# IN THIS ISSUE

## Modeling Identifies Drug Combinations that Reduce Tumor Heterogeneity

- Chemotherapeutic combinations were predicted using an RNAi-based model of tumor heterogeneity.
- Computational modeling based on single-drug efficacies defined non-intuitive optimal combinations.
- Optimized combinations minimize resistant subpopulation outgrowth and improve survival in mice.

Dynamic clonal evolution during tumor progression results in significant intratumoral heterogeneity, which remains a challenge for the design of clinically effective chemotherapeutic drug regimens. To address this problem, Zhao and colleagues used RNA interference (RNAi) in murine lymphoma cells to generate distinct genetic subpopulations and applied a mathematical algorithm based on known single-drug efficacies to predict the most effective two-drug combinations for this heterogeneous tumor model. Intriguingly, in many cases this computational approach identified nonintuitive combinations consisting of drugs that were not the most effective against independent subpopulations and not predicted given the predominant subpopulation; rather, these optimal chemotherapeutic drug combinations were predicted only when considering the entire heterogeneous tumor. For example, the combination of vorinostat (also known as suberoylanilide hydroxamic acid or SAHA) and vincristine was validated both *in vitro* and *in vivo* as the optimal therapy for lymphoma cells expressing short hairpin RNAs against checkpoint kinase 2 (*Chk2*) and the proapoptotic gene BCL2-related ovarian killer (*Bok*). This combination minimized the outgrowth of resistant subpopulations, maximized tumor cell death, and enhanced tumor-free survival in a mouse model of *Eμ-Myc*–driven lymphoma. Furthermore, experimental modeling of drug combinations facilitated assessment of therapeutic efficacy across different proportions of tumor cell subpopulations and multiple rounds of therapy. These results provide a strategy to incorporate intratumoral heterogeneity and tumor evolution in the design and optimization of antitumor drug combinations. ■

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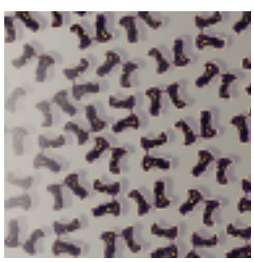
## Genome Doubling Facilitates Cancer Genome Evolution

- Genome doubling occurs early in colorectal cancer evolution and is associated with genomic complexity.
- Tetraploid cells tolerate chromosome segregation errors better than diploid cells.
- A genome-doubling event in early-stage colorectal tumors is predictive of poor relapse-free survival.

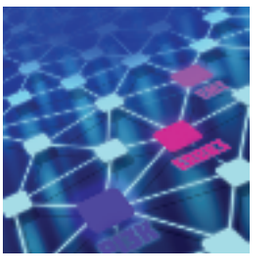


Tetraploidy is frequently observed in human tumors, but the impact of genome duplication on chromosomal instability and cancer genome evolution is unclear. By analyzing single-nucleotide polymorphism array data from colorectal cancers, Dewhurst and colleagues noted that genome doubling can occur prior to most other copy-number changes and that the total number of chromosomal aberrations was significantly increased in tumors that underwent a genome-doubling event, suggesting that genome doubling might be an early event in colorectal cancer development that promotes chromosomal instability. To directly assess the effects of genome doubling on genome stability, the authors isolated single cells from a small tetraploid subpopulation of a stable diploid colorectal cancer cell line and found that tetraploid clones had a greater cell-to-cell chromosome number variation than diploid clones. Only tetraploid clones accumulated and propagated chromosomal abnormalities over long-term culture, and aneuploidy was significantly more prevalent in colonies grown from tetraploid cells. Live-cell imaging showed that chromosome segregation errors during mitosis were far less likely to result in death or cell-cycle arrest of daughter cells in tetraploid clones than in diploid clones. In early-stage colorectal tumors, the occurrence of a genome-doubling event was significantly associated with poor relapse-free survival, and increasing genome complexity in genome-doubled tumors was associated with higher tumor stage. Taken together, these findings suggest that increased tolerance of chromosomal instability in tetraploid cells may allow the evolution of additional genetic changes that accelerate cancer evolution and drive tumor progression. ■

*See article, p. 175.*

## EMT Drives Alternative Mechanisms of PI3K-Dependent Growth in NSCLC

- Epithelial cells maintain serum-independent growth through ERBB3/PI3K autocrine signaling.
- Post-EMT, mesenchymal cells have reduced PI3K-dependent growth due to loss of ERBB3 expression.
- Mesenchymal tumors harbor *PIK3CA* changes that maintain PI3K signaling despite ERBB3 loss.

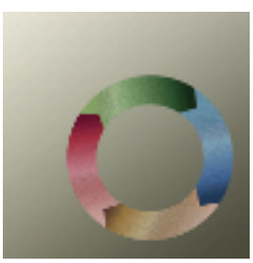


Epithelial-to-mesenchymal transition (EMT) is a developmental process associated with greater metastatic capability and poor prognosis in human malignancy. To more fully examine the molecular consequences of EMT, Salt and colleagues constructed an inducible *in vitro* model system of EMT in *KRAS*-mutant non–small cell lung cancer (NSCLC) cell lines. Upon the conversion from an epithelial to a mesenchymal state, cells displayed reduced phosphoinositide-3-kinase (PI3K)–AKT pathway activation, resulting in impaired serum-independent proliferation. This transition was accompanied by downregulation of the receptor tyrosine kinase ERBB3 in mesenchymal cells. Autocrine stimulation of ERBB3 and its heterodimerization partner ERBB2 by neuregulin-1 was required for maintenance of PI3K–AKT pathway activation and proliferation in epithelial-type cells. Despite ERBB3 loss in mesenchymal cells, serum-independent growth could be rescued by expression of mutant p110α or growth factors such as EGF and insulin-like growth factor-1, and was dependent on restoration of PI3K–AKT signaling *in vitro*. Importantly, reduced ERBB3 expression was correlated with a mesenchymal gene signature, increased expression or amplification of *PIK3CA*, which encodes the catalytic p110α subunit of PI3K, and maintenance of PI3K downstream signaling in primary human lung tumors. Furthermore, mesenchymal cells were more resistant to EGF receptor inhibition; however, there was no correlation between PI3K inhibitor sensitivity and EMT status. These results highlight the fact that although PI3K signaling is crucial for both epithelial and mesenchymal cell growth, rerouting of signaling through alternative circuits following EMT may affect therapeutic strategies. ■

*See article, p. 186.*

## MEK-Dependent Negative Feedback Drives BCR–ABL Oncogene Addiction

- BCR–ABL subverts growth factor receptor signaling in myeloid cells.
- BCR–ABL induces MEK-driven negative feedback that inhibits growth factor receptor signaling.
- Upon BCR–ABL inhibition, cells commit to apoptosis prior to relief of negative feedback.

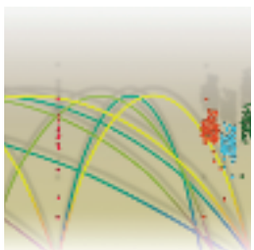


Chronic myelogenous leukemia (CML) cells expressing BCR–ABL undergo apoptosis when exposed to BCR–ABL-targeted tyrosine kinase inhibitors. To characterize the mechanisms of BCR–ABL oncogene addiction, Asmussen and colleagues performed phosphoproteomic analysis of CML cells transiently exposed to dasatinib and found that growth factor receptor effector proteins were persistently dephosphorylated, suggesting that sustained downstream activation of growth factor signaling pathways by BCR–ABL may underlie BCR–ABL-mediated oncogene addiction. When exogenously expressed in myeloid cells, BCR–ABL suppressed and rewired physiologic growth factor receptor signaling such that treatment with the BCR–ABL inhibitor dasatinib failed to fully restore growth factor receptor signaling, and the addition of growth factor did not fully rescue cells from dasatinib-induced apoptosis. Gene expression analysis indicated that BCR–ABL activates MEK-dependent negative feedback of myeloid growth factor receptor signaling that is relieved only after prolonged BCR–ABL inhibition. The failure of growth factors to completely rescue dasatinib-induced apoptosis suggests that CML cells are sensitive to BCR–ABL inhibition because they commit to apoptosis before negative feedback is diminished and prosurvival growth factor signaling is fully restored. In contrast, although oncogenic FLT3 creates a similar state of oncogene dependence, growth factor treatment sufficiently restored downstream signaling after FLT3 inhibition such that FLT3-mutant cells were more fully rescued from quizartinib-induced apoptosis. Together, these data indicate that BCR–ABL-mediated oncogene addiction is attributable to sustained MEK-dependent negative feedback and suggest that prolonging negative feedback may enhance tyrosine kinase inhibitor effectiveness. ■

*See article, p. 200.*

## Alternative Mechanisms Disrupt a Common Axis in Rhabdomyosarcoma

- *PAX* fusion status may more accurately distinguish tumor subtypes than alveolar/embryonal histology.
- RAS pathway mutations are present in 45% of *PAX* fusion-negative rhabdomyosarcomas.
- Mutations in PAX fusion protein target genes are enriched in *PAX* fusion-negative tumors.

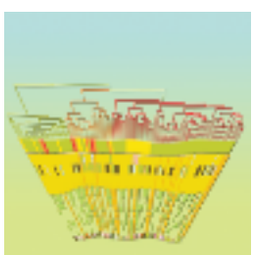


Optimization of chemotherapy regimens has improved outcomes in children with localized rhabdomyosarcoma, the most common pediatric soft-tissue sarcoma, but the prognosis for patients with metastatic rhabdomyosarcoma remains poor. Shern and colleagues performed an integrative genomic analysis of 147 rhabdomyosarcomas to gain insight into genetic differences between rhabdomyosarcoma subtypes and identify potential therapeutic targets. Expectedly, the most common recurrent alterations were rearrangements involving *PAX3* or *PAX7*, which were found in approximately 38% of tumors and most frequently led to fusions with *FOXO1*. Unsupervised clustering based on RNA sequencing clearly separated tumors with and without *PAX* gene fusions, although the finding that some tumors with alveolar and embryonal histology clustered together suggests that *PAX* fusion status may more accurately reflect the underlying biology of rhabdomyosarcomas. Compared with *PAX* fusion-positive tumors, *PAX* fusion-negative tumors had a significantly higher mutational burden and increased aneuploidy. *RAS* gene mutations were also found only in *PAX* fusion-negative tumors, but receptor tyrosine kinase–RAS–PI3K pathway mutations were present in 45% of *PAX* fusion-negative tumors and over 90% of all rhabdomyosarcomas, suggesting that targeting this signaling axis might be an effective therapeutic strategy for both *PAX* fusion-negative and *PAX* fusion-positive rhabdomyosarcomas despite their genetic heterogeneity. Notably, genes mutated in *PAX* fusion-negative tumors were enriched for direct and indirect targets of PAX fusion proteins, suggesting that alternative mechanisms exist to deregulate a common set of genes in rhabdomyosarcoma. ■

*See article, p. 216.*

## Residual Triple-Negative Breast Cancers Harbor Actionable Mutations

- Triple-negative breast cancers that do not fully respond to neoadjuvant chemotherapy very often recur.
- The majority of post-chemotherapy residual triple-negative breast cancers have targetable alterations.
- Genomic profiling after chemotherapy may guide rational use of adjuvant targeted therapies.



Patients with triple-negative breast cancer are treated with neoadjuvant chemotherapy to increase the chance of breast-conserving surgery and the prompt elimination of undetectable micrometastases. Approximately 30% of patients who experience a pathologic complete response following neoadjuvant chemotherapy have a favorable clinical outcome; however, patients with residual tumors have a poor prognosis due to a high rate of metastatic recurrences. Despite the high probability of recurrence, watchful waiting remains the standard of care for patients with residual triple-negative breast cancer after neoadjuvant chemotherapy. To identify potential targets for adjuvant therapy aimed at preventing disease recurrence, Balko and colleagues performed gene expression profiling, copy-number analysis, and targeted next-generation sequencing in residual triple-negative breast cancers after neoadjuvant chemotherapy. Several recurrent alterations, such as amplification of the antiapoptotic gene *MCL1* and mutations affecting the PI3K–mTOR pathway and cell cycle regulators, were enriched in post–neoadjuvant chemotherapy residual tumors compared with pretreatment tumors or with primary triple-negative breast cancers included in The Cancer Genome Atlas, suggesting the possibility that these genetic lesions contribute to chemoresistance. Overall, over 90% of residual triple-negative breast cancers harbored an actionable alteration in a targetable pathway, suggesting that molecular profiling could be performed on triple-negative breast cancers that do not achieve a pathologic complete response to neoadjuvant chemotherapy to potentially guide the use of targeted therapies in the immediate postoperative setting. ■

*See article, p. 232.*

# FGFR Inhibitor Sensitivity Is Modulated by FGF Ligand and MYC Expression

- The 8p12 locus containing *FGFR1* is heterogeneously amplified in squamous-cell lung cancers.
- *FGFR1*-amplified cancer cells are dependent on FGF ligands for survival *in vitro* and *in vivo*.
- High MYC expression augments FGFR1 oncogenicity and sensitizes cells to FGFR inhibition.

Amplification of the 8p12 locus, containing the fibroblast growth factor receptor 1 (*FGFR1*) kinase gene, frequently occurs in squamous cell lung cancer and has been suggested to confer sensitivity to FGFR inhibitors; however, it remains unclear which 8p12-amplified tumors will respond to therapy. To further understand FGFR1-mediated oncogenesis and define the molecular determinants of FGFR dependency, Malchers and colleagues characterized the 8p12 amplicon in primary squamous cell lung cancer samples via single-nucleotide polymorphism analysis. Amplification of 8p12 was highly heterogeneous, with amplicons from only 28% of samples centered on *FGFR1*. *FGFR1*-amplified, FGFR inhibitor–sensitive lung tumor cells were dependent on FGF ligand–mediated activation of FGFR1 signaling for survival and tumor formation both *in vitro* and *in vivo*. In addition, co-expression of MYC enhanced the ability of FGFR1 to promote oncogenic transformation and tumor growth in a cell-autonomous manner. Moreover, FGFR inhibitor treatment induced apoptosis and regression of tumors expressing both MYC and FGFR1, but not those expressing FGFR1 alone, suggesting that MYC overexpression sensitizes *FGFR1*-amplified cells to kinase inhibition. In support of this idea, depletion of MYC conferred FGFR inhibitor resistance in *FGFR1*-amplified cells, and elevated MYC expression correlated with clinical responses to FGFR inhibition in two patients with *FGFR1*-amplified lung tumors. These results suggest that high MYC expression may enable selection of a subset of patients with *FGFR1*-amplified lung tumors who will benefit from FGFR inhibition. ■

*See article, p. 246.*

**Note:** *In This Issue* is written by *Cancer Discovery* Science Writers. Readers are encouraged to consult the original articles for full details.