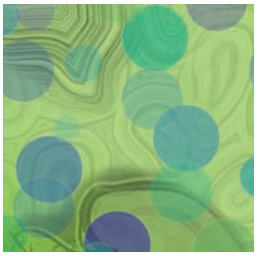
Cultured Human Mucosal Organoids Recapitulate Oral Cancer

- Patient-derived HNSCC organoids recapitulate histologic and molecular characteristics of patient HNSCC.
- The responses of HNSCC organoids to current therapies, including radiotherapy, mimic patient responses.
- This organoid system provides the potential for a personalized approach to the treatment of HNSCC.



Recent advances in three-dimensional (3-D) tumor models and organoid cultures have facilitated more accurate recapitulation of *in vivo* tumor conditions, particularly in response to drug treatment, compared with 2-D adherent cancer cell lines. Driehuis and colleagues demonstrate this advantage in a model of patient-derived head and neck squamous cell carcinoma (HNSCC) organoids. Culture conditions for surgically resected human epithelial cells were modified to establish HNSCC organoids from 31 patient-derived tumor samples, which exhibited histologic characteristics and transcriptional profiles similar to their *in vivo* counterparts. Genetic alterations detected in these HNSCC organoids were similar to those commonly found in this tumor type. Importantly, these genetic alterations were not detected in the matched wild-type organoids, derived from the same patient. HNSCC organoids retained their tumorigenic potential, forming tumors after subcutaneous injection into mice. Organoids exhibited differential sensitivity to therapies commonly used to treat patients with HNSCC including cisplatin, carboplatin, cetuximab, and ionizing radiation; organoid sensitivity to radiation correlated with patient response to postoperative radiotherapy. Additionally, cisplatin or the second mitochondria-derived activator of caspase (SMAC) mimetic L161 could serve as radiosensitizers in a subset of patient-derived lines. Moreover, the organoids were used to test sensitivity to a panel of drugs not commonly used in patients with HNSCC. These findings demonstrate the potential to harness this system for rapid and accurate *in vitro* characterization of patient-derived tumor samples, which may help guide personalized therapy in HNSCC. ■

See article, p. 852.

Targeting Enhancers and Promoters Rescues Aberrant Gene Silencing in AML

- An *ex vivo* co-culture system for primary AML samples allowed long-term evaluation of epigenetic drugs.
- *TET2* mutation was most significantly associated with response to LSD1 inhibition plus 5-azacytidine.
- Promoter demethylation and enhancer activation are both required to reactivate silenced genes in AML.

Efforts to evaluate epigenetic therapies in acute myeloid leukemia (AML) have been hindered by the inability to keep primary samples alive *in vitro* long enough to observe the effects of these agents. Duy and colleagues developed an *ex vivo* platform wherein primary AML samples were propagated in a cytokine cocktail on a feeder layer of irradiated stromal cells. Inhibition of DNA methylation with 5-azacytidine had only a limited effect on AML proliferation and viability, whereas inhibition of the histone demethylase LSD1 had a more pronounced effect, though a subset of samples remained resistant. Combined treatment with 5-azacytidine and an LSD1 inhibitor was more effective than either agent alone, including in monotherapy-resistant samples. Targeted sequencing of genes mutated in myeloid malignancies revealed that mutation of *TET2*, which encodes a dioxygenase that oxidizes 5-methylcytosine to 5-hydroxymethylcytosine, was most significantly associated with response to combination therapy. TET2 loss of function resulted in depletion of 5-hydroxymethylcytosine at LSD1-bound enhancers and facilitated LSD1-mediated enhancer silencing. Combination therapy induced differentiation and suppressed leukemia stem cell transcriptional programs in *TET2*-mutant AML cells, with LSD1 inhibition primarily leading to activation of gene enhancers and 5-azacytidine primarily leading to decreased CpG methylation at gene promoters. Combined promoter demethylation and enhancer activation led to greater induction of key target genes and facilitated enhancer–promoter looping at tumor suppressor loci such as *GATA2*. These findings suggest that rational combinations of epigenetic therapies, particularly in certain genetic contexts, may be needed for maximum antitumor activity through targeting of both promoters and enhancers. ■

See article, p. 872.

Targeting Mitochondria Is Synthetically Lethal with Venetoclax in AML

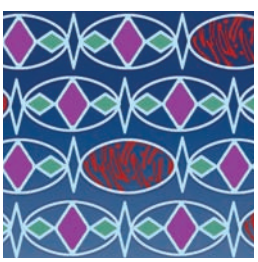
- Depleting genes involved in mitochondrial organization and structure sensitizes AML cells to venetoclax.
- The mitochondrial chaperonin CLPB is upregulated in AML and is induced further in venetoclax-resistant cells.
- CLPB depletion overcomes venetoclax resistance by inducing mitochondrial stress and apoptosis.

BCL2 supports cancer cell survival by suppressing mitochondria-mediated apoptosis. Venetoclax is a selective BCL2 inhibitor, recently approved by the FDA to treat acute myeloid leukemia (AML). However, intrinsic and acquired venetoclax resistance remains a significant problem. Chen, Glytsou, and colleagues performed a genome-wide CRISPR/Cas9 loss-of-function screen in human AML cells to identify genes whose loss would sensitize AML cells to venetoclax. Ablation of mitochondrial proteins, including CLPB, BAX, and PMAIP1, as well as p53 loss of function sensitized AML cells to venetoclax treatment. CLPB is a mitochondrial chaperonin that sustained functional mitochondrial cristae morphology in wild-type cells by interacting with OPA1 and HAX1, regulators of mitochondrial function. Expression of CLPB was upregulated in patients with AML and protected AML cells against mitochondrial-mediated apoptosis. Loss of CLPB disrupted mitochondrial cristae structure, increased sensitivity to oxidative stress, and led to decreased growth of AML cells both *in vitro* and *in vivo*. CLPB loss altered the cellular transcriptome and metabolome and induced a mitochondrial stress response leading to cell-cycle arrest and apoptosis. Sensitizing venetoclax-resistant AML cells by CLPB ablation was p53-independent, and thus overcame p53-mediated venetoclax resistance. Targeting CLPB also enhanced the efficacy of combination treatment of venetoclax and azacitidine. Collectively, these results propose targeting proteins involved in mitochondrial structure and homeostasis as a promising strategy to overcome resistance to venetoclax in patients with AML. ■

See article, p. 890.

p53 Network Proteins Mediate AML Sensitivity to BCL2 inhibition

- Inactivation of p53 and proapoptotic proteins promotes resistance to venetoclax.
- Resistance to venetoclax is accompanied by changes in mitochondrial function and cellular metabolism.
- Loss of p53 function confers acquired sensitivity to TRK inhibitors in AML.

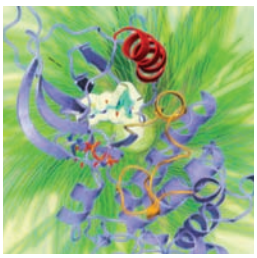


Upregulation of antiapoptotic proteins from the BCL2 family contributes to intrinsic and acquired drug resistance in cancer. Venetoclax is a BH3 mimetic that selectively targets BCL2 and has been approved in combination with the hypomethylating agent azacytidine for the treatment of acute myeloid leukemia (AML). To identify genes and pathways contributing to venetoclax resistance in AML, Nechiporuk and colleagues performed a genome-wide CRISPR/Cas9 screen in human AML cells. The screen revealed genes whose inactivation promotes resistance to venetoclax, namely *TP53* and several p53 transcriptional targets with proapoptotic activity such as BAX and PMAIP1 (NOXA). Inactivation of p53 and BAX led to deregulation of the mitochondrial apoptotic network and loss of apoptotic response to venetoclax, and was accompanied by changes in cellular metabolism and proliferation as well as increased cellular respiration and ROS production. In addition, inactivation of p53 but not BAX led to a number of compensatory changes in cellular prosurvival machinery. Consistent with the findings from the CRISPR screen, AML patient samples from the Beat AML dataset with *TP53* mutation or with low expression levels of p53 and BAX showed decreased sensitivity to venetoclax *ex vivo*. Inactivation of *TP53* also conferred sensitivity to TRK inhibitors in correlation with increased expression of NTRK3 and decreased expression of the putative NTRK regulator RUNX1A. Collectively, these findings indicate that disruption of a p53-dependent apoptotic network, including proteins involved in mitochondrial function and specific metabolic pathways, can drive resistance to venetoclax in AML and suggest potential therapeutic strategies to overcome venetoclax resistance. ■

See article, p. 910.

Allosteric Inhibition of Mutant EGFR Bypasses Resistance Mutations

- JBJ-04-125-02 allosterically inhibits mutant EGFR in NSCLC models *in vitro* and *in vivo*.
- JBJ-04-125-02 can bind EGFR simultaneously with the ATP-competitive EGFR inhibitor osimertinib.
- Dual targeting of mutant EGFR is a potential strategy to overcome resistance mutations in NSCLC.

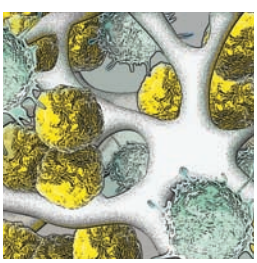


Inhibition of mutant EGFR is an effective treatment strategy in patients with non–small cell lung cancer (NSCLC); however, resistance mutations often develop in the ATP-binding pocket, contributing to tumor progression. To circumvent these resistance mutations, To, Jang, and colleagues identified and characterized a novel allosteric inhibitor of EGFR, JBJ-04-125-02, as a single agent and as a combinatorial therapy with mutant-selective ATP-competitive EGFR inhibitors. *In vitro*, JBJ-04-125-02 bound the allosteric pocket of mutant EGFR at nanomolar potency and with high selectivity. JBJ-04-125-02 inhibited EGFR phosphorylation and proliferation in cells expressing mutant EGFR, with no effect on cells expressing wild-type EGFR. JBJ-04-125-02 was similarly effective *in vivo*, inducing tumor regression in a genetically engineered mouse model of EGFR-mutant lung cancer and EGFR-mutant xenografts without overt toxicity. Notably, the efficacy of JBJ-04-125-02 was limited by EGFR dimer formation, as cell lines with high rates of EGFR dimerization were resistant to JBJ-04-125-02. Combined treatment with JBJ-04-125-02 and the covalent ATP-competitive EGFR inhibitor osimertinib increased the amount of JBJ-04-125-02 bound to EGFR, independent of EGFR dimerization. Dual treatment inhibited EGFR phosphorylation, reduced cell proliferation, and increased apoptosis more effectively than either treatment alone and delayed the emergence of drug-resistant clones. Furthermore, combined treatment with JBJ-04-125-02 and osimertinib induced tumor regression and extended median overall survival *in vivo*. Collectively, these findings demonstrate the therapeutic potential for allosteric inhibitors of mutant EGFR to be used either alone or in combination with ATP-competitive EGFR inhibitors. ■

See article, p. 926.

Alkylating Agents Induce ER Stress to Drive Lymphoma Clearance

- High lymphoma:macrophage ratios in bone marrow promote resistance to antibody-based therapies.
- High-dose alkylating agents induce ER stress to promote phagocytosis of opsonized lymphoma cells.
- Treatment with alkylating agents and monoclonal antibodies elicits synergistic antitumor effects.

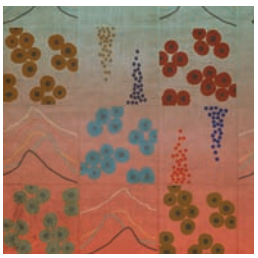


Although monoclonal antibodies such as rituximab or alemtuzumab (Alem) are standard therapies for high-grade lymphomas, they have demonstrated limited efficacy. Conversely, cyclophosphamide (CTX) is a highly potent therapy against lymphoma, yet the cellular and molecular mechanisms underlying its anticancer activity remain unclear. Lossos and colleagues sought to characterize the mechanism underlying CTX efficacy in a mouse model of human double-hit lymphoma. Treatment of mice harboring rituximab-resistant lymphoma patient-derived xenografts with Alem eliminated tumor cells from the blood and spleen but not the bone marrow (BM). In contrast, treatment with CTX or other alkylating agents reduced lymphoma burden in the BM and were highly synergistic in combination with Alem; the efficacy of these combinations required a low lymphoma:macrophage ratio. Moreover, resistance to Alem in the BM occurred only after extended accumulation of lymphoma, where tumor cells exhibited increased expression of antiphagocytic factors. CTX treatment of BM lymphoma cells resulted in upregulation of the ER stress response transcription factor ATF4, driving expression and secretion of VEGFA, which in turn increased phosphorylation of the SYK kinase in macrophages and promoted phagocytosis of Alem-treated BM lymphoma cells; inhibition of either VEGFA or SYK abrogated this signaling mechanism. Single-cell RNA sequencing of macrophages subjected to CTX treatment identified a subpopulation of highly phagocytic macrophages marked by increased expression of Fc gamma receptor 4 and CD36. These data describe the ability of CTX to induce macrophage-dependent clearance of lymphoma and demonstrate the potential of CTX to overcome lymphoma resistance to antibody-based therapies. ■

See article, p. 944.

FOXH1 Mediates Mutant p53 Gain-of-Function in Acute Myeloid Leukemia

- Mutant gain-of-function $p53^{R172H}$ endows AML with increased capacity for self-renewal.
- Hematopoietic stem cells depend on gain-of-function p53 signaling even prior to transformation.
- Mutant p53 requires FOXH1 to maintain stem cell–associated transcriptional programs.



Although the majority of cancer-related mutations in *TP53* render this canonical tumor suppressor inactive, gain-of-function mutations can confer the ability to regulate pro-oncogenic transcriptional programs. In complex karyotype acute myeloid leukemia (CK-AML), mutations in *TP53* are associated with poor prognosis, yet it remains unknown whether these mutations have gain-of-function activity. Loizou and colleagues utilized a mouse model of AML harboring $p53^{R172H}$, the most common mutation in AML, to characterize the mechanisms underlying mutant p53 activity. Expression of $p53^{R172H}$ accelerated CK-AML initiation compared with p53-deficient mice, suggesting that mutant p53 contributes to AML progression. Depletion of mutant p53 promoted differentiation and induced apoptosis of AML cells and increased survival in mice. *In vitro*, $p53^{R172H}$-expressing hematopoietic stem and progenitor cells (HSPC) exhibited enhanced self-renewal compared with p53-deficient cells. Consistent with this finding, transplanted $p53^{R172H}$ cells outcompeted wild-type and p53-deficient HSPCs *in vivo*, and downstream hematopoietic progenitors were increased in $p53^{R172H}$ mice. Among the most upregulated genes in $p53^{R172H}$ AML cells were the transcription factor forkhead box H1 (FOXH1) as well as many of its target genes. In turn, FOXH1 induced a number of transcriptional targets involved in self-renewal and hematopoietic stem cell identity. Depletion of FOXH1 eliminated the self-renewal capacity of $p53^{R172H}$ cells, whereas overexpression of FOXH1 either in p53–wild-type cells or p53-deficient cells conferred increased self-renewal capacity. Taken together, these findings reveal that gain-of-function oncogenic mutations in p53 create a dependency that promotes CK-AML pathogenesis. ■

See article, p. 962.

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