In glioblastoma (GBM) and several other malignancies, cancer cell stemness has been associated with immunosuppression in the tumor microenvironment (TME), yielding poor prognosis. To identify epigenetic regulators that may be involved in self-renewing, immunosuppressive characteristics in GBM cells, Chen and colleagues performed a gain-of-function screen of genes encoding epigenetic regulators in human neural stem cells. Of the genes tested, the circadian-rhythm gene CLOCK emerged as the top hit. shRNA-mediated depletion of CLOCK or BMAL1 (encoding the CLOCK protein’s canonical partner) in glioma stem cells (GSC) reduced their capacity for cellular self-renewal. CLOCK-depleted cells also exhibited evidence of reduced migration of immune-suppressive microglia. Mechanistically, CLOCK depletion in GSCs led to a specific and substantial reduction in levels of OLFML3, which encodes a secreted extracellular matrix glycoprotein involved in intercellular interactions, and CLOCK and BMAL1 appeared to directly bind the OLFML3 promoter to regulate its transcription. Further supporting CLOCK’s role in GBM, immunocompromised mice implanted with tumors grown from CLOCK-depleted GSCs had extended survival compared with those implanted with control GSCs. Additionally, tumors in a mouse model of glioma with a GSC-like phenotype were less rapidly lethal and exhibited reduced stemness and microglia infiltration when CLOCK was depleted. Supporting the proposed mechanism, OLFML3 depletion in the GSC-derived tumor model also extended survival. Collectively, these data suggest a role for CLOCK in GBM maintenance mediated by increased stemness and recruitment of microglia to the TME and pinpoint OLFML3 as a potential target in GBM.

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The production of type I interferons (IFN) activates the JAK–STAT pathway, increasing transcription of hundreds of interferon-stimulated genes (ISG) involved in immunity. Type I IFNs promote immune responses to several malignancies, including breast cancer, and Fan and colleagues found that expression of the ISG UBA7 was positively associated with improved survival in patients with breast cancer. UBA7 encodes a protein that performs ISGylation, which is the conjugation of another ISG’s protein product, ISG15, to cellular proteins to help perpetuate the IFN-mediated signal. In a mouse model of breast cancer, tumors exhibited heightened Uba7 and Isg15 expression, and tumors in Uba7-knockout (KO) mice developed more rapidly and were more prone to metastasize to the lungs than tumors in mice with wild-type Uba7. Tumors from Uba7-KO mice also exhibited a reduction in infiltrating CD3⁺CD8⁺ and CD3⁺CD4⁺ T cells, perhaps explaining UBA7’s antitumor effects, and deeper investigation indicated a tumor cell–autonomous function for UBA7. UBA7-mediated protein ISGylation synergized with TLR3 and TLR4 signaling in vitro, leading to enhanced expression of ligands of the chemokine receptor CXCR3. Supporting this proposed mechanism in vivo, CXCR3 blockade reduced the antitumor effects of ISGylation in mice. Additional experiments revealed a role for ISGylation in modulating chemokine expression mediated by STAT1/2, which themselves are ISG products and subject to ISGylation. Further, ISGylation promoted condensation of STAT1 and STAT2 around type I IFN–induced PML bodies in vitro. In summary, this work provides evidence for a previously unknown mechanism that may partially underlie ISGs’ roles in cancer.

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Chronic lymphocytic leukemia (CLL) is characterized by constitutive activation of Bruton tyrosine kinase (BTK), which is crucial for proliferation and survival of CLL and some other B-cell cancers. Woyach and colleagues conducted a phase Ib/II clinical trial in patients with treatment-naïve or relapsed/refractory CLL to test treatment with obinutuzumab, a monoclonal antibody to CD20 that is currently used as a first-line therapy for CLL, plus acalabrutinib, a selective BTK inhibitor that has shown positive results as monotherapy for CLL in early trials. The trial included 19 patients with treatment-naïve CLL and 26 patients with relapsed/refractory CLL; among the latter group, the median number of prior therapies was one, and none had previously received a BTK inhibitor. The overall response rate for patients with treatment-naïve or relapsed/refractory CLL was 95% and 92%, respectively, with 32% of treatment-naïve patients and 8% of patients with relapsed/refractory disease attaining complete responses. Median progression-free survival and median overall survival had not been reached by the end of treatment follow-up at 39 months for treatment-naïve patients and 42 months for patients with relapsed/refractory CLL. The combination was generally tolerable, with quality-of-life (QOL) assessments indicating that most patients perceived improved QOL over the course of treatment; however, one patient in the treatment-naïve cohort and four patients in the relapsed/refractory cohort discontinued study treatment due to adverse events. This study demonstrates that obinutuzumab with acalabrutinib can produce durable responses in patients with CLL, indicating that further research on this combination is warranted, perhaps also in other lymphoid malignancies.

The tumor microenvironment (TME) in classic Hodgkin lymphoma is extensive, comprised primarily of benign immune cells; only approximately 1% of the cell population are malignant Hodgkin and Reed–Sternberg (HRS) cells. To characterize the TME in Hodgkin lymphoma, Aoki, Chong, and colleagues performed single-cell RNA-sequencing (scRNA-seq), multicolor immunohistochemistry (IHC), and imaging mass cytometry (IMC) analyses on Hodgkin lymphoma tumors. The scRNA-seq data revealed multiple immune-cell clusters, with a notable enrichment of LAG3+ T cells, and further experiments implied that these cells may play an immunosuppressive role. The multicolor IHC experiments showed that there was a high density of LAG3+ T cells in the areas surrounding the malignant HRS cells in MHC class II+ tumors. This spatial relationship was not affected by MHC class I status, Epstein–Barr virus status, or pathologic subtype and was confirmed by the IMC analysis. In tumor tissue from an independent cohort of 166 patients with classic Hodgkin lymphoma treated with first-line doxorubicin (aka adriamycin), bleomycin, vinblastine, and dacarbazine (commonly known as ABVD therapy), LAG3+ T cells were enriched in the areas surrounding HRS cells in MHC class II+ tumors. Further, increased numbers of LAG3+ T cells were correlated with reduced disease-specific survival. Mechanistically, in vitro experiments showed that whereas IL6 expressed in MHC class II+ cells induced LAG3+ T cells, MHC class II+ cells depleted LAG3+ T cells. In summary, this work functionally and spatially characterized the TME of Hodgkin lymphoma at the single-cell scale and identified LAG3+ T cells as a potential target in this malignancy.
In contrast to prior findings, Treg depletion sped up carcinogenesis in a mouse model of pancreatic cancer. The presence of an abundance of regulatory T cells (Tregs) in the tumor microenvironment (TME) of pancreatic ductal adenocarcinoma (PDAC) has been correlated with metastasis and poor prognosis in patients, and mouse experiments have indicated that Treg depletion may trigger a CD8+ T cell–mediated antitumor response. In samples from human PDAC tumors and pancreatic precancerous lesions [pancreatic intraepithelial neoplasia (PanIN)], Zhang and colleagues found large populations of Tregs—often located close to the tumor cells—and relatively few CD8+ T cells, whereas the Treg population in pancreatic samples from patients with the nonmalignant condition chronic pancreatitis was small. Contrasting with the aforementioned prior findings, in a mouse model of mutant Kras-driven PDAC, Treg depletion hastened the development of PanIN. Treg depletion altered the fibroblast populations in mouse pancreata, with a marked decrease in α-smooth muscle actin–expressing fibroblasts—which normally restrain tumor growth—but no change in total fibroblast numbers. Additionally, many extracellular-matrix genes (including Tgfβ1) were downregulated in the Treg-depleted pancreata. The immune microenvironment was also altered by Treg depletion, with an increase in immunosuppressive myeloid cells associated with increased levels of Cx3, Ccl6, and Ccl8, genes encoding chemokines that bind CCR1 and recruit myeloid cells. Correspondingly, pharmacologic CCR1 inhibition or CD4+ T-cell depletion prevented the PanIN progression associated with Treg depletion. Together, these findings provide new insight into the complexities of the immune TME in PDAC and further imply that targeting CCR1 may be worth investigating.

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**Treg Depletion Promotes Pancreatic Cancer via Microenvironment Modulation**

- In contrast to prior findings, Treg depletion sped up carcinogenesis in a mouse model of pancreatic cancer.
- Treg depletion reduced tumor-restraining fibroblast numbers and increased immunosuppressive myeloid populations.
- Expression of CCR1-binding chemokines may have promoted myeloid-cell recruitment, making CCR1 a possible target.

**HDAC3 Inhibition Synergizes with PD-L1 Blockade in CREBBP-Mutant DLBCL**

- Hotspot mutations in the histone acetyltransferase–encoding gene CREBBP increase repression of BCL6 target genes.
- Inhibition of BCL6 partner HDAC3 restored normal epigenetic and transcriptional profiles in CREBBP-mutant cells.
- HDAC3 inhibition synergized with PD-L1 blockade to eradicate DLBCL cells in a mouse model.

CREBBP, encoding a histone acetyltransferase that primarily acetylates lysine residue 27 of histone 3 (H3K27Ac) to activate transcription, is the second most commonly mutated gene in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. In lymphoma cells, Mondello, Tadros, and colleagues found that the catalytically inactivating hotspot mutation CREBBP<sup>R1446C</sup> strengthened the transcriptional repression of genes coregulated by the transcription factor BCL6, which is essential for the development of the germinal center B cells from which DLBCL and follicular lymphoma originate. CREBBP<sup>R1446C</sup>-mutant and CREBBP<sup>WT</sup>-knockout B cells were more sensitive than wild-type (WT) cells to treatment with BRD3308, a selective inhibitor of HDAC3, which BCL6 recruits to enact gene silencing, and to shRNA-mediated HDAC3 knockdown. In patient-derived xenograft models of both CREBBP<sup>R1446C</sup>-mutant and CREBBP<sup>WT</sup> DLBCL, BRD3308 treatment led to a substantial reduction in tumor growth rate. Treatment of CREBBP<sup>R1446C</sup>-mutant cells with BRD3308 caused reversion to the epigenetic and transcriptional programs observed in CREBBP<sup>WT</sup> cells. Further, regardless of CREBBP mutational status, selective inhibition of HDAC3 in lymphoma cells countered the repression of BCL6-target genes, including many linked to interferon signaling, PD-1 signaling, and antigen presentation via MHC class II. Experiments using a mouse model of DLBCL showed that HDAC3 inhibitor–mediated reactivation of these genes enabled MHC class I/II–dependent destruction of DLBCL cells by tumor-infiltrating lymphocytes, an effect that was synergistic with that of PD-L1 blockade. Thus, selective HDAC3 inhibition may represent a useful approach in combination with immune-checkpoint blockade in the large subset of lymphoma patients who have CREBBP-mutant DLBCL.

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Inactivating mutations in the tumor-suppressor gene von Hippel-Lindau (VHL) cause stabilization of hypoxia-inducible factor 2 α (HIF2α), predisposing to clear-cell renal cell carcinoma (ccRCC). However, not all VHL-mutant ccRCCs respond to HIF2α inhibition, suggesting that additional mechanisms may drive VHL-mutant ccRCC. Hu and colleagues found that VHL loss and hypoxia can each increase phosphorylation of TANK-binding kinase 1 (TBK1) at serine residue 172 in a HIF-independent manner in ccRCC cells, leading to TBK1 hyperactivation. Validating this finding’s in vivo relevance, VHL-mutant patient ccRCCs also exhibited increased TBK1 phosphorylation. Further in vitro experiments revealed that functional VHL’s physical interaction with TBK1 and the consequent reduction in TBK1 phosphorylation was dependent on hydroxylation of TBK1’s proline residue 48 by the prolyl hydroxylase EGLN1 (also known as PHD2). Additionally, functional VHL promoted interactions between the phosphatase PPM1B and TBK1, further reducing levels of phosphorylated TBK1. TBK1-knockdown VHL-null ccRCC cells orthotopically injected into mouse renal capsules failed to proliferate, indicating the importance of TBK1 in this context. Intriguingly, TBK1’s role in kidney cancer appeared to be distinct from its previously established function in innate immunity. Instead, in vitro experiments showed that TBK1 can directly phosphorylate the proto-oncoprotein p62 at serine residue 366, increasing p62’s stability and thus potentially contributing to tumorigenesis. Collectively, these findings provide a detailed mechanistic understanding of the previously unknown role of TBK1 in VHL-mutant ccRCC and identify TBK1 as a possible drug target in this malignancy.

See article, p. 460.