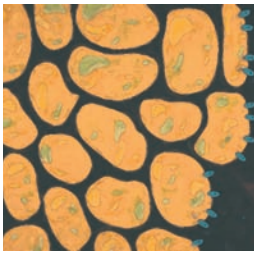
Lipid Uptake via FATP2 Influences Age Dependency of Melanoma Response

- Aged fibroblasts had an altered lipid secretome, and FATP2 mediated uptake of these lipids by melanoma cells.
- Treatment with a FATP2 inhibitor restored sensitivity to BRAF and MEK inhibition in resistant melanoma cells.
- FATP2-inhibitor treatment synergized with BRAF and MEK inhibition in aged but not young mice with melanomas.



Compared with younger patients with melanoma, patients age 50 and older have worse prognoses and poorer responses to BRAF- and MEK-targeted therapies, even when controlling for tumor grade and stage at diagnosis. Alicea and colleagues found that the lipid secretome of dermal fibroblasts from older donors differed substantially from that of dermal fibroblasts from younger donors, containing significantly more ceramides, and melanoma cells grown in conditioned medium from aged dermal fibroblasts took up these lipids, increasing their total lipid concentration and the concentrations of specific ceramides secreted by the aged fibroblasts. Levels of the fatty acid transporter FATP2 were elevated in melanoma cells grown in conditioned medium from aged but not young dermal fibroblasts, in melanoma cells grown in aged but not young skin reconstructs, and in melanoma tumors grown intradermally in aged but not young mice. *In vitro*, treatment with a selective FATP2 inhibitor rendered melanoma cells resistant to BRAF and MEK inhibitors sensitive to combination treatment. Intradermally grown melanomas in young and aged mice initially responded to combined BRAF and MEK inhibition but relapsed after 55 days and 10 to 15 days, respectively; the addition of a FATP2 inhibitor aided survival in aged mice but had no effect on young mice. Collectively, this work demonstrates that alterations to the lipid secretome of aged dermal fibroblasts contribute to a microenvironment that is protumorigenic due to FATP2-mediated uptake of fatty acids by melanoma cells and suggests that FATP2 inhibition may be a strategy worth investigating in older patients with melanoma. ■

See article, p. 1282.

Fbxw7 Deficiency Decreases dsRNA Sensing to Cause Anti–PD-1 Resistance

- *Fbxw7* deficiency or loss of function in melanomas conferred anti–PD-1 resistance in immunocompetent mice.
- Loss of *Fbxw7* lowered expression of dsRNA sensing–related genes and altered the tumor immune microenvironment.
- Reactivating viral sensing in *FBXW7*-mutant melanomas, which occur in humans, may be of therapeutic interest.

Immunotherapy with PD-1 blockade has become a standard-of-care treatment for melanoma, but some tumors do not respond, in some cases exhibiting a heterogeneous pattern of response within the same patient. In a 74-year-old patient with diffusely metastatic melanoma in whom all tumors except a single right adrenal mass completely responded to PD-1 blockade with pembrolizumab within 11 months, Gstalder and colleagues found that the nonresponsive tumor had a loss-of-function mutation in the tumor-suppressor gene *FBXW7*. In immunocompetent mice, melanomas that were deficient or had loss-of-function mutations in *Fbxw7* were resistant to anti–PD-1 treatment. Mechanistically, *Fbxw7* deficiency altered the tumor immune microenvironment, decreasing the transcription of IFNγ-related genes and genes involving viral sensing as well as reducing the $CD8^+$ T-cell infiltration normally elicited by PD-1 blockade; correspondingly, *FBXW7* expression was correlated with $CD8^+$ T-cell infiltration in many human tumor types. Detection of double-stranded RNA (dsRNA) was impaired in *Fbxw7*-deficient tumors due to decreased expression of the dsRNA sensors MDA5 and RIG1, and restoration of dsRNA sensing enhanced IFN signaling and increased tumor response to PD-1 blockade. In summary, this work identifies a previously unknown role for the tumor-suppressor gene *FBXW7* in resistance to PD-1 blockade in melanoma and establishes a mechanism through which resistance can occur. Further, this study suggests that investigating methods to reactivate of dsRNA-sensing pathways in *FBXW7*-mutant melanomas may be of therapeutic interest. ■

See article, p. 1296.

EZH2 Is a Vulnerability in Resistant Triple-Negative Breast Cancer

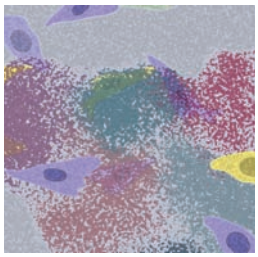
- Methionine metabolism and the epigenome were altered in taxane-resistant triple-negative breast cancer cells.
- Epigenomic changes repressed transposable elements, preventing an antiviral response to double-stranded RNA.
- EZH2 inhibition blocked repression, making EZH2 a therapeutic vulnerability in cells, organoids, and xenografts.

Cytotoxic chemotherapeutic agents such as taxanes are the only FDA-approved treatments for triple-negative breast cancer (TNBC), and resistance to these agents poses a major barrier to treatment success. Deblois and colleagues generated taxane-resistant TNBC cell lines and performed metabolomic analyses and isotope-labeling experiments, which revealed a marked decrease in the levels of most methionine-cycle metabolites and a decrease in the rate of *S*-adenosylmethionine (SAM) synthesis in resistant cells. Taxane-resistant cells also exhibited global alterations in DNA methylation, with an overall decrease in methylation that particularly affected transposable elements called long interspersed nuclear elements (LINE) such as LINE1. Although this hypomethylation would be expected to increase LINE1 retrotransposition activity, it was coupled with histone 3 lysine residue 27 trimethylation (H3K27me3), which should repress LINE1 activity. H3K27me3 clustered into large domains reminiscent of large organized chromatin lysine (LOCK) domains previously described for H3K27me2. These LOCKs preferentially included transposable elements such as LINE1, maintaining their repression and preventing activation of the viral-mimicry response, a process by which cells recognize double-stranded RNA produced during retrotransposition as evidence of viral infection and mount an IFN response. This implied that taxane-resistant TNBC cells may be sensitive to inhibition of EZH2, the PRC2 component responsible for trimethylating H3K27; indeed, susceptibility to pharmacologic EZH2 inhibition was observed in taxane-resistant TNBC cells, organoids grown from patient-derived xenografts (PDX), and PDXs grown *in vivo*. Collectively, these findings elucidate a mechanism linking altered methionine metabolism to epigenetic regulation of transposable elements in taxane-resistant TNBC, uncovering a possible therapeutic vulnerability. ■

See article, p. 1312.

Specific Cancer-Associated Fibroblasts Modulate Immunotherapy Response

- A previously identified kind of cancer-associated fibroblast (CAF) was divisible into up to eight subtypes.
- Two CAF subtypes—myofibroblasts expressing extracellular matrix or TGFβ pathway genes—were immunosuppressive.
- The presence of these and one other CAF subtype predicted poor response to anti–PD-1 in metastatic melanoma.



Certain cancer-associated fibroblasts (CAF) in the tumor microenvironment (TME) of breast cancers are known to mediate immunosuppression, but whether this group of CAFs is heterogeneous and whether they influence response to immunotherapy remains unknown. Kieffer, Hocine, and colleagues performed a single-cell analysis of more than 19,000 breast cancer–derived CAFs of this subtype, called FAP^+ CAF or CAF-S1, revealing that these CAFs are heterogeneous and can be divided further into eight transcriptomically defined subtypes. Deeper analysis of the five most abundant subtypes using multicolor flow cytometry confirmed the presence of these five distinct subsets of cells, which were also shown to be present in head and neck squamous cell carcinoma and non–small cell lung cancer by analyzing publicly available single-cell RNA-sequencing data from these two cancers. In breast cancer tumors, inflammatory CAFs expressing high levels of genes involved in detoxification pathways (detoxiCAF) or interleukin signaling (IL-iCAF) were associated with an immunocompetent TME, whereas CAFs defined by being myofibroblasts expressing high levels of extracellular matrix genes (ECM-myCAF) or TGFβ pathway genes (TGFβ-myCAF) were associated with an immunosuppressive microenvironment having low $CD8^+$ T-cell and high $CD4^+$ T-cell infiltration along with high PD-1 and CTLA4 expression. In patients with metastatic melanoma, expression of genes representative of the latter two CAF subtypes and one other CAF-S1 subtype (myCAFs expressing wound-healing pathway genes) were associated with poor response to anti–PD-1. In summary, this work identifies previously unknown CAF subtypes and defines their roles in shaping the TME to modulate immunotherapy response. ■

See article, p. 1330.

The Serine-Synthesis Enzyme PHGDH Mediates Metastasis to the Brain

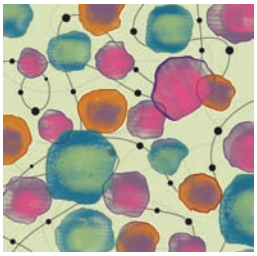
- Brain-metastatic cancer cells had increased expression of PHGDH, an enzyme in the serine-synthesis pathway.
- PHGDH expression was required for proliferation in low-serine conditions *in vitro* and brain metastasis *in vivo*.
- Pharmacologic PHGDH inhibition suppressed brain metastasis, so PHGDH inhibitors may be worth investigating.

The environment in the brain is restrictive to growth due in part to a lack of amino acids, raising the question of how cancer cells overcome this barrier to brain metastasis. Ngo, Kim, Osorio-Vasquez, and colleagues found that 3-phosphoglycerate dehydrogenase (PHGDH)—the rate-limiting first enzyme in the canonical pathway by which serine is synthesized from glucose—was the most upregulated protein in highly metastatic triple-negative breast cancer (TNBC) cells compared with parental or indolent cells. Radiolabeling experiments showed that a HER2$^+$ breast cancer cell line implanted in the brain parenchyma had higher serine synthesis and PHGDH expression than the same line implanted in the mammary fat pad, and complementary results were obtained in melanoma. *PHGDH* knockdown or expression of catalytically inactive PHGDH inhibited growth of highly brain-metastatic TNBC cells in medium with low levels of serine and glycine (which can be synthesized from serine) similar to the concentrations of these amino acids found in the cerebrospinal fluid, and these results were confirmed *in vivo*, where PHGDH expression was not only necessary but also sufficient for brain metastasis. Pharmacologic PHGDH inhibition suppressed proliferation of brain-metastatic cells in serine- and glycine-poor conditions *in vitro* and suppressed brain metastasis *in vivo*. Mechanistically, PHGDH depleted nucleotide pools, causing a DNA-damage response. Together, these findings identify PHGDH as a key regulator of brain metastasis and suggest that PHGDH inhibition, which has shown limited efficacy in tumors outside the brain, may be of interest in the brain-metastatic cancer setting. ■

See article, p. 1352.

CHD1 and IL6 Mediate Immunosuppression in *Pten*-Null Prostate Cancer

- CHD1 increased IL6 expression in *Pten*-null prostate cancer, altering the tumor immune microenvironment.
- IL6 recruited immunosuppressive myeloid-derived suppressor cells, which hinder immunotherapy response.
- CHD1 depletion or IL6 inhibition enhanced response to immune-checkpoint blockade in mouse prostate cancer models.

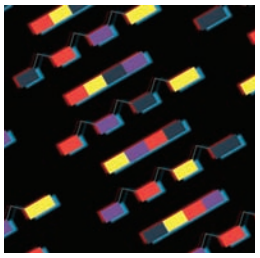


Loss of the tumor-suppressor gene *CHD1*, encoding a chromatin remodeler, occurs in approximately 7% of prostate cancers; however, it rarely coincides with *PTEN* mutations, and its downstream targets are unknown. Zhao, Cai, Lu, and colleagues found that prostate-specific deletion of *Chd1* had no effect on the prostate in a mouse model of prostate cancer, whereas in *Pten*-null prostate cancer *Chd1* deletion resulted in less-aggressive tumor growth and increased overall survival. Compared with prostate tumors in which *Pten* alone was deleted, prostate tumors with codeletion of *Pten* and *Chd1* had decreased levels of immunosuppressive myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages and increased levels of T, B, and natural killer cells. *In vitro* assays suggested that CHD1 may regulate MDSC recruitment, possibly through a cytokine-dependent mechanism, and further analysis showed that this effect was mediated by IL6. Mechanistically, CHD1 directly bound the *Il6* promoter at a conserved CHD1-binding motif, making the *Il6* promoter more accessible for transcription and increasing its expression, consistent with CHD1's known role in establishing open chromatin, leading to MDSC recruitment. CHD1's role in increasing IL6 expression and consequent MDSC recruitment suggested that inhibiting the CHD1–IL6 pathway could enhance the poor response of prostate cancer to immune-checkpoint blockade (ICB). Indeed, CHD1 depletion or treatment with an IL6 inhibitor improved the efficacy of ICB in *Pten*-null murine prostate cancer. Collectively, these results elucidate the mechanism behind CHD1's role in prostate cancer and suggest that inhibition of the CHD1–IL6 pathway could enhance ICB's efficacy in this disease. ■

See article, p. 1374.

SRSF6 Mediates Exon Skipping in T-cell Acute Lymphoblastic Leukemia

- Exon skipping was elevated in T-cell acute lymphoblastic leukemia (T-ALL) due to increased levels of SRSF6.
- T-ALL was dependent on the splicing factor SRSF6, which altered splicing of proteasome-related transcripts.
- Combination treatment with proteasome and splicing inhibitors acted synergistically against T-ALL cells.

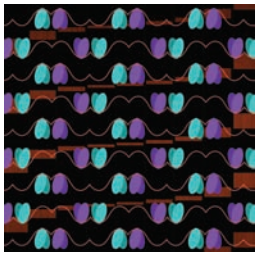


Mutations in or amplification or dysregulation of genes encoding splicing factors, leading to aberrant splicing and the presence of atypical splice variants, are common in many cancers; however, this phenomenon has not been well characterized in T-cell acute lymphoblastic leukemia (T-ALL). Zhou and colleagues found that exon skipping was substantially elevated in T-ALL cells compared with normal T cells, and the most common sequence preceding skipped exons was the serine/arginine-rich splicing factor 6 (SRSF6)–binding motif; correspondingly, a CRISPR–Cas9 screen showed that T-ALL cells exhibited strong dependency on *SRSF6*. Levels of SRSF6 protein were elevated in T-ALL patient samples, as were levels of the deubiquitinase USP7, and treatment of T cells with a selective USP7 inhibitor or silencing of *USP7* in these cells led to increased SRSF6 ubiquitination. SRSF6 was essential for T-ALL growth, with *SRSF6* silencing leading to decreased leukemic burden and increased survival in xenografted mice, and even mild (25 to 40%) knockdown of *SRSF6* hindering T-ALL cell growth *in vitro*. Treatment with a splicing inhibitor decreased growth of T-ALL cell lines and patient samples and altered splicing of proteasome-related transcripts, with the transcript encoding the proteasome assembly chaperone PSMG1 being most markedly altered, leading to decreased proteasome activity. Notably, combination treatment with a proteasome inhibitor plus a splicing inhibitor synergistically acted on T-ALL cells. Collectively, this work elucidates the role of aberrant splicing and exon skipping in T-ALL and provides a rationale for investigating the combination of proteasome inhibitors and splicing inhibitors in this disease. ■

See article, p. 1388.

The RNA-Binding Protein RBMS1 Suppresses Colorectal Cancer Metastasis

- An analytic method was developed to identify RNA-binding proteins that act as posttranscriptional regulators.
- RBMS1 bound and stabilized target mRNAs to reduce the propensity of colorectal cancer cells to metastasize.
- In patients with colorectal cancer, decreased expression of *RBMS1* correlated with progression and metastasis.



Abnormal function of posttranscriptional gene-regulatory mechanisms is common in cancer, but identifying previously unknown regulatory interactions remains a challenge due to a dearth of predictive computational methods. To address this, Yu, Navickas, Asgharian, and colleagues developed PRADA (Prioritization of Regulatory Pathways based on Analysis of RNA Dynamic Alterations), an analytic method that can pinpoint RNA-binding proteins that modulate mRNA stability and gene expression. Applying PRADA to data about the metastatic propensity of colorectal cancer cell lines revealed that RBMS1 was the RNA-binding protein with the greatest regulatory potential, and xenograft experiments along with analyses of matched patient primary and metastatic tumors showed that downregulation of RBMS1 and its mRNA regulon was positively correlated with metastasis. *RBMS1* knockdown led to destabilization of its predicted target mRNAs, consistent for a role for RBMS1 in posttranscriptional regulation. RBMS1 binding was enriched in the 3′ untranslated region of target mRNAs, where it interacted with ELAVL1, an RNA-binding protein known to regulate gene expression by stabilizing mRNAs. *RBMS1* knockdown increased the potential of colon cancer cells to metastasize to the liver, and colon cancer cells that do not normally express *RBMS1* had decreased potential to metastasize to the liver upon expression of *RBMS1*. Gene-expression data from patients with colorectal cancer showed that decreased expression of *RBMS1* (and *AKAP12*, identified as a major mRNA targeted by RBMS1) was correlated with disease progression and metastasis. In summary, this study identifies RBMS1 as a protein of interest in colorectal cancer metastasis and demonstrates the utility of PRADA. ■

See article, p. 1410.

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