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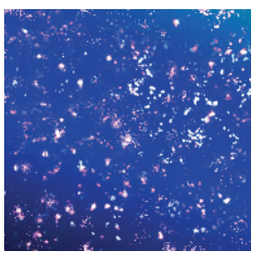
Bisphosphonates Target Extraskeletal Tumor-Associated Macrophages

- Bisphosphonates localize to mouse mammary tumors via leaky tumor blood vessels.
- TAMs, but not tumor cells, internalize bisphosphonates bound to granular microcalcifications.
- Bisphosphonate uptake was observed in a primary human breast tumor with TAMs and microcalcifications.



Nitrogen-containing bisphosphonate (N-BP) drugs such as zoledronic acid inhibit protein prenylation and target skeletal tissue to prevent osteoclastic bone resorption and skeletal-related events in patients with cancer. In addition, zoledronic acid exhibits antitumor activity in preclinical models of extraskeletal tumors and has been shown to increase survival and decrease tumor relapse in women with early breast cancer. However, the cellular targets and mechanisms by which N-BPs inhibit tumor growth outside of bone have not been defined. Using real-time intravital two-photon microscopy of a syngeneic mouse model of breast cancer, Junankar and colleagues found that fluorescently labeled N-BP localized to the periphery of mammary tumors via the leaky tumor vasculature. N-BPs rapidly bound granular microcalcifications, which were detected in 40% of human breast tumor samples analyzed, and were internalized by F4/80$^+$ tumor-associated macrophages (TAM), but not tumor epithelial cells, via phagocytosis. Consistent with this finding, uptake of radiolabeled bisphosphonate was observed in the primary tumor of a patient with grade 1 invasive ductal carcinoma. Furthermore, analysis of the resected mammary carcinoma from this patient revealed extensive TAM infiltration and the presence of granular microcalcifications within the tumor tissue, similar to those detected in mouse mammary tumors. These findings indicate that N-BPs target TAMs outside the skeleton to mediate their antitumor effects and further support the use of these drugs as adjuvant therapy for early-stage breast cancer. ■

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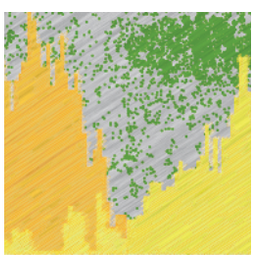
Microsatellite-Instable Colon Cancers Display Activated Immune Checkpoints

- Colon cancers with microsatellite instability exhibit strong Th1/CTL-mediated immune responses.
- Microsatellite-instable tumors evade immune clearance by upregulating immune-checkpoint proteins.
- Tumors with mismatch repair defects may benefit clinically from immune-checkpoint inhibitors.

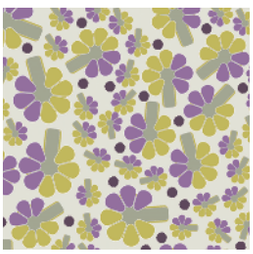


In some cancers, including colorectal cancer, the degree of immune-cell infiltration in the tumor microenvironment has been suggested to have prognostic value. Generation of tumor-specific immune signatures by expression profiling may predict which patients will respond to therapies targeting immune checkpoints, such as the programmed cell death 1 (PD-1) pathway. However, previous studies indicated that colorectal cancers do not respond well to PD-1 pathway blockade, prompting Llosa and colleagues to comprehensively characterize the immune microenvironment of colorectal cancers. Quantitative immunohistochemistry of standard T-cell markers revealed that a subset of colorectal tumors displayed high levels of T-cell infiltration, in particular by CD8$^+$ CTLs and T helper 1 (Th1) cells. Expression profiling of genes encoding inflammatory cytokines, transcription factors, and checkpoint-related proteins indicated that Th1 and CTL responses were selectively activated in the microenvironment of mismatch repair–deficient microsatellite-instable colorectal tumors. Intriguingly, infiltrating lymphocytes in microsatellite-instable tumors expressed high levels of the immune checkpoint–related proteins PD-1, its ligand PD-L1, cytotoxic T-lymphocyte–associated protein 4, lymphocyte-activation gene 3, and indoleamine 2,3-dioxygenase, and an increased number of PD-L1–positive myeloid cells was observed at the invasive front and in the stroma of microsatellite-instable tumors, suggesting a potential mechanism of adaptive immune evasion. Together, these findings support the notion that mismatch repair defects are associated with activated immune-checkpoint responses and provide a rationale for the treatment of the genetically defined subset of microsatellite-instable colorectal cancers with PD-1 pathway blockers and inhibitors of other immune-checkpoint proteins. ■

See article, p. 43.

KRAS^{G12D} Drives Formation of Precancerous Pancreatic Lesions via ICAM1

- ICAM1 produced by *KRAS*-mutant pancreatic acinar cells attracts macrophages to areas of ADM.
- M1 macrophages promote ADM via secretion of cytokines such as TNF and matrix metalloproteinases.
- Macrophage depletion or ICAM1 neutralization slows the progression of murine pancreatic lesions.



The inflammatory microenvironment is a prominent feature of mutant KRAS–driven pancreatic ductal adenocarcinoma, but the mechanisms that mediate crosstalk between pancreatic cells and infiltrating immune cells are unknown. Liou and colleagues determined that infiltrating macrophages preferentially localized to regions of acinar-to-ductal metaplasia (ADM) as compared with pancreatic intraepithelial neoplastic (PanIN) lesions in mice expressing oncogenic KRAS^{G12D} specifically in pancreatic acinar cells. Depletion of macrophages in this model reduced the overall number of abnormal pancreatic structures and slowed the progression of ADM to PanIN lesions. The attraction of macrophages was dependent on KRAS^{G12D}-induced expression of intercellular adhesion molecule 1 (ICAM1) in acinar cells. M1-polarized, but not M2-polarized, primary murine macrophages migrated toward recombinant soluble ICAM1 and primary murine acinar cells expressing mutant KRAS *in vitro*, and this chemoattraction was ablated by an ICAM1-neutralizing antibody. Consistent with these findings, ICAM1 expression localized to regions of ADM in human and murine samples, and correlated with the presence of macrophages and expression of macrophage-derived TNF and matrix metalloproteinase 9 (MMP9), suggesting that local inflammation and extracellular matrix degradation synergize with KRAS^{G12D} to drive ADM. Similar to the effects of macrophage depletion, treatment of mice expressing acinar cell–specific KRAS^{G12D} with ICAM1-neutralizing antibody reduced macrophage infiltration, the formation of abnormal lesions, and progression of ADM to PanIN lesions. These results indicate that mutant KRAS–induced ICAM1 expression promotes macrophage infiltration to promote the initiation of precancerous lesions, which may be blocked by targeted immunotherapies. ■

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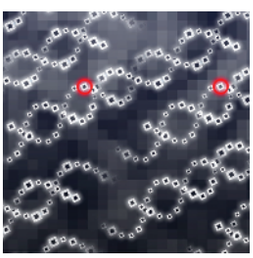
Cell-Free DNA Analysis Detects *BRAF*^{V600E} Mutation in Histiocytic Disorders

- Analysis of urine and plasma cfDNA detects *BRAF*^{V600E} mutations in patients with histiocytic disorders.
- Serial urinary cfDNA analyses monitor response to immunomodulatory and RAF inhibitor therapies.
- cfDNA genotyping identified a previously undescribed *KRAS*^{G12S} mutation in a *BRAF* wild-type patient.

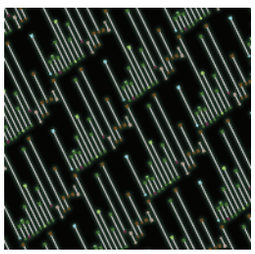


Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD) are heterogeneous systemic disorders in which histiocytes accumulate throughout the body, compromising organ function. These orphan diseases are frequently characterized by somatic *BRAF*^{V600E} mutation and respond to RAF inhibitors; however, identification of the *BRAF*^{V600E} mutation is challenging due to low histiocyte content, stromal contamination, and suboptimal tissue samples generated from bone biopsies. Hyman, Diamond, and colleagues carried out a first-in-kind prospective, blinded study using a droplet-digital PCR assay to quantitatively detect this mutation in circulating tumor cell-free DNA (cfDNA) obtained from the urine and plasma of 30 patients with ECD or LCH. In contrast to tissue-based genotyping, which definitively assigned *BRAF* status to 21 (70%) patients, urinary cfDNA analysis defined the *BRAF* genotype of all 30 patients, including two patients not previously known to have this mutation. Among treatment-naïve patients, there was 100% concordance between tissue and urinary cfDNA genotypes, and comparable results were obtained with plasma cfDNA. In addition, serial urinary cfDNA analyses in patients treated with a BRAF inhibitor or immunomodulatory therapy showed a progressive decrease in the *BRAF*^{V600E} allele burden, consistent with radiographic evidence of disease improvement. Furthermore, genotyping analysis of tissue and cfDNA identified a previously unreported somatic *KRAS*^{G12S} mutation in a *BRAF* wild-type patient with ECD. Together, these data suggest cfDNA testing as a reliable, noninvasive method of detecting *BRAF*^{V600E} mutations and monitoring response to therapy in histiocytic disorders. ■

See article, p. 64.

Residual Estrogen Receptor Activity Predicts Fulvestrant Response

- Incomplete reduction in estrogen availability during treatment correlates with early progression.
- Optimal effects of fulvestrant on tumor ER availability are observed after only two doses.
- FES-PET scans represent a useful tool for measuring ER availability in patients with breast cancer.



Fulvestrant is an estrogen receptor (ER) antagonist that competitively binds and downregulates ER and inhibits estrogen-mediated tumor growth in patients with metastatic ER-positive breast cancer. However, despite favorable clinical outcomes and low toxicity with higher dosing regimens, a subset of patients do not benefit from fulvestrant treatment, prompting van Kruchten and colleagues to use PET/CT with the tracer [^{18}F]fluoroestradiol (FES) to determine whether the standard 500 mg dosing of fulvestrant is sufficient to inhibit tumor ER availability. Sixteen patients with metastatic breast cancer received fulvestrant and underwent follow-up PET/CT scans at days 28 and 84 prior to subsequent fulvestrant administrations. Eight of the nine patients who displayed a reduction in FES of greater than or equal to 75% from baseline exhibited clinical benefit, with a progression-free survival of 11.7 months as compared with 3.3 months in the six (38%) patients with incomplete reduction in ER availability. Importantly, this longitudinal study also revealed that maximum fulvestrant effects occurred after only two doses, patients with multiple lesions displayed heterogeneity in FES uptake, and plasma fulvestrant levels were not predictive of tumor FES uptake. In addition, prior treatment with tamoxifen was associated with lower baseline FES levels and may need to be corrected for in analyses of FES uptake. Together, this work highlights FES-PET as a useful tool to measure ER availability to optimize fulvestrant dosing and predict response in patients with metastatic breast cancer. ■

See article, p. 72.

A Telomerase Substrate Precursor Induces Telomere Dysfunction

- 6-thio-dG is converted into 6-thio-dGTP, which can be incorporated into telomeres by telomerase.
- 6-thio-dG is selectively cytotoxic to telomerase-expressing cancer cells independent of telomere length.
- 6-thio-dG safely reduces tumor growth *in vivo* in association with increased telomeric DNA damage.

Telomerase, the ribonucleoprotein enzyme complex that counteracts telomere shortening and prevents replicative senescence by adding telomeric DNA repeats to chromosome ends, is considered an attractive therapeutic target because it is expressed in the majority of human cancers but is not expressed in most normal cells. Existing telomerase inhibitors work by causing progressive telomere shortening over successive cell divisions and thus require prolonged treatment periods to be effective, but prolonged telomerase inhibition has been found to cause hematologic, hepatic, and gastrointestinal toxicity. Building on the knowledge that telomerase has high affinity for guanine bases containing 2′-deoxyguanosine 5′-triphosphate, Mender and colleagues designed an analogue of the antileukemic agent 6-thioguanine, 6-thio-2′-deoxyguanosine (6-thio-dG), that is converted into 6-thio-2′-deoxyguanosine 5′-triphosphate (6-thio-dGTP) and incorporated into de novo telomeric products. Both 6-thio-dG and 6-thioguanine induced cell death in telomerase-expressing cancer cell lines in a dose-dependent manner regardless of telomere length while sparing normal cell types. However, 6-thio-dG induced significantly greater telomeric DNA damage and more enhanced telomere shortening than 6-thioguanine without directly inhibiting telomerase activity. 6-thio-dG treatment also led to marked telomere dysfunction and suppression of tumor growth *in vivo* without any obvious hematologic toxicity, impairment of liver function, or effects on organ histology. This approach to selectively disrupt telomere structure and function in cancer cells independent of initial telomere length by exploiting the activity of telomerase itself to "poison" telomeres could ultimately lead to a less toxic and more effective strategy for targeting telomerase-expressing cancer cells. ■

See article, p. 82.

Note: *In This Issue* is written by *Cancer Discovery* Science Writers. Readers are encouraged to consult the original articles for full details.

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