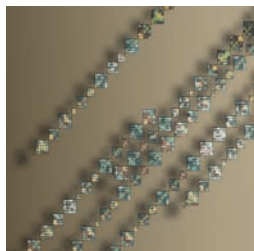


TCR Repertoire Intratumor Heterogeneity Is Linked to NSCLC Relapse

- T-cell receptor (TCR) sequencing in 45 tumor regions from 11 NSCLCs reveals intratumor heterogeneity.
- Patients with NSCLC exhibit intratumor differences in T-cell density, clonality, and repertoire.
- T-cell repertoire heterogeneity may be linked to genomic intratumor heterogeneity and recurrence.



A high proportion of heterogeneous neoantigens is associated with a poor response to checkpoint blockade in patients with non-small cell lung cancer (NSCLC). A previous report used whole-exome sequencing to characterize the genomic intratumor heterogeneity in 11 patients with NSCLC, and found that

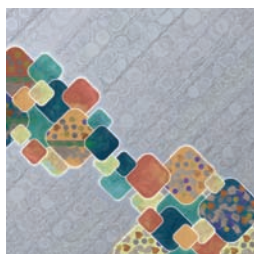
high levels of genomic heterogeneity were associated with an increased risk of disease relapse. Reuben and colleagues characterized the T-cell repertoire in the same patient cohort to determine how intratumor heterogeneity in the T-cell landscape correlates with the genomic landscape and with patient outcome in NSCLC. T-cell receptor (TCR) profiling of

45 tumor regions using ImmunoSEQ revealed a high level of intratumor heterogeneity in T-cell infiltration, clonality, and repertoire. Assessment of T-cell clones from different regions of individual tumors showed substantial intratumor heterogeneity, with most clones restricted to individual tumor regions. However, the most abundant T-cell clones in each patient were detectable throughout the tumor. The tumor-infiltrating lymphocytes were predominantly CD4-positive across patients. An increased degree of TCR intratumor heterogeneity correlated with neoantigen heterogeneity and was associated with disease relapse and reduced disease-free survival. Taken together, these findings suggest a relationship between genomic and immune intratumor heterogeneity and disease relapse in patients with NSCLC. ■

See article, p. 1088.

Transition to Invasive Breast Cancer Is Associated with Immune Escape

- IDCs contain more tumor-infiltrating leukocytes but fewer activated T cells compared with DCIS.
- TIGIT expression is more common in DCIS, whereas triple-negative IDC shows overexpression of PD-L1.
- Amplification of a chemokine cluster inversely correlates with activated T cells in HER2⁺ER⁺ tumors.



Breast cancer progresses from ductal carcinoma *in situ* (DCIS) lesions, in which the intact basement membrane and myoepithelial cell layer separate cancer cells from the immune microenvironment, to invasive ductal carcinomas (IDC), in which cancer cells are exposed to immune cells in the stroma. However, the

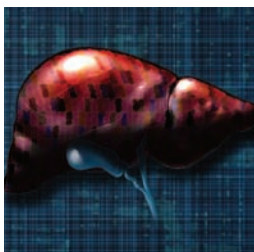
changes in the immune landscape during this transition and the mechanisms that underlie immune escape in breast cancer remain unclear, prompting Gil Del Alcazar and colleagues to characterize the composition and activation status of leukocytes in normal breast tissue, DCIS, and HER2⁺ and triple-negative IDCs. IDCs contained relatively more tumor-infiltrating T cells compared with DCIS samples. Of note, however, DCIS samples showed enrichment for cyto-

toxic T-cell gene signatures, more activated CD8⁺ T cells, and a higher T-cell receptor clonotype diversity compared with IDCs, which were instead enriched for regulatory T-cell gene sets, suggestive of a switch to a more immunosuppressive microenvironment in IDCs. Expression of the immune checkpoint protein TIGIT was more common in DCIS T cells, whereas the gene encoding PD-L1 was preferentially amplified and overexpressed in a subset of triple-negative IDCs. Furthermore, coamplification of a chemokine gene cluster on chromosome 17q12 with *ERBB2* in HER2⁺ER⁺ tumors negatively correlated with the presence of activated T cells, indicative of coevolution of cancer cells and leukocytes. These results identify the *in situ* to invasive breast carcinoma transition as an important step for immune escape and tumor progression and suggest that immunotherapies may be more effective in early-stage disease. ■

See article, p. 1098.

Fluke-Positive Cholangiocarcinoma Exhibits a Distinct Genome Landscape

- The genomic landscapes of cholangiocarcinomas were defined by whole-exome and epigenomic analysis.
- Etiologically distinct cholangiocarcinoma subtypes exhibit unique molecular landscapes.
- Integrated genomic analyses of carcinogen-driven cancer provide insights into cancer etiology.



Cholangiocarcinomas are highly lethal bile-duct malignancies with poor prognosis, associated with endemic liver fluke infection in Southeast Asia, and primary sclerosing cholangitis and hepatolithiasis in non-Asian countries. Because previous studies have genomically characterized fluke-negative cholangiocarcinoma, Jusakul and colleagues performed, as part of the International Cancer Genome Consortium, integrated genomic, epigenomic, and transcriptomic analyses of 489 cholangiocarcinomas from 10 countries to molecularly characterize both fluke-positive and fluke-negative cholangiocarcinoma. Whole-genome sequencing of 71 pairs of cholangiocarcinoma tumors, including 22 fluke-positive cases, and matched normal tissues revealed that fluke-positive cholangiocarcinoma exhibited significantly more mutations than fluke-negative cholangiocarcinoma. Analysis of

sequencing data from these tumors and an additional 428 cases identified four distinct cholangiocarcinoma subtypes characterized by distinct disease etiology and genomic alterations: Clusters 1 and 2 were associated with *TP53* mutations, *ERBB2* amplifications, fluke-positive cholangiocarcinoma, and poor patient survival, while Clusters 3 and 4 were associated with fluke-negative cholangiocarcinoma and better prognosis. Further, cholangiocarcinomas belonging to Clusters 1 and 4 exhibited distinct DNA hypermethylation patterns, demonstrating the existence of distinct epigenomic subtypes. *RASA1*, *STK11*, *MAP2K4*, and *SF3B1* were identified as potential cholangiocarcinoma driver genes, and mutations in H3K27me3-associated binding sites were identified in all four clusters, particularly in Cluster 1 tumors. These findings describe the genomic landscapes of both liver fluke-positive and -negative cholangiocarcinomas and identify a molecular taxonomy that may provide insight into the biology of cholangiocarcinoma. ■

See article, p. 1116.

An *RARA* Superenhancer May Sensitize AML to the *RARα* Agonist SY-1425

- Analysis of superenhancers in 66 patients with non-APL AML defined 6 epigenomic subtypes.
- A subset of AMLs harbored an *RARA* superenhancer that conferred sensitivity to SY-1425 *in vivo*.
- The *RARA* superenhancer may serve as a biomarker for patients who may respond to *RARα* agonism.



New therapeutic options are needed for patients with acute myeloid leukemia (AML) to improve response rates. To identify potential therapeutic targets, McKeown, Corces, Eaton, and colleagues used H3K27ac chromatin immunoprecipitation sequencing to characterize the enhancer landscape of 66 patients with AML, 28 AML cell lines, 4 hematopoietic stem and progenitor cell samples, and 6 monocyte samples. A median of 807 superenhancers were identified per sample, and the superenhancer maps stratified AML into 6 epigenetic state defined subgroups independent of mutational status. Differences in overall survival were observed between the superenhancer subtypes based on projection to public datasets. A superenhancer was identified at the retinoic acid receptor

alpha (*RARA*; encoding *RARα*) locus in a subset of patients, including 2 patients with *RARA* fusions, and it was associated with high expression of *RARA*, suggesting a potentially critical cell state role for *RARα*. Accordingly, in patients lacking the acute promyelocytic leukemia (APL) *RARA* translocation, the *RARA* superenhancer was associated with increased sensitivity to the selective *RARα* agonist SY-1425 *in vitro*. *In vivo*, *RARA*-high AML patient-derived xenografts (PDX) responded to SY-1425, but *RARA*-low PDXs did not. In *RARA*-high AML cells, SY-1425 induced differentiation, suppressed proliferation, and induced expression of retinoic acid response genes, a response similar to that observed in APL treated with retinoids. In addition to identifying AML subtypes characterized by superenhancers, these findings suggest that patients with AML with a superenhancer at *RARA* may respond to *RARα* agonists. These findings support further investigation of SY-1425 in biomarker-selected patients. ■

See article, p. 1136.

CAR T Cells Targeting Tumor Cells and TAMs Overcome Immunosuppression

- CART directed against CD123 (CART123) target Hodgkin lymphoma cells *in vitro* and *in vivo*.
- CART123 also targets tumor-associated macrophages (TAM) to overcome immunosuppression.
- CART therapies targeting both tumor cells and the microenvironment may have enhanced efficacy.



In Hodgkin lymphoma an immunosuppressive tumor microenvironment (TME), including tumor associated macrophages (TAM), limits the efficacy of immunotherapies such as chimeric antigen receptor T cells (CART) targeting antigens expressed on Hodgkin lymphoma cells. To potentially enhance the

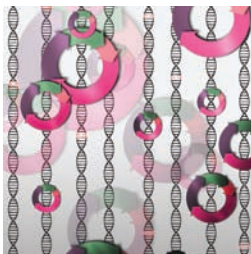
efficacy of CART therapy, Ruella, Klichinsky, and colleagues aimed to develop CART that would target both the tumor cells and the TME in Hodgkin lymphoma. In tumor samples from patients with Hodgkin lymphoma, the IL3 receptor CD123 was highly expressed on both tumor cells and TAMs, nominating it as a potential target. CART directed against CD123 (CART123) had previously been developed for the potential

treatment of acute myeloid leukemia, and CART123 was also able to kill Hodgkin lymphoma cells *in vitro* and *in vivo* in Hodgkin lymphoma xenografts. Further, when CART123-treated immunodeficient mice who entered remission were rechallenged with Hodgkin lymphoma cells, the tumor was rejected, indicating that CART123 established long-term immunologic memory. The presence of Hodgkin lymphoma cells polarized macrophages to an M2-like immunosuppressive phenotype, thereby suppressing T-cell proliferation, but CART123 was also able to kill TAMs to overcome immunosuppression. Taken together, these findings suggest that CART cell therapies, such as CART123, that target both the tumor cells and TAMs may overcome immunosuppression to improve their efficacy, and warrant further investigation for the treatment of patients with Hodgkin lymphoma. ■

See article, p. 1154.

Loss of MutL Promotes Intrinsic Endocrine Therapy Resistance

- Loss of the mismatch repair complex MutL sensitizes ER⁺ breast cancer cells to CDK4/6 inhibitors.
- MutL loss downregulates CHK2, relieving inhibition of CDK4 to promote endocrine therapy resistance.
- CDK4/6 inhibitors may overcome endocrine therapy resistance in patients with MutL-deficient ER⁺ tumors.



Although mechanisms of acquired resistance to endocrine therapy have been identified in estrogen receptor-positive (ER⁺) breast cancer, the causes of intrinsic resistance, present at the time of diagnosis, are less well understood. Haricharan and colleagues linked loss of the MutL mismatch repair (MMR)

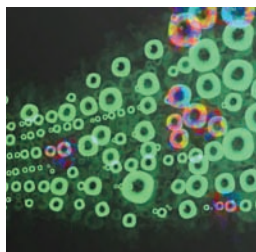
complex to intrinsic resistance to endocrine therapy. MMR has two damage-sensing complexes: MutS and MutL. In patients with ER⁺ breast cancer, endocrine therapy resistance was associated with dysregulation of MutL but not MutS. In ER⁺ breast cancer cell lines, silencing of MutL complex genes promoted resistance to endocrine therapy (including estrogen deprivation, a surrogate for aromatase inhibitor exposure, fulvestrant, and tamoxifen) by preventing CHK2-mediated inhibition of CDK4/6, resulting in deregulated CDK4/6 activity that drove endocrine therapy

resistance. Further, mutations that dysregulated MutL promoted resistance to endocrine therapy in patient-derived xenograft models. Response to endocrine therapy required MutL-mediated activation of ATM/CHK2 and inhibition of CDK4. Thus, MutL-deficient ER⁺ breast cancer cells exhibited CDK4 upregulation and were sensitive to CDK4/6 inhibitors. Moreover, MutL-deficient xenografts regressed when treated with estrogen deprivation in combination with CDK4/6 inhibition, but not with estrogen deprivation alone. In patients with ER⁺HER2⁻ breast cancer treated with an aromatase inhibitor, MutL deficiency was associated with failure to fully suppress Ki67, but the addition of the CDK4/6 inhibitor palbociclib increased Ki67 inhibition and the incidence of complete cell-cycle arrest. Collectively, these findings show that MutL dysregulation may be a mechanism of intrinsic resistance to endocrine therapy, and predict that, for patients with MutL-deficient ER⁺ breast cancer, adjuvant treatment with CDK4/6 inhibitors may be beneficial. ■

See article, p. 1168.

Hypoxia Upregulates *BLIMP1* to Drive Pancreatic Cancer Metastasis

- Hypoxic induction of *BLIMP1* drives the metastatic potential of pancreatic cancer cells.
- *BLIMP1* regulates expression of hypoxia-regulated prometastatic and cell-cycle arrest genes.
- Local tumor microenvironmental cues promote the metastatic heterogeneity of pancreatic cancer cells.



Pancreatic ductal adenocarcinoma (PDAC) is characterized by a desmoplastic stromal response, which promotes tumor development and prevents drug delivery; however, the role of the local tumor microenvironment in PDAC metastasis remains incompletely understood. To identify potential factors that drive PDAC metastasis, Chiou and colleagues added an HMGA2-GFP reporter to the *Kras*^{LSL-G12D/+}; *Trp53*^{LSL-R172H/+} (KPC) genetically engineered PDAC mouse model. GFP⁺ PDAC cells were highly metastatic, gave rise to metastases with heterogeneous GFP expression, and had a transcriptional signature that was enriched for genes induced by hypoxia/HIF that was predictive of poor survival for patients with PDAC. Significant gene expression changes were observed between GFP⁺ and

GFP⁻ PDAC cells, and knockdown of genes upregulated in GFP⁺ PDAC cells revealed that the transcription factor *BLIMP1* drives metastatic ability. Hypoxia induced *BLIMP1* expression in a partially HIF1 α -dependent manner in human and mouse PDAC cell lines, and hypoxia-response elements upstream of *Blimp1* were bound by HIF1 α . *BLIMP1* was required for both the upregulation and suppression of hypoxia-regulated genes, including prometastatic factors and cell-cycle arrest genes, respectively. Knockdown of the *BLIMP1*-dependent prometastatic gene *Hilpda* reduced metastasis *in vivo*. Further, a *BLIMP1*-dependent hypoxia-induced gene signature correlated with *BLIMP1* expression and worse prognosis in human PDAC. Together, these results provide insights into the role of the tumor microenvironment in promoting PDAC metastasis and the development of potential therapeutic strategies. ■

See article, p. 1184.

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