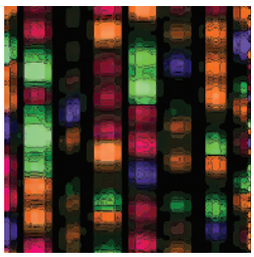
Genomic Analysis Identifies Mutations in Esophageal Cancer Subtypes

- Loss-of-function mutations in *NOTCH* genes were detected specifically in ESCC tumors.
- *NOTCH1* mutations were more common in ESCC from North American patients than those from China.
- Most EAC mutations were also present in matched, benign Barrett esophagus precursor lesions.

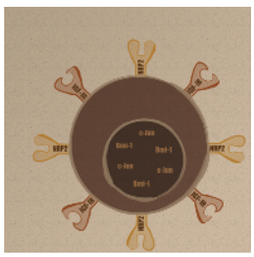


Esophageal cancer can be classified based on histology and environmental risk factors into esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) subtypes. However, the mutations that contribute to the development of each subtype remain largely unknown. To comprehensively characterize the genetic differences between these tumors, Agrawal and colleagues performed exomic sequencing of 11 EAC and 12 ESCC tumors and matched normal tissues. This genomic analysis identified high-confidence somatic mutations, including mutations in the most commonly altered esophageal cancer gene, *TP53*. EAC and ESCC tumors differed significantly in the frequency and type of mutations. Furthermore, inactivating mutations in the *NOTCH1* tumor suppressor and several related genes were specific to the ESCC subtype and were confirmed to be frequently mutated in 41 additional ESCCs. Interestingly, the prevalence of *NOTCH1* mutation was significantly higher in ESCCs from North American patients compared to Chinese patients, supporting the existence of disparate genetic and environmental factors. In addition, comparison of EACs and matched Barrett esophagus tissue showed that most EAC mutations were already present in benign Barrett esophagus samples, providing further genetic evidence that Barrett esophagus represents a precursor lesion that progresses to malignant EAC. These findings offer insights into esophageal cancer pathogenesis and emphasize the importance of understanding the genetic and geographic variability of this disease in order to develop effective therapeutic strategies. ■

See article, p. 899.

Neuropilin-2 Suppresses IGF-IR in High-Grade Prostate Cancer

- NRP2 expression correlates with aggressive prostate cancer and is activated by c-Jun.
- VEGF signaling through NRP2 activates BMI-1, which represses IGF-IR expression.
- NRP2 predicts response to IGF-IR therapy and inhibition of both abrogates tumor growth.



Advanced prostate cancer is not amenable to current therapeutic strategies, emphasizing the need to elucidate the molecular mechanisms underlying tumor progression. Although VEGF expression is elevated in high-grade prostate tumors, little is known about its functional contribution to this disease. Goel and colleagues found that increased expression of the VEGF receptor neuropilin 2 (NRP2) was correlated with high Gleason grade and absence of PTEN expression in prostate cancer cell lines and tumor samples. NRP2 expression was induced by PTEN loss via activation of c-Jun, and depletion of NRP2 in prostate cancer cell lines modestly decreased anchorage-independent growth and slowed tumor formation, supporting a protumor role for NRP2. However, NRP2 loss also triggered compensatory activation of IGF-IR expression, suggesting that NRP2 negatively regulates this receptor. Indeed, in response to VEGF stimulation, NRP2 activated focal adhesion kinase and promoted the expression of BMI-1, a Polycomb group transcriptional repressor, which directly inhibited *IGF1R* transcription in aggressive prostate cancer cell lines. Consistent with the inverse relationship between NRP2 and IGF-IR expression, NRP2 depletion resulted in enhanced sensitivity to IGF-IR inhibitor treatment, implicating NRP2 expression as a predictive biomarker of IGF-IR therapy response. In addition, combined inhibition of both NRP2 and IGF-IR synergized to more effectively suppress tumor growth. Together, these results delineate a mechanism by which the VEGF–NRP2 pathway promotes prostate cancer progression and suggest a combinatorial approach to overcome resistance in advanced tumors. ■

See article, p. 906.

HER2 Mediates Drug Resistance in *EGFR*-Mutant Lung Cancer

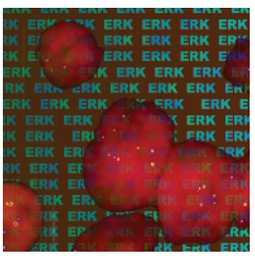
- HER2 phosphorylation is reduced by treatment with afatinib in *EGFR*-mutant lung cancer cells.
- Modulation of HER2 expression alters the drug sensitivity of *EGFR*-mutant lung cancer cells.
- *HER2* is amplified in resistant lung tumors independent of the EGFR T790M mutation.

Acquired resistance to EGF receptor (EGFR) tyrosine kinase inhibitors (TKI) is common in *EGFR*-mutant non–small cell lung cancer (NSCLC). Drug resistance often results from a secondary EGFR mutation at T790M, which can be overcome by combined treatment with the anti-EGFR antibody cetuximab and the EGFR-TKI afatinib. Because afatinib also strongly inhibits the related receptor HER2, Takezawa and colleagues investigated whether HER2 modulated the sensitivity of *EGFR*-mutant NSCLC to EGFR-TKIs. Treatment with afatinib, either alone or more significantly in combination with cetuximab, diminished the phosphorylation of both EGFR and HER2 in resistant cell lines and *in vivo* in *EGFR*-mutant tumors harboring the T790M mutation. Depletion of HER2 enhanced the sensitivity of EGFR-TKI–resistant cells to afatinib and resulted in growth inhibition, indicating that downregulation of HER2 activity contributes to the effectiveness of these anti-EGFR treatments. Conversely, overexpression of HER2 in EGFR-TKI–sensitive cells was sufficient to confer resistance to erlotinib but not afatinib, and to sustain phosphorylation of the downstream targets AKT and ERK, suggesting that HER2 may mediate drug resistance in *EGFR*-mutant lung adenocarcinoma. In support of this idea, *HER2* amplification was detected in a subset of mouse and human tumor samples with acquired resistance to erlotinib; in patient samples, *HER2* amplification occurred independently of the EGFR T790M mutation. These results identify HER2 as an important mediator of EGFR-TKI resistance and suggest that HER2 inhibition may improve clinical outcome in NSCLC. ■

See article, p. 922.

ERK Reactivation Underlies EGFR Inhibitor Resistance

- *MAPK1* amplification or *DUSP6* downregulation confers resistance to EGFR inhibitors.
- MEK inhibition restores sensitivity to EGFR inhibitors and prevents resistant clone formation.
- *MAPK1*-amplified NSCLC cells have increased EGFR internalization and decreased chemosensitivity.

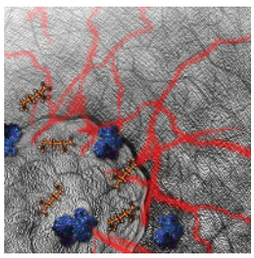


EGF receptor (EGFR) inhibitors are initially effective in patients with *EGFR*-mutant non–small cell lung cancer (NSCLC), but resistance ultimately develops, most often due to a secondary EGFR T790M mutation. Irreversible pyrimidine-based EGFR kinase inhibitors that selectively inhibit EGFR T790M are in clinical development, but mechanisms of acquired resistance to such inhibitors remain uncharacterized. Ercan and colleagues generated NSCLC cell lines that were resistant to the irreversible pyrimidine-based EGFR inhibitor WZ4002 but did not observe additional *EGFR* mutations or reactivation of EGFR. Instead, ERK phosphorylation was aberrantly upregulated via amplification of *MAPK1*, the gene encoding ERK2, or downregulation of *DUSP6*, which encodes a negative regulator of the ERK pathway. Knockdown of *MAPK1* was sufficient to restore sensitivity to WZ4002 in resistant cells, whereas knockdown of *DUSP6* was sufficient to confer resistance to WZ4002-sensitive cells. Increased ERK signaling in WZ4002-resistant cells was associated with increased EGFR internalization and decreased sensitivity to chemotherapeutic agents. Downstream inhibition of ERK signaling with a MEK inhibitor restored WZ4002 sensitivity in resistant NSCLC cell lines and mouse models, and combined use of WZ4002 and a MEK inhibitor prevented the emergence of resistant clones. These findings, together with the observation that a patient with *EGFR*-mutant NSCLC acquired a *MAPK1* amplification following erlotinib treatment, suggest that ERK reactivation may be a general mechanism of EGFR inhibitor resistance and that combining EGFR and MEK inhibitors may be clinically effective. ■

See article, p. 934.

A Secretome Rescue Screen Identifies Compensatory RTKs

- More than 2,800 secreted proteins were tested for their capacity to induce drug resistance.
- A compensatory relationship exists between MET, FGFRs, and HER ligands following kinase inhibition.
- Combined FGFR and MET inhibition prevents ligand-mediated rescue and induces tumor regression.



One potential mechanism of acquired resistance to receptor tyrosine kinase (RTK) inhibition is activation of compensatory pathways that bypass the drug target, either as a result of activating mutations or due to autocrine or paracrine ligand-dependent RTK stimulation. To systematically evaluate ligand-mediated mechanisms of drug resistance, Harbinski and colleagues individually transfected cDNAs encoding more than 2,800 predicted secreted proteins into cells, and each supernatant was subsequently tested for its ability to rescue the growth of RTK-addicted cancer cells treated with corresponding RTK inhibitors. Interestingly, the growth of multiple hepatocyte growth factor (HGF) receptor (MET)–dependent cell lines treated with a MET inhibitor could be rescued by several HER and fibroblast growth factor (FGF) ligands, and FGFR-addicted cell lines treated with FGFR inhibitors could be rescued by HER ligands or HGF. Of note, several cell lines in the Cancer Cell Line Encyclopedia and a human lung cancer primary xenograft model showed evidence of dual FGFR and MET autocrine activation, indicating that this compensatory relationship may be clinically relevant. Combined FGFR and MET inhibition blocked the growth of these cell lines more effectively than inhibition of either RTK alone and induced xenograft tumor regression *in vivo*. This high-throughput screen of the human secretome thus underscores the flexibility of oncogene addiction bypass mechanisms, and establishes that MET, FGFRs, and HER ligands may have broad compensatory potential that can be targeted with combination therapy. ■

See article, p. 948.

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