Although it is established that many types of activating KRAS mutations are oncogenic, a full characterization of how mutation types affect tumorigenesis, tumor progression, and treatment response is lacking. To study this, Zafra and colleagues used CRISPR–Cas9-based genome editing to develop new genetically engineered mouse models with conditional expression of a range of common oncogenic Kras variants. In the pancreas, after 12 weeks, Kras\textsuperscript{G12D} expression caused 90% of each pancreas to be overtaken by pancreatic intraepithelial neoplasia (PanIN); Kras\textsuperscript{G12C} expression led to high levels of PanIN (comprising 50% of the organ), with most of the rest of each pancreas exhibiting acinar-to-ductal metaplasia (ADM); and Kras\textsuperscript{G13D} expression produced pancreata that were only 33% ADM or PanIN, although these organs were 50% PanIN by one year, suggesting that Kras\textsuperscript{G13D} expression simply has a slower pace of progression. In contrast, pancreata expressing Kras\textsuperscript{G12R} exhibited almost no PanIN even by one year. Notably, preneoplastic lesions in Kras\textsuperscript{G12C}- and Kras\textsuperscript{G13D}-mutant pancreata progressed in response to acute pancreatitis, but those in Kras\textsuperscript{G12R}-expressing pancreata did not. Additionally, Kras\textsuperscript{G13D}-expressing pancreatic organoids were sensitive to EGFR inhibition, and Kras\textsuperscript{G12C}-expressing pancreatic organoids were sensitive to KRAS\textsuperscript{G12C}-specific inhibitors only in the context of EGFR suppression. Collectively, these results highlight how different Kras mutations, despite all being expected to increase KRAS activity, may have markedly different effects in vivo.

See article, p. 1654.

Different Types of Oncogenic Kras Mutations Yield Distinct Phenotypes

- Oncogenic Kras mutations are all expected to increase KRAS activity, but they produced distinct phenotypes.
- Pancreatic Kras\textsuperscript{G12C} or Kras\textsuperscript{G12D} expression was rapidly oncogenic, but expression of some other mutants was not.
- The Kras variant expressed by pancreatic organoids influenced sensitivity to EGFR or KRAS\textsuperscript{G12C} inhibition.

The use of genetically engineered adoptive cell therapies (ACT) involving retroviral or lentiviral transduction of patient T cells with DNA encoding a chimeric antigen receptor or a T-cell receptor (TCR) specific to a cancer-associated antigen has yielded great benefits for the treatment of several malignancies; however, relapse can occur. Extending preclinical findings that expression of transgenic TCRs by circulating T cells diminishes rapidly after reinfusion and that the commonly used murine stem-cell virus (MSCV) is subject to DNA methylation-based epigenetic silencing, Nowicki and colleagues investigated the applicability of these observations in patients. In 16 patients treated with TCR ACT, although the engineered T cells retained the transgenic DNA encoding the TCR, RNA- and protein-level expression of the transgene was markedly reduced by 70 days following infusion. Patients with the lowest expression of the transgene exhibited substantially increased levels of CpG methylation at the MSCV 5’ long terminal repeat promoter region, an epigenetic mark associated with reduced transcription. All patients had increased CpG methylation across the entire MSCV vector—a factor also associated with reduced transgene transcription—over the 70 days following infusion, but this effect was particularly pronounced in patients who had the greatest reduction in TCR expression over time. In summary, this study reveals the role of CpG methylation-based epigenetic silencing of transgenic material in reduced TCR expression in patients, a major hurdle for long-term efficacy of ACTs, and suggests that approaches to mitigate or prevent this problem are needed.

See article, p. 1645.

Engineered T cells in patients receiving adoptive cell therapy retained the inserted T-cell receptor transgene.

Transgene methylation (an epigenetic mark) occurred, especially in T cells with low transgene expression.

This epigenetic silencing of T-cell receptor transgenes may be a cause of treatment resistance or relapse.
IDH-mutant tumors exhibit metabolic derangement, including low NAD⁺ levels, and are often treated with alkylating agents such as temozolomide, further reducing NAD⁺ pools due to upregulation of poly(ADP-ribose) (PAR) polymerase, which consumes NAD⁺ to produce PAR. To investigate whether IDH-mutant cancers depleted of NAD⁺ via treatment with alkylating agents could be further damaged by inhibiting PAR glycohydrolase (PARG), which depolymerizes PAR to restore NAD⁺ pools, Nagashima and colleagues first established that a selective small-molecule PARG inhibitor augmented temozolomide’s efficacy against IDH-mutant cancer cell lines. Supporting the proposed mechanism, IDH-mutant cancer cells treated with the combination of a PARG inhibitor and temozolomide had low NAD⁺ levels, and cell viability in the presence of temozolomide and the PARG inhibitor was restored by supplementation with NAD⁺ precursors. Sensitivity to temozolomide in IDH-mutant fibrosarcoma and glioma cells made resistant to the drug was restored by the addition of the PARG inhibitor; again, supplementation with NAD⁺ precursors abolished the cytotoxic effects of the combination. In vivo, IDH-mutant, PARG-knockout fibrosarcoma xenografts were more sensitive to temozolomide than PARG-wild-type xenografts and had low NAD⁺ levels at baseline that were further depleted after one cycle of temozolomide treatment. In summary, this work demonstrates that low basal NAD⁺ levels are a therapeutically exploitable vulnerability in IDH-mutant cancer cells, providing an avenue toward rationally designed treatment combinations to enhance the efficacy of existing therapies.

See article, p. 1672.

Some WNT Pathway–Activated Colorectal Cancers Are Asparaginase-Sensitive

- WNT pathway activation upstream of GSK3, which inhibits this kinase, conferred asparaginase sensitivity.
- GSK3 inhibition impairs protein degradation to limit amino acid generation and trigger asparaginase sensitivity.
- Activating mutations upstream of GSK3 are common in colorectal cancers, making asparaginase promising.

Activation of WNT signaling upstream of GSK3—which, when not inactivated by WNT signaling, phosphorylates β-catenin and many other proteins to promote their degradation—occurs in 10% to 15% of colorectal cancers, whereas activation of the WNT pathway downstream of GSK3 occurs in 85% of cases. In acute leukemias, WNT pathway activation upstream, but not downstream, of GSK3 causes sensitization to asparaginase, prompting Hinze and colleagues to investigate whether this was also the case in colorectal cancer. Indeed, in human colorectal cancer cell lines and mouse organoids, activation of the WNT pathway upstream, but not downstream, of GSK3 was associated with hypersensitivity to asparaginase. Consistent with the mechanism proposed in leukemia, further in vitro and organoid experiments showed that this hypersensitivity was caused by WNT-dependent protein stabilization. In vivo, asparaginase was highly effective against tumors with Rspo3 fusions (which act upstream of GSK3). In contrast, monotherapy with asparaginase or a GSK3 inhibitor was ineffective in vivo against mouse tumors with mutations that activate WNT signaling downstream of GSK3, such as activating mutations in Cmmbl (encoding β-catenin) or inactivating mutations in Apc, but the combination of both drugs was effective; further, it hindered tumor growth in a model of liver-metastatic colorectal cancer. Finally, the combination treatment, but not monotherapy, was effective in vivo against APC-mutant patient-derived xenografts. In summary, this work provides evidence that asparaginase hypersensitivity is present in a subset of colorectal cancers as a consequence of specific WNT pathway–activating mutations.

See article, p. 1690.
Recent studies have linked dysfunction of enzymes dependent on 2-oxoglutarate (2-OG; also known as α-ketoglutarate) to various malignancies, including breast cancer. Aiming to determine whether any 2-OG–dependent enzymes are involved in triple-negative breast cancer (TNBC) growth, Liao and colleagues performed an siRNA screen to ascertain whether reductions in any of these enzymes would decrease proliferation or anchorage-independent growth of a TNBC cell line. This screen identified γ-butyrobetaine hydroxylase (BBOX1), which catalyzes the last step of the L-carnitine biosynthesis pathway. Further investigation showed that BBOX1’s catalytic activity was required for it to enhance TNBC cell growth, but L-carnitine supplementation did not increase growth of BBOX1-knockdown TNBC cells. Instead, wild-type (but not catalytically inactivated) BBOX1 bound IP3R3, an endoplasmic reticulum (ER) calcium channel protein that mediates calcium transport from the ER into mitochondria, stabilizing IP3R3 by preventing its ubiquitination and subsequent proteasomal degradation. Mechanistically, loss of BBOX1-mediated stabilization of IP3R3 blocked calcium release from the ER, leading to impairments in processes that require calcium, such as mitochondrial respiration and mTORC1-dependent glycolysis, causing apoptosis and defective cell-cycle progression. In vivo, breast tumors lacking BBOX1 exhibited markedly reduced growth, as did tumors with pharmacologic inhibition of BBOX1; correspondingly, BBOX1 deficiency or inhibition led to reduced anchorage-independent growth in vitro. In summary, this work identifies BBOX1 as a targetable vulnerability in TNBC cells and uncovers the mechanism by which TNBC cells depend on BBOX1 for growth.

See article, p. 1706.

Enhancers Define Meningioma Subgroups, Revealing a Druggable Target

Epigenetic dysregulation has been implicated in meningioma, but whether epigenetic information can be used to garner clinical information or develop new therapies remains unclear. Prager and colleagues investigated the meningioma enhancer landscape by searching for genomic regions enriched in histone 3 lysine residue 27 acetylation (H3K27ac, which commonly marks enhancer elements) in 33 meningiomas, including tumors that represented all histologic grades and were obtained from male and female patients. This revealed that enhancers could differentiate meningiomas based on prognosis, with three subgroups defined by time to recurrence emerging from the dataset, and on the basis of transcriptional profile, with one group expressing adipogenesis- and cholesterol-related genes, another group expressing mesodermal- and muscle-development genes, and a final group expressing genes involved in neuronal programs. Correspondingly, transcription factor (TF) expression differentiated three subgroups characterized by expression of TFs related to adipogenesis and cholesterol, mesodermal development, or neural crest development; notably, the former two groups had higher progesterone receptor–signature scores, whereas the latter group had a higher androgen receptor–signature score, and hormone receptors have previously been linked to meningioma. Further analysis of superenhancer-associated genes related to survival revealed that, among the top ten genes, the gene encoding DUSP1 (which can dephosphorylate MAPK1/ERK2) was the only one with an available inhibitor. In vitro and in vivo, the DUSP1 inhibitor showed efficacy against meningioma. Collectively, these findings illustrate the critical role of epigenetic dysregulation in meningioma and illustrate how epigenetic information can be used to stratify patients and develop therapies for this disease.

See article, p. 1722.
PRMT5 Inhibition Has In Vivo Efficacy in Myeloproliferative Neoplasms

- Pharmacologic inhibition of PRMT5 was effective in treating polycythemia vera and myelofibrosis in vivo.
- PRMT5 inhibition reduced methylation of the transcription factor E2F1, lowering expression of E2F1 target genes.
- This work supports currently active early clinical trials of PRMT5 inhibition in myeloproliferative neoplasms.

Dysregulation of the protein arginine methyltransferase PRMT5 has been observed in a variety of malignancies, and early-phase clinical trials of PRMT5 inhibitors for treatment of multiple cancers are underway. Pastore and colleagues found that PRMT5 was overexpressed in myeloproliferative neoplasm (MPN) cells, and treatment of MPN cells with the small-molecule PRMT5 inhibitor C220 reduced proliferation in vitro and in ex vivo experiments using patient-derived samples. In a mouse model of JAK2V617F-driven polycythemia vera, a type of MPN, C220 treatment reduced hematocrit levels, leukocytosis, and splenomegaly. Similarly, in a mouse model of MPLW515L-driven myelofibrosis, another MPN, C220 treatment reduced leukocytosis, thrombocytosis, hepatosplenomegaly, and fibrosis. In both models, combination treatment with C220 plus a JAK1/2 inhibitor, which has already shown efficacy in MPNs, was more effective than treatment with either agent alone. Mechanistically, inhibition of PRMT5 with C220 reduced methylation of the transcription factor E2F1, the expression of which has been linked to multiple cancers, causing impaired E2F1 activity. This led to decreased expression of E2F1 target genes, many of which encode proteins involved in DNA-damage repair and cell-cycle progression, causing cell-cycle arrest at the G2-M transition, increased DNA double-strand breaks following radiation exposure, and defective repair of DNA single-strand breaks. Collectively, these results support the continued investigation of PRMT5 inhibition for the treatment of MPNs in clinical trials and reveal the mechanism of action of PRMT5 inhibition in these diseases.

See article, p. 1742.

DAB2+ Tumor-Associated Macrophages Promote Invasion and Metastasis

- In vivo, Dab2 expression in hematopoietic-lineage cells promoted lung metastasis in multiple tumor models.
- DAB2 expressed by tumor-associated macrophages at lesion borders supported extracellular matrix remodeling.
- This study identifies DAB2 as a key mediator of metastasis, predicting poorer outcomes in patients.

Tumor-associated macrophages (TAM) can promote metastasis, but the molecular details underlying this phenomenon have not been fully determined. Based on the prior finding that tumor-infiltrating myeloid cells in mouse tumors have high expression of the gene encoding Disabled homolog 2 (DAB2), Marigo, Trovato, and colleagues investigated the effects of knocking out Dab2 in cells of the hematopoietic lineage. In vivo, fibrosarcoma and breast cancer metastasis to the lungs were reduced in Dab2-knockout mice, whereas growth of primary tumors was unaffected. In the fibrosarcoma and breast cancer models, DAB2 protein expression was highest in TAMs at the perillesional region demarcating invasive tumor tissue from healthy tissue, with lower DAB2 expression being observed in tumor cores. Mechanistically, DAB2 promoted endocytosis of extracellular matrix (ECM) proteins via increased integrin recycling, remodeling the ECM such that DAB2+ TAMs at lesion borders promoted tumor-cell invasion. The enhancement of tumor-cell invasiveness caused by DAB2+ TAMs was dependent on a mechanotransduction pathway mediated by the Hippo pathway proto-oncoproteins and transcriptional coactivators YAP and TAZ, the roles of which in mechanosensing have recently started to be uncovered. Notably, high abundance of DAB2+ TAMs in breast and gastric tumors in patients was associated with poorer prognosis, with increased risk of tumor progression and metastasis and decreased median overall survival. Together, these findings support a role for DAB2+ TAMs in promoting tumor invasiveness and metastasis and suggest that ECM remodeling may underlie this observation.

See article, p. 1758.

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