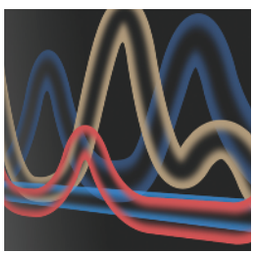
# IN THIS ISSUE

## *BRCA1* Is a Fanconi Anemia Susceptibility Gene

- Biallelic *BRCA1* mutations were found in a woman with congenital anomalies and early-onset breast cancer.
- Patient cells displayed impaired DNA damage repair and hypersensitivity to ICL-inducing agents.
- Deleterious biallelic *BRCA1* mutations predispose to a distinct Fanconi anemia subtype.



Fanconi anemia (FA) is a developmental syndrome characterized by pediatric cancer susceptibility, congenital abnormalities, and bone marrow failure that is caused by mutations in FA genes, including *BRCA2* (*FANCD1*) and *PALB2* (*FANCN*), which play critical roles in DNA interstrand crosslink (ICL) repair. However, although deleterious biallelic mutations in the related DNA repair gene *BRCA1* have recently been described, a causative role for *BRCA1* in FA has not been established. Sawyer, Tian, and colleagues describe a second female patient harboring biallelic compound heterozygous mutations in *BRCA1* who presented with multiple developmental anomalies, including growth delay and dysmorphic features similar to patients with FA, and early-onset breast cancer. Whole-exome sequencing and pedigree analysis revealed autosomal recessive inheritance of a truncating allele, *BRCA1*:c.594_597del, which was associated with a high incidence of breast and ovarian cancers in the family, and a missense mutation in the second *BRCA1* allele corresponding to an Arg1699Trp substitution in the BRCT repeats of the BRCA1 protein. Consistent with defective BRCA1 function and impaired DNA double-strand break repair, patient-derived cells exhibited increased chromosomal breakage and radial chromosomes, impaired formation of BRCA1 and RAD51 foci in response to DNA damage, and hypersensitivity to ICL-inducing agents such as the PARP inhibitor olaparib, which was rescued by ectopic expression of wild-type BRCA1. These findings demonstrate that deleterious biallelic *BRCA1* mutations cause a distinct FA subtype (termed FA-S) and have implications for genetic risk counseling. ■

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## Blocking PI3K Delays MEK1/2 Inhibitor Resistance in *BRAF*-Mutated Melanoma

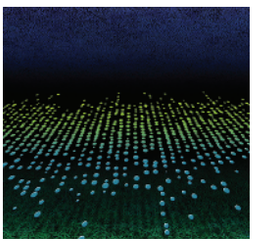
- $BRAF^{V600E}$/$PIK3CA^{H1047R}$ melanoma cells are sensitive to selective PI3Kα inhibition.
- $BRAF^{V600E}$/$PTEN^{null}$ melanoma cells require combined blockade of PI3Kα, δ, and γ for growth suppression.
- Dual treatment with PI3K inhibitors prevents MEK1/2 inhibitor resistance in *BRAF*-mutated melanoma.

Two frequently occurring molecular lesions in melanoma are oncogenic $BRAF^{V600E}$, which promotes MEK–ERK signaling, and sustained phosphatidylinositide 3′ (PI3′)-lipid production as a result of PTEN silencing or, less frequently, expression of mutationally activated $PIK3CA^{H1047R}$. Deuker and colleagues examined the effects of isoform-selective PI3K inhibitors on melanoma-derived cell lines and genetically engineered mouse models to better understand how PI3K signaling cooperates with oncogenic $BRAF^{V600E}$ to promote melanomagenesis. Treatment of $BRAF^{V600E}$/$PIK3CA^{H1047R}$, but not $BRAF^{V600E}$/$PTEN^{null}$, melanoma cells with BYL719, which selectively blocks PI3Kα, potently suppressed cell proliferation and enhanced the growth-suppressive effects of $BRAF^{V600E}$ inhibition, resulting in superior melanoma regression *in vivo*. $BRAF^{V600E}$/$PTEN^{null}$ melanoma cells were insensitive to selective PI3Kβ blockade, and instead were dependent on the combined activity of PI3Kα, δ, and/or γ for proliferation; treatment with a PI3Kβ-sparing inhibitor targeting the PI3K α, δ, and γ isoforms (GDC-0032) or combined treatment with BYL719 and a PI3Kδ/γ–selective inhibitor produced a robust antiproliferative response and sustained inhibition of downstream signaling in $BRAF^{V600E}$/$PTEN^{null}$ cells. Furthermore, dual treatment with GDC-0032 and a MEK1/2 inhibitor induced more substantial melanoma regression compared with single-agent treatment and prevented the development of MEK1/2 inhibitor–resistant tumors in $BRAF^{V600E}$/$PTEN^{null}$ mice, suggesting that PI3K blockade augments the duration of response to $BRAF^{V600E}$ pathway inhibition. Together, these data suggest that combining $BRAF^{V600E}$ pathway–targeted agents with PI3K inhibitors may be clinically beneficial for a subset of patients with *BRAF*-mutated melanoma and may potentially delay the onset of drug-resistant disease. ■

*See article, p. 143.*

## An Isogenic Cell Screen Predicts Genotype-Specific Drug Responses

- Isogenic cell lines expressing a panel of oncogenes were screened against a library of cancer drugs.
- Isogenic cell line screens allow analysis of rare mutations and direct analysis of gene–drug interactions.
- This approach revealed that MYC overexpression confers dasatinib sensitivity in TNBC models.

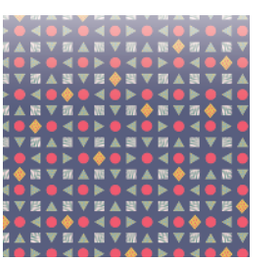


The goal of personalized cancer therapy is to devise therapeutic strategies based on the genomic aberrations in a given tumor. Large-scale cancer cell line screens have provided useful information on genetic determinants of drug sensitivity and resistance, but it remains difficult to distinguish the effect of single mutations and evaluate the impact of rare mutations. Martins, Zhou, and colleagues developed a strategy to systematically and quantitatively assess the effect of specific recurrent mutation, amplification, or overexpression events on proliferation of triple-negative breast cancer (TNBC) in response to a library of cancer drugs using an isogenic cell model system in which over 50 wild-type and mutant genes were individually expressed in receptor-negative, non-transformed MCF10A cells. The resulting chemical–genetic interaction map revealed both known and unsuspected links between gene alterations and drug response, and showed that some undruggable alterations, such as MYC overexpression, conferred sensitivity to compounds in clinical use or development. The drug with the strongest "synthetic lethal" interaction with MYC overexpression was the tyrosine kinase inhibitor dasatinib, and MYC overexpression predicted dasatinib sensitivity in a large panel of cancer cell lines. Among known dasatinib targets, the SRC family tyrosine kinase LYN was upregulated in a MYC-dependent manner in MCF10A cells and mediated dasatinib sensitivity in MYC-overexpressing TNBC cells. These findings demonstrate the potential of this approach, which may be scalable and adaptable to other tumor types, to uncover unexpected actionable interactions and facilitate genotype-directed therapy. ■

*See article, p. 154.*

## Glycosylation-Dependent Leukocyte Binding Drives Lung Tumor Metastasis

- Tumor-derived soluble factors promote mobilization of galectin-3$^+$ leukocytes into the circulation.
- Aberrant glycosylation increases lung cancer cell presentation of T-Antigen, a galectin-3 ligand.
- T-Antigen binds to galectin-3 expressed by leukocytes in the metastatic niche to promote metastasis.

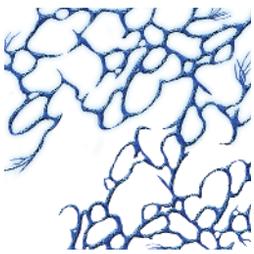


Crosstalk between primary tumors and tumor-recruited CD11b$^+$ myeloid cells in the early metastatic niche is critical for metastatic progression, but the role of cell-surface galectins in this process is largely unknown. Utilizing a mouse model of lung adenocarcinoma metastasis, Reticker-Flynn and Bhatia determined that, whereas galectin-3 expression was unchanged across tumor cells of varying metastatic potential, tumor-bearing mice exhibited an increase in CD11b$^+$ galectin-3$^+$ leukocyte mobilization into the circulation. Although galectin-3 can act as a chemoattractant for macrophages, manipulation of tumor-derived galectin-3 did not affect myeloid-cell mobilization or tumor metastasis. However, tumor-derived secretion of IL6, which was found to be genetically amplified in patients with lung adenocarcinoma, induced CD11b$^+$galectin-3$^+$ leukocyte mobilization from the bone marrow. Importantly, metastatic cell lines and human non–small cell lung cancer samples exhibited increased surface presentation of the Thomsen-Friedenreich Antigen (T-Antigen) disaccharide, a galectin-3 carbohydrate ligand expressed on glycoproteins in many tumors. Elevated T-Antigen surface presentation resulted from downregulation of C2GnT2 (encoded by *Gcnt3*) and upregulation of St6GalNAc4 (encoded by *St6galnac4*) glycosyltransferase expression, which prevented T-Antigen glycan elongation. Restoration of glycosyltransferase-mediated T-Antigen glycan chain elongation decreased T-Antigen presentation, reduced tumor-cell galectin-3 binding, and almost completely abrogated experimental metastases *in vivo*. These results indicate that aberrant glycosyltransferase activities play a critical role in the metastatic progression of lung tumors by promoting the interaction between T-Antigen$^+$ tumor cells and galectin-3$^+$ leukocytes in the early metastatic niche. ■

*See article, p. 168.*

## NOTCH Decoys Inhibit Angiogenesis via DLL- or JAGGED-Specific Mechanisms

- NOTCH1 EGF-like repeats 1-13 and 10-24 elicit DLL- and JAG-specific activities, respectively.
- NOTCH1 decoys targeting JAG1/2 inhibit tumor angiogenesis via increased soluble VEGFR1 levels.
- NOTCH1 decoys targeting JAG ligands may represent an effective and safe anti-angiogenic therapy.

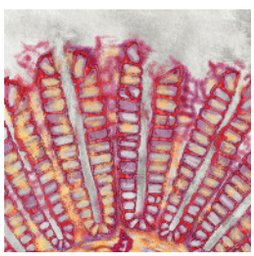


NOTCH receptors interact with two classes of ligands, JAGGED (JAG) and Delta-like (DLL), via EGF-like repeats in their extracellular domain. NOTCH signaling has been implicated in the regulation of tumor angiogenesis; however, therapeutic targeting of this pathway has been challenging. To address whether certain NOTCH EGF-like repeats exhibit ligand-specific interactions, Kangsamaksin and colleagues designed soluble NOTCH inhibitor decoys composed of various NOTCH1 EGF-like repeats. Whereas decoys consisting of EGF-like repeats 1-24 acted as pan-ligand inhibitors, decoys harboring repeats 1-13 ($N1_{1\text{-}13}$) specifically inhibited the DLL class, and decoys spanning repeats 10-24 ($N1_{10\text{-}24}$) exhibited JAG-specific inhibition. Functionally, inhibition of DLL with the $N1_{1\text{-}13}$ decoy induced a hypersprouting phenotype *in vitro*, whereas both the pan-ligand and JAG-specific decoys elicited an anti-angiogenic response. Furthermore, the $N1_{10\text{-}24}$ decoy reduced retinal angiogenesis *in vivo*, whereas the pan-ligand and DLL-specific decoys enhanced retinal vascular density. Importantly, the DLL and JAG classes of decoys both reduced tumor perfusion, promoted hypoxia, and suppressed xenograft tumor growth *in vivo* with limited gastrointestinal toxicity, albeit via different mechanisms. In contrast to DLL decoys, which enhanced endothelial cell density and dysfunctional tumor angiogenesis, JAG decoys reduced endothelial cell density, inhibited pericyte and smooth muscle coverage, and specifically increased expression of soluble VEGF receptor 1 (sVEGFR1), a competitive VEGF antagonist. Genetic experiments showed that the anti-angiogenic effects of JAG blockade were dependent on increased sVEGFR-1 secretion. These results highlight specific NOTCH1 interaction domains that may be therapeutically exploited to block JAG-induced angiogenesis mechanisms. ■

*See article, p. 182.*

## NOTCH Drives Colon Cancer Metastasis via the DAB1–ABL–TRIO RHOGEF Axis

- NOTCH-driven metastasis in a mouse colorectal cancer model requires DAB1-mediated ABL activation.
- ABL-mediated phosphorylation of TRIO Y2681 promotes cell invasion via RHO activation.
- Phosphorylation of TRIO Y2681 correlates with poor prognosis and may represent a useful biomarker.



Amino-terminal enhancer of split (AES) functions as an inhibitor of NOTCH signaling and has been implicated in colon cancer metastasis suppression. In an effort to dissect the contributions of the NOTCH pathway in colorectal cancer progression, Sonoshita and colleagues showed that, in a mouse model of colorectal cancer driven by *Aes* loss, the NOTCH signaling transcription factor RBPJ was required for tumor invasion and transcriptionally induced *DAB1*, a gene previously implicated in neuronal cell motility. Depletion of DAB1 in tumor cells with high DAB1 expression inhibited tumor invasion and metastasis *in vivo*, whereas exogenous DAB1 expression in cells with low endogenous DAB1 promoted cell invasion both *in vitro* and *in vivo*. Mechanistically, DAB1 interacted with and was phosphorylated by the ABL tyrosine kinase, which led to autophosphorylation and activation of ABL in colon cancer cells. In line with the notion that ABL kinase activity fuels NOTCH-driven metastasis, inhibition of ABL reduced tumor metastasis induced by *Aes* loss or DAB1 overexpression. ABL specifically phosphorylated the RAC/RHO guanine nucleotide exchange factor TRIO at tyrosine 2681 (Y2681), resulting in RHO activation in colon cancer cells. Inhibition of RHO reduced colon cancer cell invasiveness, and high levels of TRIO Y2681 phosphorylation were associated with poor prognosis in patients with colorectal cancer. Together, these results highlight TRIO-driven RHO activation as a critical downstream effector of NOTCH-driven metastasis in colon cancer and provide a rationale for developing phosphorylated TRIO Y2681 as a prognostic biomarker. ■

*See article, p. 198.*

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