Identified tumor-specific neoantigens and tumor-associated antigens (TAA), which are differentially expressed in tumor tissue compared to normal tissue, are promising targets for immunotherapy. A comprehensive characterization of T-cell antigenic targets is needed to develop personalized cancer vaccines. To this end, Kalaora and colleagues combined whole-exome sequencing, RNA sequencing, and human leukocyte antigen (HLA) peptidomics to identify TAAs and neoantigens in 16 tumors from 7 patients with melanoma and performed T-cell receptor (TCR) sequencing on isolated tumor-infiltrating lymphocytes. In metastatic lesions from the same patient there was substantial overlap in the presented peptides between metastatic lesions, with 25% to 80% of peptides detected in at least two metastases from the same patient. Moreover, TCR clonotypes were found at comparable frequencies in two metastases from the same patient. Evaluation of tumor-infiltrating lymphocytes (TIL) from a sample that presented three neoantigens, two of which induced T-cell reactivity, revealed a high specificity toward autologous melanoma cells. HLA-peptidomic and antigen prediction identified immunogenic neoantigens and TAAs, and two neoantigen-specific clonotypes were responsible for the killing of the large majority (90%) of autologous melanoma cells both in vitro and in vivo. In addition to characterizing the neoantigen and TAA repertoire in patients with melanoma, these findings demonstrate that a limited set of neoantigen-specific T cells may be largely responsible for tumor rejection in melanoma. These data suggest that identification of a few targetable tumor antigens and their corresponding TIL clones may be sufficient to guide personalized cancer immunotherapy.

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MTF2 Deficiency Is Linked to Chemoresistance and Poor Survival in AML

- MTF2 promoter hypermethylation downregulates its expression in AML, reducing global H3K27 trimethylation.
- MTF2 promotes transcriptional repression of MDM2 to increase p53 expression and chemosensitivity.
- MDM2 inhibition may be beneficial in combination with induction chemotherapy in patients with AML.

Genetic markers are not entirely sufficient to stratify patients with acute myeloid leukemia (AML) based on prognosis, suggesting an important role for epigenetic regulation. Indeed, epigenetic modifiers are frequently mutated in patients with AML, but the mechanism by which epigenetic dysregulation promotes AML remains poorly understood. Maganti, Jrade, and colleagues analyzed global levels of H3K27me3, a repressive histone mark established by the enzymatic EZH1 or EZH2 subunits of the polycomb repressive complex 2 (PRC2), in 32 samples from patients with AML who underwent induction chemotherapy. Reduced H3K27me3 levels were associated with poor survival and downregulation of MTF2, which encodes a protein that recruits PRC2 to DNA regions with unmethylated CpGs. MTF2 is rarely mutated in AML, but its promoter was hypermethylated in MTF2-deficient AML samples. Ectopic overexpression of MTF2 in deficient cells was sufficient to restore global H3K27me3. Loss of MTF2 expression conferred resistance to standard induction chemotherapy in patient-derived AML cells, and a similar effect was achieved by dual inhibition of the methyltransferases EZH1 and EZH2. Mechanistically, MTF2 transcriptionally repressed the negative regulator of p53 MDM2, thereby increasing p53 expression. Thus, MDM2 inhibitors could reactivate the p53 program in MTF2-deficient cells without reactivating other PRC2 target genes, and MDM2 inhibition sensitized MTF2-deficient refractory cells to induction chemotherapy drugs. In patient-derived xenograft models of AML, combination treatment with an MDM2 inhibitor and induction chemotherapy extended survival. Altogether, these findings reveal an epigenetic mechanism by which MTF2 deficiency promotes chemoresistance in AML and suggest the potential for combination therapy with MDM2 inhibitors.

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The standard-of-care treatment for patients with estrogen receptor-positive (ER+)/HER2− breast cancer has previously been based upon sequencing endocrine therapy, targeted therapy, and/or chemotherapy and now includes CDK4/6 inhibitors. Recent studies have identified potential mechanisms of resistance to CDK4/6 inhibitors, including RB loss and CDK6 amplification, in preclinical models, but it is unclear whether these resistance mechanisms occur in patients. To identify drivers of resistance to CDK4/6 inhibitors in patients with ER+ breast cancer, O'Leary and colleagues performed whole-exome sequencing of matched baseline and post-treatment plasma samples from 16 patients enrolled in the phase III PALOMA-3 trial treated with either placebo + fulvestrant or palbociclib + fulvestrant and found that clonal evolution was common in patients receiving palbociclib + fulvestrant. ctDNA profiling of matched baseline and post-treatment plasma samples from 195 patients in the PALOMA-3 trial showed that although RB1 mutations arise in 6 of 127 (5%) patients after palbociclib + fulvestrant treatment, these RB1 mutations were often subclonal. Further, there were no significant differences in exome copy-number profiles. Mutations in known drivers, particularly the ESRI Y537S mutation, arose in 60 of 195 (30.8%) patients treated with either placebo + fulvestrant or palbociclib + fulvestrant, more commonly in patients on treatment for longer. These results show that clonal evolution occurs in response to combination therapy in patients with ER+/HER− breast cancer and suggest that the ESRI Y537S mutation may mediate resistance to fulvestrant.

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Genomic alterations affecting components of the DNA-damage response occur frequently in patients with high-grade serous ovarian cancer (HGSC), leading to potential DNA-repair defects that may render tumors sensitive to therapies targeting repair defects. However, the functional consequences of these defects in DNA-repair genes remain incompletely characterized. Hill and colleagues used patient-derived HGSC organoids to profile DNA-repair activity and identify potential therapeutic vulnerabilities. In total, 33 organoid cultures derived from 22 patients with HGSC were assessed for defects in homologous recombination (HR) and replication fork protection. Whole-exome sequencing and morphologic analysis confirmed that the organoids resembled the tumors from which they were derived. The majority of HGSC organoids exhibited functional HR and were insensitive to agents targeting HR defects including the PARP inhibitor olaparib. Only 2 of 34 (6%) organoid cultures were olaparib-sensitive, indicating a lack of functional HR defects despite more frequent genetic alterations that would predict HR deficiency. In contrast, replication fork instability, which occurred in 61% of tested cultures, was associated with sensitivity to carboplatin, prexasertib, and VE-822. In addition, fork instability and replication stress could be induced in fork-stable lines by combining prexasertib with carboplatin or gemcitabine. Collectively, these findings suggest that functional organoid profiling in combination with genomic analysis may enhance the identification of targetable DNA damage repair defects in patients with HGSC.

See article, p. 1404.
The acetyltransferase CREB binding protein (CREBBP) is among the most frequently mutated genes in human small cell lung cancer (SCLC), a highly lethal neuroendocrine lung tumor, but its role in SCLC is unknown. To elucidate the role of Crebbp loss in SCLC tumorigenesis, Jia and colleagues generated multiple genetically engineered mouse models in which Crebbp was deleted in different Trp53-deficient in vivo models of SCLC as well as two other neuroendocrine tumors, pituitary and thyroid neuroendocrine tumors. Ablation of Crebbp enhanced transformation and tumorigenesis of preneoplastic SCLC cells and promoted the accelerated development of SCLC or pituitary and thyroid tumors in mice with targeted inactivation of RB and p53 in lung or neuroendocrine cells, respectively. Consistent with these findings, expression of CREBBP in CREBBP-deleted human SCLC cells resulted in reduced CDH1 expression and proliferation. CREBBP was shown to promote the histone acetylation and subsequently the upregulated expression of tight junction and adhesion genes such as Cdh1 to prevent the acquisition of mesenchymal features. Treatment with the clinical HDAC inhibitor pracinostat restored histone acetylation and the expression of Cdh1, resulting in the regression of Crebbp-null SCLC in vivo. Taken together, these results describe an autochthonous mouse model of Crebbp-deleted SCLC, characterize CREBBP as a tumor suppressor in SCLC, and suggest that patients with CREBBP-mutant SCLC may benefit from Pracinostat treatment.

See article, p. 1422.

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of disorders characterized by inadequate hematopoiesis that results in dysplastic or cytopenic phenotypes and increased risk of leukemic transformation. A number of genetic alterations have been implicated in MDS development; however, it remains unclear whether these driver mutations converge to induce MDS via a common mechanism. Hayashi, Zhang, Yokota, and colleagues found increased expression of the transcription factor hypoxia-inducible factor 1α (HIF1α) and induction of a HIF1α target gene signature across a broad spectrum of patients with MDS, suggesting that this pathway may represent a critical downstream effector of MDS-associated mutations. Consistent with this idea, expression of constitutively active HIF1α conferred a clonal advantage in hematopoietic stem and progenitor cells and was sufficient to induce the development of cytopenias and multilineage bone marrow dysplasia in mice. MDS-associated alterations, including partial tandem duplication of Mll and Runx1 mutation, cooperatively stimulated HIF1α protein stabilization and activation of HIF1α signaling in a hypoxia-independent manner. This aberrant activation of HIF1α signaling was required for the development of MDS phenotypes, as genetic deletion of Hif1a or treatment with echinomycin, an inhibitor of HIF1α-mediated target gene activation, abrogated disease development and prolonged survival in mouse models of MDS without significant effects on normal hematopoiesis. These results identify dysregulation of HIF1α signaling as a central mediator of MDS and a potential therapeutic target for a broad spectrum of MDS.

See article, p. 1438.
The parasympathetic nervous system is a key regulator of organ function, and induction of parasympathetic cholinergic signaling through acetylcholine-mediated stimulation of muscarinic receptors can promote tumor growth in some contexts. The pancreas is innervated by parasympathetic nerve fibers supplied by the vagus nerve, but the roles of vagal innervation, cholinergic signaling, and muscarinic receptors in pancreatic tumorigenesis are unknown. Renz, Tanaka, Sunagawa, Takahashi, and colleagues observed that surgical sub-diaphragmatic transection of the vagus nerve (vagotomy) accelerated pancreatic intraepithelial neoplasia and PDAC development in \( \text{Kras} \)-mutant (KC) mice in association with increased expansion of CD44\(^+\) cancer stem cells (CSC) and immunosuppressive CD11b\(^+\) myeloid cells, whereas a muscarinic agonist reduced tumor incidence in mice that had undergone vagotomy, suggesting that cholinergic signaling in the pancreas suppresses tumor progression. Consistent with these findings, the combination of a muscarinic agonist with chemotherapy significantly extended the survival of \( \text{Kras-mutant;Trp53-mutant (KPC)} \) mice that develop PDAC and suppressed the CSC compartment. These effects were dependent on the muscarinic receptor CHRM1, as knock-out of \( \text{Chrm1} \) significantly increased tumor incidence and decreased overall survival in KC and KPC mice and increased stemness of \( \text{Kras-mutant} \) pancreatic spheres via increased EGFR/MAPK and PI3K/AKT signaling. Additional studies in a model of hepatic metastasis confirmed these results, as treatment with a muscarinic agonist significantly prolonged survival and selective hepatic vagotomy significantly reduced survival. These findings point to a tumor suppressor role of parasympathetic cholinergic signaling in the pancreas and suggest that muscarinic agonists may be an effective therapeutic strategy for PDAC.

See article, p. 1458.

### The HPV–Host Protein Interactome Reveals Additional Oncogenic Roles of HPV

Most previous efforts to investigate human papillomavirus (HPV)-driven tumorigenesis have centered on the high-risk HPV proteins E6 and E7, which exert inhibitory activity on human tumor suppressor proteins such as RB and p53. However, HPV genomes encode up to 7 additional proteins, and the complete HPV–human protein interactome has not been characterized. Eckhardt, Zhang, and colleagues expressed all 9 HPV proteins in several human cell lines and performed affinity purification and mass spectrometry to systematically map HPV–human protein–protein interactions. Integration with mutation profiles from cervical squamous cell carcinomas, endocervical adenocarcinomas, and head and neck squamous cell carcinomas revealed that HPV proteins target human proteins encoded by genes that are preferentially mutated in HPV-negative but not HPV-positive tumors, suggesting that HPV hijacks the same oncogenic cellular pathways that are disrupted by genetic alterations in non–virally induced cancers. These pathways included several not previously implicated in viral oncogenesis, such as the KEAP1–NRF2–antioxidant response element pathway (identified through an inhibitory interaction between the HPV E1 protein and KEAP1) and the RNF20/RNF40 ubiquitin ligase complex (identified through an interaction with the HPV L2 protein required for cell invasion). The systematic mapping of HPV–host interactions across all HPV proteins in multiple cellular contexts thus suggests not only that other HPV proteins beyond the well-studied E6 and E7 can play roles in HPV-driven tumorigenesis but also that viral phenocopying of recurrent mutations is pervasive in HPV-positive cancers.

See article, p. 1474.

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