellular (20%) domains. Most mutations in the kinase domain were distinct from those found in other cancers and did not occur in the activation loop. Comparison of HER2 and EGF receptor (EGFR) protein structures showed that several mutations were located in regions predicted to activate HER2 or promote its dimerization with EGFR. Indeed, 7 of 13 mutations were activating mutations, including V777L, D769H/Y, V842I, G309A, and R896C. These mutations enhanced HER2 kinase activity and downstream signaling, promoted the growth of invasive structures in Matrigel, augmented anchorage-independent growth, and accelerated xenograft tumor formation, suggesting that HER2 mutations may be driver events in breast cancer. Several mutations, particularly L755S, were associated with lapatinib resistance; however, all of these mutations retained sensitivity to the irreversible HER2 kinase inhibitor neratinib, which potently suppressed growth and HER2 signaling. These results support clinical screening for HER2 mutations to identify additional patients who may benefit from treatment with HER2-targeted drugs. ■

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