Adoptive transfer of T cells expressing chimeric antigen receptors (CAR) has led to striking clinical responses in some patients with refractory leukemia and lymphoma, but the clinical efficacy of this approach is limited by the expansion and persistence of CAR-T cells, particularly in solid tumors. Efforts have been made to engineer CAR-T cells to coexpress immunostimulatory cytokines (so-called “armored” CAR-T cells) because CARs cannot recapitulate the function of cytokines in T-cell activation, but systemic accumulation of cytokines secreted by armored CAR-T cells has the potential to cause severe adverse events such as neurotoxicity or cytokine release syndrome.

Shum and colleagues devised a method to selectively deliver cytokine signals to CAR-T cells through coexpression of a constitutively active interleukin 7 receptor (C7R), which triggers the IL7 signaling axis in CAR-T cells without a requirement for extracellular ligand and without stimulating bystander lymphocytes. Consistent with the known stimulatory effect of IL7 signaling on tumor-specific T cells, expression of C7R significantly increased the survival, proliferation, and antitumor activity of several types of CAR-T cells in vitro and in vivo without promoting autonomous or antigen-independent T-cell growth. In addition to demonstrating a requirement for immunostimulatory cytokine signaling for sustained activity of CAR-T cells against solid tumors, these findings suggest a strategy for safer delivery of cytokine signals to CAR-T cells that could potentially reduce the risk of severe adverse events associated with CAR-T cell therapy.

See article, p. 1238.

Residual melanomas exhibit tumor cell–intrinsic and –extrinsic alterations during anti-MAPK therapy. Transcriptomic, epigenomic and immune landscapes were generated from pre- and on-treatment melanomas. Targeting recurrent alterations may enhance response to anti-MAPK therapy in residual melanoma.

Patients with BRAF mutant melanoma receive combined MEK inhibitor and BRAF inhibitor (BRAFi) therapy to overcome acquired BRAFi resistance, but partially responding tumors often progress. To identify early adaptations critical for eventual tumor regrowth, Song, Piva, Sun, and colleagues performed transcriptomic profiling of patient-matched tumors at baseline and after clinical response to MAPKi and of human melanoma cell lines or murine melanoma tumors at several timepoints before and during continuous MAPKi. MAPKi-resistant cell lines exhibited either a MAPK reactivation/addiction (Ra) phenotype or a MAPK redundant (Rr) phenotype: Ra cells display few gene expression changes compared to their MAPKi-naive parental cells, whereas both Rr cells and regressing patient tumors display large-scale and recurrent expression alterations in genes associated with processes such as mesenchymal transition and wound-healing. Further analyses revealed an epigenome-basis of the mesenchymal-invasive-angiogenic switch in residual patient melanoma and Rr cell lines. Methylation of a CpG site in PD-L2 (encoded by PDCD1LG2) was increasingly lost concomitant with its dramatic overexpression during MAPKi treatment. PD-L2 knockdown in Rr cell lines induced apoptosis in vitro, which was rescued by co-culture with PD-1 expressing cells. MAPKi treatment also induced recurrent transcriptomic alterations associated with remodeling of the stromal/immune compartment and upregulation of PDCD1LG2 in CD11c+ immune cells. Combined BRAFi and anti-PD-L2 antibody treatment inhibited resistance development and prolonged intratumoral CD8+ T-cell inflammation in vivo. Moreover, Pdcd1lg2 overexpression in murine melanoma cells promoted BRAFi resistance in immunodeficient mice. These results catalogue clinically recurrent alterations that may drive adaptive resistance to MAPKi and may represent potential therapeutic targets.

See article, p. 1248.
In cancer metabolism lysosomes execute both catabolism, degrading organelles via autophagy and micropinocytosis, and mTORC1-dependent anabolism to promote tumorigenesis. Existing antimalarial compounds [including chloroquine (CQ) and quinacrine (QN)] inhibit the catabolic lysosomal functions, but do not suppress mTORC1 signaling. Therefore, Rebecca, Nicastri, McLaughlin, and colleagues sought to develop lysosomal inhibitors that could concurrently inhibit lysosomal catabolism and mTORC1 signaling as anticancer compounds. A dimeric CQ, Lys05, had previously been shown to be a more potent inhibitor than monomeric derivatives; thus, a series of dimeric CQ compounds were developed and tested in proliferation assays. Dimeric QN (DQ) had the most potent antiproliferative effects. A library of methylated and unmethylated DQs with a range of linker lengths was synthesized and further evaluated. The compounds with longer linkers were more potent, and central nitrogen methylation promoted lysosomal localization; DQ661 emerged as the top lysosomal inhibitor. DQ661 bound to the lysosomal enzyme palmitoyl-protein thioesterase 1 (PPT1), which cleaves thioester bonds from palmitoylated proteins to free them from membranes and promote proteolysis. PPT1 inhibition phenocopied DQ661 treatment, promoting palmitoylated protein accumulation to suppress mTORC1 signaling and lysosomal catabolism. Further, DQ661 suppressed the growth of melanoma xenografts, had antitumor activity in mouse models of colon and pancreatic cancers, and could be combined with chemotherapy. This study identifies dimer CQ inhibitors, including DQ661, that block both mTORC1 signaling and lysosomal catabolism to suppress tumor growth.

See article, p. 1266.

VHL Inactivation Promotes Enhancer Malfunction in Renal Cell Carcinoma

The VHL tumor suppressor gene is commonly inactivated in patients with clear cell renal cell carcinoma (ccRCC), but the effects of VHL loss on the tumor epigenome are not well defined. Yao, Tan, Lim, and colleagues characterized the cis-regulatory landscape of 10 matched primary tumors and normal samples, 7 ccRCC cell lines, and 2 normal kidney cell lines. H3K27 acetylation identified promoters and enhancers that distinguished tumor samples from normal samples. Enhancers and superenhancers gained in ccRCC were enriched for disease-specific features including HIFα signaling, proangiogenic pathways, and SLC-mediated transmembrane transport. ZNF395 was identified as a putative target of one of the ccRCC-gained superenhancers and was overexpressed in ccRCC but not other tumor types. Depletion of ZNF395 suppressed colony formation in ccRCC cell lines, but had little effect on normal kidney cells. In vivo, ZNF395 depletion suppressed tumorigenesis in xenografts. VHL deficiency led to enhancer remodeling, and in cell lines with VHL mutations, VHL restoration depleted H3K27ac at enhancers and superenhancers selectively activated in VHL-mutant ccRCC, resulting in downregulation of target genes. These VHL-responsive enhancers gained in ccRCC, including the ZNF395 superenhancer, had increased occupancy of the HIF2α–HIF1β heterodimer, which promoted recruitment of the histone acetyltransferase p300 to facilitate chromatin remodeling and modulate gene expression. The finding that VHL deficiency can alter the epigenomic landscape to facilitate oncogenic gene expression in ccRCC suggests potential therapeutic targets such as ZNF395.
Inducible MyD88/CD40 Signaling Activation Enhances CAR T-cell Efficacy

- MyD88 and CD40 signaling promote CAR T-cell IL2 production, proliferation, and tumor cell killing.
- Activation of MyD88 and CD40 in CAR T cells extends survival in solid tumor xenograft models.
- An inducible costimulatory molecule may improve the efficacy of adoptive immunotherapy in solid tumors.

T cells engineered to express chimeric antigen receptors (CAR) have demonstrated clinical activity in adoptive immunotherapy in CD19+ malignancies, but have had little success in patients with solid tumors. To potentially enhance their antitumor activity, Mata and colleagues generated CAR T cells engineered with an inducible co-stimulatory (iCO) molecule comprising a chemical inducer of dimerization (CID)–binding domain and the signaling domains of MyD88 and CD40, costimulatory molecules that improve T-cell activation (iMyD88.CD40). In the presence of CID, the CID-binding domain promotes inducible activation of MyD88 and CD40 signaling that results in increased production of cytokines, including IL2, following stimulation. Based on these findings, T cells expressing iMyD88.CD40 and a HER2 CAR were generated (HER2ζ.iCO). These HER2ζ.iCO cells exhibited superior effector function compared with control HER2.CD28ζ cells, with increased proliferation, cytokine production, and tumor cell killing following stimulation. The tumor cell killing was dependent on the CID and a functional CAR. In vivo, HER2ζ.iCO CAR T cells displayed superior antitumor activity in metastatic osteosarcoma and non–small cell lung cancer xenograft models. Multiple doses of CID increased the antitumor activity, extending survival in non–small cell lung cancer xenografts. Taken together, these findings suggest that inducible activation of MyD88 and CD40 signaling may enhance the efficacy of CAR T-cell therapy, and this approach may warrant further investigation for the potential treatment of patients with solid tumors.

See article, p. 1306.

Notch-Dependent Paracrine Signaling Drives Breast Cancer

- Activated Notch promotes a protumorigenic tumor microenvironment in basal-like breast cancer.
- Activated Notch recruits TAMs via IL1β and CCL2 and sensitizes BLBC to TAM-derived TGFβ.
- Targeted inhibition of IL1β and CCL2 may be a potential therapeutic strategy for patients with BLBC.

Basal-like breast cancer (BLBC), which is characterized by activated Notch signaling, is an aggressive breast cancer subtype for which there currently is no effective targeted therapy. Notch mediates intercellular signaling to regulate numerous cellular processes such as differentiation and proliferation, but its role in tumor–stroma interactions is less clear. To evaluate the role of activated Notch signaling in the BLBC microenvironment, Shen and colleagues depleted BLBC cell lines of Notch ligand or receptors and identified IL1β and CCL2 as potential NOTCH target genes. Activated, cleaved Notch receptor bound to the IL1β promoter and upregulated IL1β expression, while the inflammasome drove IL1β production in a Notch-independent manner. In vivo, Notch promoted the recruitment of monocytes via IL1β and CCL2. Further, Notch regulated the expression of TGFβR1 to enhance tumor-associated macrophage (TAM)–derived TGFβ signaling, which promoted tumor Notch signaling and the production of IL1β and CCL2, closing a protumorigenic feedback loop between BLBC cells and TAMs. In vivo ablation of Notch ligand or receptor reduced tumor growth, the expression of IL1β and CCL2, and TAM recruitment in syngeneic and autochthonous mouse models of breast cancer. Increased expression of inflammasome genes was associated with Notch-activated human BLBC cell lines and primary tumors, and bioinformatic analysis identified a correlation among Notch signaling, IL1β and CCL2 expression, macrophage infiltration, and the BLBC subtype. These results elucidate the role of Notch in mediating tumor–stroma interactions to promote an aggressive breast cancer subtype and support the development of potential therapeutic strategies targeting the innate immune response.

See article, p. 1320.
TOX Suppresses NHEJ to Promote Genomic Instability and T-ALL

The KU70/KU80 dimer gets recruited to sites of DNA damage to initiate DNA double-strand break (DSB) repair by nonhomologous end joining (NHEJ). Thus, mice lacking KU70 or KU80 develop increased genomic instability and T-cell malignancies. However, the mechanisms by which genomic instability is increased and NHEJ is altered have not been established in patients with T-cell acute lymphoblastic leukemia (T-ALL). Lobbardi and colleagues screened for oncogenic drivers required for leukemic initiation in a zebrafish model of MYC-driven T-ALL. Thymocyte selection-associated high mobility box protein (TOX) cooperated with MYC to accelerate malignant transformation, although TOX alone was insufficient to transform thymocytes. TOX was highly expressed in the majority of primary human T-ALLs, and its expression promoted growth of T-ALL cells. Further, depleting TOX suppressed T-ALL xenograft growth in mice. TOX expression also induced genomic instability in zebrafish and in mouse embryonic fibroblasts. Mechanistically, the HMG box domain of TOX bound directly to KU70/KU80, sequestering it away from sites of active DNA repair to suppress NHEJ at DSBs and promote genomic instability. Accordingly, depletion of TOX resulted in elevated NHEJ repair in T-ALL cells. Taken together, these findings identify TOX as an oncogenic driver in T-ALL and elucidate a mechanism by which it suppresses NHEJ to promote genomic instability.

See article, p. 1336.

In This Issue is written by Cancer Discovery editorial staff. Readers are encouraged to consult the original articles for full details.