There is preclinical evidence that fibroblast growth factor receptor 4 (FGFR4), the receptor for fibroblast growth factor 19 (FGF19), may drive oncogenesis in hepatocellular carcinoma (HCC); however, a causal link in humans has not been established. Building on a clinical trial of the small-molecule FGFR4 inhibitor fisogatinib in HCC, Hatlen, Schmidt-Kittler, and colleagues investigated mechanisms of resistance to the drug. In two patients from the trial who initially responded to fisogatinib treatment before developing resistance, only wild-type FGFR4 was present prior to treatment with fisogatinib. However, at resistance, one patient had a mutation affecting the FGFR4 gatekeeper residue and the other had mutations affecting both the gatekeeper residue and the hinge-1 residue. An in vitro resistance screen corroborated the notion that these mutations were responsible for the observed resistance, and ultraperformance liquid chromatography–tandem mass spectrometry confirmed that the mutations prevented covalent binding of the drug to FGFR4. Mouse xenograft experiments provided additional evidence that mutations that enable tumor escape from fisogatinib occurred in the gatekeeper and hinge-1 residues of FGFR4. Notably, a crystal structure of an additional FGFR4 inhibitor bound to the FGFR4 kinase domain hinted that this second inhibitor did not bind the gatekeeper pocket and thus may not be affected by the same resistance mutations, an idea that was confirmed using a mouse HCC model. Together, these findings provide evidence that FGFR4 is a bona fide HCC driver and suggest that second-generation FGFR4 inhibitors that do not bind the gatekeeper pocket may be worth developing further.

See article, p. 1686.

Ferroptosis Mediates Synergy between Radiotherapy and Immunotherapy

- Ferroptosis was triggered by and required for the efficacy of radiotherapy in mouse cancer models.
- Adding ferroptosis inducers to radiotherapy caused greater tumor control than radiotherapy alone.
- An association between ferroptosis and radiotherapy as well as immunotherapy was observed.

Immune-checkpoint blockade with anti–PD-L1 anti–CTLA4 may be used in conjunction with traditional cancer therapies, such as radiotherapy, but the optimal way to combine these treatments has not been established. Lang, Green, and colleagues investigated the potential relationship between ferroptosis (an iron-dependent form of programmed cell death) and radiotherapy response. Unexpectedly, they found evidence that radiotherapy triggered tumor ferroptosis in mouse models of multiple cancers; in fact, experiments using tumors derived from ferroptosis-resistant subclones revealed that ferroptosis was required for the efficacy of radiotherapy in this context. Combined treatment with ferroptosis inducers controlled tumor growth to a greater extent than either treatment alone, implying that modulating ferroptosis may be of interest in improving the efficacy of radiotherapy. Radiotherapy and IFNγ treatment appeared to act synergistically to cause downregulation of SLC7A11, a vital ferroptosis regulator, at both the RNA and protein levels in tumor cells; this effect was dependent on expression of the genes ATM and STAT1. Notably, a link between ferroptosis and the efficacy of immunotherapy and radiotherapy (which are often combined) was also noted, with an association appearing between the therapies and ferroptosis along with T cell–mediated immunity. Together, these results support a role for ferroptosis in the observed synergy between immunotherapy and radiotherapy and suggest that it may be of interest to develop drugs targeting this pathway.

See article, p. 1673.

Aberrant FGFR4 Activation Drives Hepatocellular Carcinoma

- Some patients with hepatocellular carcinoma developed resistance to the FGFR4 inhibitor fisogatinib.
- Resistance seemed to be caused by mutations in FGFR4’s gatekeeper or hinge-1 residues.
- A second-generation FGFR4 inhibitor appears unaffected by the resistance mutations that thwart fisogatinib.

There are preclinical data that indicate that fibroblast growth factor receptor 4 (FGFR4), the receptor for fibroblast growth factor 19 (FGF19), may drive oncogenesis in hepatocellular carcinoma (HCC); however, a causal link in humans has not been fully established. Building on a clinical trial of the small-molecule FGFR4 inhibitor fisogatinib in HCC, Hatlen, Schmidt-Kittler, and colleagues investigated mechanisms of resistance to the drug. In two patients from the trial who initially responded to fisogatinib treatment before developing resistance, only wild-type FGFR4 was present prior to treatment with fisogatinib. However, at resistance, one patient had a mutation affecting the FGFR4 gatekeeper residue and the other had mutations affecting both the gatekeeper residue and the hinge-1 residue. An in vitro resistance screen corroborated the notion that these mutations were responsible for the observed resistance, and ultraperformance liquid chromatography–tandem mass spectrometry confirmed that the mutations prevented covalent binding of the drug to FGFR4. Mouse xenograft experiments provided additional evidence that mutations that enable tumor escape from fisogatinib occurred in the gatekeeper and hinge-1 residues of FGFR4. Notably, a crystal structure of an additional FGFR4 inhibitor bound to the FGFR4 kinase domain hinted that this second inhibitor did not bind the gatekeeper pocket and thus may not be affected by the same resistance mutations, an idea that was confirmed using a mouse HCC model. Together, these findings provide evidence that FGFR4 is a bona fide HCC driver and suggest that second-generation FGFR4 inhibitors that do not bind the gatekeeper pocket may be worth developing further.

See article, p. 1686.
Mutations affecting fibroblast growth factor 19 (FGF19) are potential drivers of hepatocellular carcinoma (HCC), prompting development of therapies targeting fibroblast growth factor receptor 4 (FGFR4). In a first-in-human, phase I clinical trial, Kim and colleagues evaluated the small-molecule FGFR4 inhibitor fisogatinib in patients with HCC. One hundred fifteen patients were enrolled in the study. Sixty-three percent of enrolled patients were positive for FGF19, as measured by immunohistochemistry, and FGFR4 pathway integrity was confirmed using RNA sequencing for FGFR4 and KLB expression. The most common reasons for discontinuing treatment were progressive disease (75%) and adverse events (13%). No deaths deemed to be related to treatment occurred. In the 106 patients dosed once daily, 98 were evaluable for response, including 66 patients with FGF19-positive tumors. Among patients with FGF19-positive tumors, 17% experienced an overall response, including 1 (2%) complete response and 10 (15%) partial responses. For responding patients in this group, the median duration of response was 5.3 months, and the median progression-free survival was 3.3 months. Of the 29 FGF19-negative and five FGF19-unknown patients, 0% experienced an overall response, 50% had stable disease, and 50% had progressive disease; the median progression-free survival in FGF19-negative patients was 2.3 months. Because most enrolled patients had metastatic disease with poor prognostic characteristics, the favorable results seen in a subset of patients demonstrates that it may be feasible to target the FGF19–FGFR4 pathway in HCC using small molecules, and the authors have initiated further study of fisogatinib alone and in combination with immunotherapy in FGF19-positive patients.

See article, p. 1696.

Glioma stem cells from patient glioblastoma samples fell on a spectrum from proneural (pGSC) to mesenchymal (mGSC). In silico lineage tracing indicated that mGSCs are the progenitors of pGSCs. A drug screen revealed that only drugs that target both pGSC- and mGSC-specific targets acted synergistically. The intratumor heterogeneity observed with glioblastomas is thought to be at least in part responsible for the relative lack of utility of targeted therapies in treating these cancers. Using single-cell mRNA sequencing and DNA profiling of several untreated human glioma specimens, Wang, Babikir, Müller, and colleagues identified more than 20,000 neoplastic cells from the tumor milieu (which also contained stromal and immune cells) on which to conduct further experiments. The glioma stem cells (GSC) could be defined using a single axis of variation illustrating a range from proneural (pGSC) to mesenchymal (mGSC). In silico lineage tracing analysis supported the idea of a cellular hierarchy in which mGSCs are the progenitors of pGSCs, and the authors suggest that the proportions of mGSCs, pGSCs, and their progeny along with stromal and immune cells are sufficient to account for the observed intratumor heterogeneity in glioblastoma. Notably, the authors also screened FDA-approved drugs that target genes they found to be specific to pGSCs or mGSCs in their single-cell studies for synergistic interactions and determined that only drugs that act on both pGSC- and mGSC-specific targets exhibited synergy. This study lays the groundwork for future research on intratumor heterogeneity in glioblastoma and its impact on treatment.

See article, p. 1708.
Astrocyte-Induced PPARγ Signaling Promotes Brain Metastasis

- In mouse models, increased PPARγ signaling was associated with greater likelihood of brain metastasis.
- Release of polyunsaturated fatty acids by astrocytes enhanced brain metastasis by triggering PPARγ signaling.
- A PPARγ inhibitor restricted growth of brain metastases but not other tumors in mice.

Although the role of peroxisome proliferator-activated receptor γ (PPARγ) signaling in cancer is controversial, some studies indicate that activation of the pathway inhibits cancer development. In mouse models of brain metastases from breast cancer and melanoma, Zou and colleagues discovered a role for PPARγ in promoting brain metastasis. Experiments using mouse models of melanoma that had metastasized to the brain as well as in vitro studies revealed that astrocytes promoted growth and survival of metastatic cancer cells in the brain. PPARγ signaling mediated brain metastasis, with increased PPARγ activity being associated with a greater likelihood of metastasis to the brain and PPARγ depletion reducing brain metastatic burden. Activation of PPARγ did not increase survival of brain-metastatic cells, but it increased their proliferation, an effect that was enhanced in the presence of astrocytes. This appeared to be due to the astrocytes’ release of specific polyunsaturated fatty acids, including arachidonic acid and mead acid, which are taken up by the metastatic cells and activate PPARγ signaling. Interestingly, in samples from human patients with melanoma, expression and nuclear localization of PPARγ was higher in brain metastases than in primary tumors or metastases in the lymph nodes or gastrointestinal tract. Returning to the mouse models, administration of a PPARγ antagonist restricted the growth of brain metastases but not subcutaneously implanted tumors or lung metastases. Together, these results provide insight into the complex role of PPARγ in cancer and suggest that investigating PPARγ antagonists for the control of brain metastases may be worthwhile.

See article, p. 1720.

Susceptibility to Some Pediatric Leukemias May Depend on Ontogeny

- Acute megakaryoblastic leukemia (AMKL) tends to occur at a younger age than other acute myeloid leukemias.
- Susceptibility to pediatric AMKL caused by oncogenic gene fusions is ontogeny dependent.
- A novel mouse model recapitulates human ETO2-GLIS2-positive AMKL and may be used in further experiments.

Age of onset, driving oncogene, and disease phenotype appear to be correlated in pediatric cancers, but the precise details remain murky. Lopez and colleagues found that acute megakaryoblastic leukemia (AMKL), a type of acute myeloid leukemia (AML), is typically diagnosed at a younger age than other types of AML regardless of the driver oncogene, and experiments using patient-derived xenografts of AMKL positive for the ETO2-GLIS2 gene fusion (EG+) revealed that such fusions may result in transformation of hematopoietic stem cells (HSC) or multipotent progenitors. An inducible transgenic mouse model of EG+ AMKL that accurately recapitulates many aspects of AMKL in humans, which was developed for this study, was used for further experiments. Interestingly, the developmental stage of cells expressing ETO2-GLIS2 appeared to be responsible for the predominance of AMKL relative to AML. Further, expression of ETO2-GLIS2 in fetal long-term HSCs resulted in an exclusively megakaryoblastic phenotype by altering transcriptional programs in long-term HSCs in an ontogeny-dependent fashion. Additional experiments provided evidence that the higher incidence of EG+ AMKL in young children compared to members of other age groups may result at least in part from differences in the activities of the transcription factors GATA1 and CEBPA that occur during normal ontogeny, as well as deregulation of genes associated with self-renewal in fetal HSC caused by ETO2-GLIS2 expression. Collectively, these findings suggest that pediatric AMKL may originate in fetal HSCs and that cellular changes that occur during hematopoietic ontogeny may explain some age- and phenotype-related observations in AMKL.

See article, p. 1736.
Experiments using gene-targeted mice have provided evidence that CD39 may be a checkpoint mediator worth targeting in various cancers, but stronger evidence is needed. Li, Moesta, and colleagues employed novel antibodies that block mouse and human CD39, allowing them to probe the effects of targeting the checkpoint mediator. In a mouse model of colon adenocarcinoma, one antibody against mouse CD39 exhibited single-agent efficacy in slowing tumor growth that was as potent as anti–PD-1. In tests of mouse models bearing tumors sensitive to anti–PD-1, the combination of anti–PD-1 and anti-CD39 was more effective than either agent alone at delaying tumor growth. Mechanistically, the antitumor efficacy of anti-CD39 appeared to be dependent on host lymphocytes and host CD39. Blocking CD39 caused substantial changes in the tumor microenvironment, with an induction of type-I interferon response and downregulation of genes related to immune suppression as well as a reduction in intratumor macrophages and an increase in intratumor T-cell activation. This and the results of several other experiments support a model in which anti-CD39 leads to release of extracellular ATP (eATP), which triggers pyroptosis (an inflammatory form of programmed cell death) and corresponding inflammasome activation. Further experiments implied that this liberation of eATP by anti-CD39 may result from binding between anti-CD39 and CD39 on the surfaces of P2X7-expressing intratumor macrocytes, explaining the activation of the NALP3 inflammasome. Collectively, this study deepens our understanding of the potential utility of anti-CD39 in cancer treatment; notably, first-in-human trials are under way.

See article, p. 1754.

Inflammasome Activation Underlies Antitumor Effects of CD39 Blockade

- Antibodies against the checkpoint mediator CD39 slowed tumor growth in mouse models of colon adenocarcinoma.
- CD39 blockade altered the tumor microenvironment and induced a type-I interferon response.
- This study provides mechanistic understanding of the effects of anti-CD39; first-in-human trials are under way.

Aberrant Nucleosome Eviction at a MYC Enhancer May Drive T-ALL

- Chromatin accessibility at the MYC enhancer N-Me was aberrantly increased in mouse and human T-ALL.
- This chromatin state was caused by aberrant nucleosome eviction by the transcription factor GATA3.
- GATA3 was required for initiation and maintenance of T-ALL in NOTCH1-driven T-ALL in mice.

More than 60% of cases of T-cell acute lymphoblastic leukemia (T-ALL) are characterized by constitutive activation of NOTCH1, which promotes transformation of T cells into leukemic cells in T-ALL via activation of genes driving cell growth and proliferation. Notably, NOTCH1 activity increases the transcription of the proto-oncogene MYC through N-Me, a T cell–specific, long-range distal MYC enhancer that appears to be active only during early T-cell development and in T-ALL. Belver and colleagues found evidence for aberrant increases in chromatin accessibility at N-Me in T-ALL cells in mice and humans. Circularized chromatin conformation capture experiments on human and mouse T-ALL lymphoblasts revealed interactions between N-Me and the MYC proximal promoter, as anticipated, but also unexpectedly provided evidence for chromatin loops connecting N-Me and the MYC promoter, with distal elements on both sides of the MYC transcription start site. This suggests that the transcriptional regulation involves a mechanism more complex than a simple promoter–enhancer interaction. Notably, it appeared that nucleosome eviction by the transcription factor GATA3 contributed to N-Me activity, and GATA3 was required for initiation of maintenance of NOTCH1-driven T-ALL in a mouse model. These findings support a model in which abnormally increased chromatin accessibility at the MYC enhancer N-Me as a result of GATA3-mediated nucleosome eviction leads to transcriptional activation of MYC, which may promote NOTCH1-driven T-ALL.

See article, p. 1774.

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