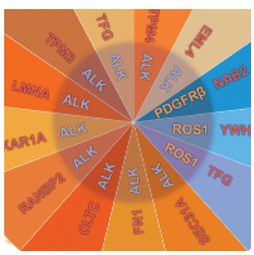
Kinase Fusions Are Common in Inflammatory Myofibroblastic Tumor

- A patient with refractory ALK-negative IMT had a *ROS1* fusion and responded to crizotinib.
- Most patients with IMT harbor potentially actionable kinase fusions involving *ALK*, *ROS1*, or *PDGFRB*.
- Molecular profiling should be incorporated into standard of care for patients with IMT.

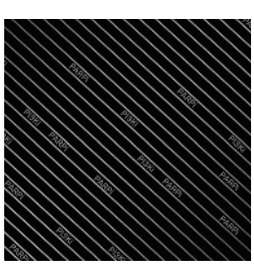


Inflammatory myofibroblastic tumor (IMT) is a rare soft-tissue tumor that predominantly affects children and young adults. IMT is usually treated by surgical resection, but there is no standard therapy for patients with unresectable or advanced IMT. Activating *ALK* gene fusions occur in approximately half of IMTs, and a patient with ALK-positive IMT was recently shown to have a partial response to the ALK inhibitor crizotinib. However, no actionable genetic alterations have been reported in ALK-negative IMT. Lovly and colleagues performed targeted next-generation–based genomic profiling on archival tissue from an 8-year-old patient with unresectable, treatment-refractory ALK-negative IMT and identified a *TFG–ROS1* fusion. As crizotinib also inhibits ROS1, the patient was compassionately treated with crizotinib and subsequently experienced a dramatic response. Evaluation of a larger cohort of samples revealed that 8 of 11 IMTs classified as ALK-negative by immunohistochemistry harbored a kinase fusion; in addition to 2 cases that had *ALK* fusions that were not detectable by immunohistochemistry, 4 had *ROS1* fusions and 2 had *PDGFRB* fusions, which had not previously been reported in IMT. Of the 22 ALK-positive tumors tested, 20 contained *ALK* fusions, and several previously uncharacterized *ALK* breakpoints and *ALK* fusion partners were observed. The finding that 85% of IMTs are characterized by fusions affecting kinases that can be inhibited with FDA-approved targeted therapies strongly argues that genomic profiling should be part of standard of care for patients with IMT. ■

See article, p. 889.

Dual PARP/PI3K Inhibition Is Effective in Advanced Prostate Cancer

- Olaparib induces senescence or apoptosis in *PTEN*-deficient cells depending on p53 status.
- PARP inhibition alone does not reduce prostate cancer cell growth due to hyperactivation of AKT.
- PARP/PI3K inhibition reduces prostate tumor burden, improving progression-free survival in mice.



Advanced hormone-insensitive prostate cancer is a highly intractable disease often characterized by concomitant loss of the tumor suppressors PTEN and p53. PTEN loss results in DNA repair defects, suggesting that inhibition of PARP may be a potential therapeutic strategy for these tumors. To determine whether genetic alterations in *PTEN* or *TP53* predict sensitivity to the PARP inhibitor olaparib, González-Billalabeitia, Seitzer, and colleagues utilized mouse embryonic fibroblasts (MEF) that mimic different stages of prostate cancer progression. Olaparib treatment induced senescence in *Pten*-deficient, *Trp53*-proficient MEFs; however, profound apoptosis and DNA damage occurred when *Pten* loss was coupled with homozygous *Trp53* deletion. Although similar phenotypes were observed in human prostate cancer cell lines and genetically engineered mouse models of prostate cancer, tumor growth was not significantly reduced due to olaparib-induced, PI3K-dependent hyperactivation of the prosurvival protein AKT, suggesting that PI3K blockade may enhance sensitivity to olaparib. Consistent with these findings, the combination of olaparib and the PI3K inhibitor BKM120 significantly inhibited cell growth compared with single-agent treatment in all cell lines tested. Furthermore, dual PARP and PI3K inhibition synergized in an advanced prostate cancer mouse model and xenografts of human prostate cancer cells, resulting in tumor regression, markers of prostate gland normalization, and prolonged progression-free survival. These findings indicate that the combination of PARP and PI3K inhibitors may be an effective treatment approach in *PTEN*-deficient advanced prostate cancer. ■

See article, p. 896.

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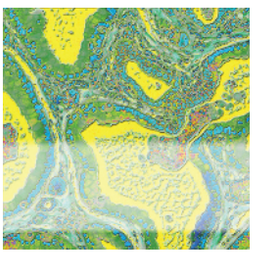
Autophagy Inhibition Blocks PDAC Growth Independent of p53 Alterations

- *Atg5* deletion in the pancreas increases tumor initiation but suppresses progression to PDAC.
- Pharmacologic or genetic autophagy inhibition decreases PDAC growth independent of p53 status.
- Hydroxychloroquine blocks the growth of *TP53*-mutant patient-derived PDAC xenografts.

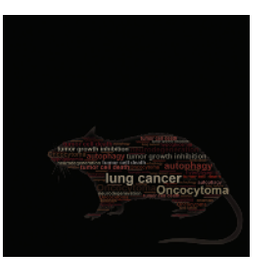


Autophagy has been suggested to function as both a suppressor of tumor initiation and a promoter of tumor progression, in particular in KRAS-driven cancers such as pancreatic ductal adenocarcinoma (PDAC). Inhibition of autophagy, which is elevated in human PDAC, induces antitumor responses; however, recent studies suggest that p53 status may modulate the role of autophagy in pancreatic cancer. To further investigate the importance of autophagy in PDAC progression, Yang, Rajeshkumar, and colleagues used an autochthonous mouse model of pancreatic cancer driven by *Kras* mutation and *Trp53* LOH, similar to human PDAC. Genetic inactivation of autophagy in this model via deletion of autophagy-related 5 (*Atg5*) in the pancreas increased the development of premalignant pancreatic intraepithelial neoplasias, but impaired the progression of these precursor lesions to invasive PDAC and prolonged survival, consistent with a dual role of autophagy in pancreatic cancer. Acute inhibition of autophagy with chloroquine or through depletion of autophagy genes diminished the growth of murine PDAC cell lines with heterozygous loss or mutation of *Trp53* as well as cell lines with homozygous *Trp53* deletion, suggesting that the antitumor activity of autophagy inhibition is independent of *Trp53* genotype. Furthermore, in a mouse preclinical trial, treatment with hydroxychloroquine decreased the growth of *KRAS*- and *TP53*-mutant patient-derived pancreatic cancer xenografts. These results provide evidence of the essential role of autophagy in pancreatic cancer development and support ongoing clinical trials of hydroxychloroquine in patients with pancreatic cancer. ■

See article, p. 905.

Systemic Autophagy Inhibition Shows Antitumor Activity in Lung Cancer

- *Atg7* deletion in adult mice results in neurodegeneration, infection, and liver and muscle damage.
- Autophagy is required to maintain fat stores and for glucose homeostasis during fasting.
- Acute autophagy inactivation selectively blocks the growth of established *Kras*-mutant NSCLC.



Autophagy is a catabolic process that prevents accumulation of damaged cellular components and is required for energy homeostasis and survival during starvation. Upregulation of autophagy in response to oncogenic transformation has been shown to sustain mitochondrial metabolism and to promote lung tumor progression, suggesting that autophagy may be a therapeutic target. To investigate the effects of acute autophagy inactivation on normal and tumor tissues, Karsli-Uzunbas and colleagues conditionally and systemically deleted autophagy-related 7 (*Atg7*), an essential autophagy gene, in adult mice. *Atg7*-deficient mice exhibited a shorter lifespan due to increased susceptibility to infection and neurodegeneration. Autophagy inhibition resulted in limited damage to normal tissues at early time points, whereas extended *Atg7* deficiency induced degenerative changes in multiple tissues and produced systemic metabolic defects, including depletion of lipid stores in white adipose tissue. In addition, *Atg7* loss impaired the ability of adult mice to tolerate starvation; this failure to survive fasting resulted from development of hypoglycemia and was accompanied by accelerated depletion of lipids and glycogen, decreased lipid mobilization, and severe muscle wasting, indicative of an essential role for autophagy in glucose homeostasis. Furthermore, acute *Atg7* deletion did not affect the initiation of *Kras*-mutant, *Trp53*-deficient lung tumors but was necessary for tumor maintenance; short-term autophagy ablation selectively suppressed established lung tumor growth and generated benign oncocytomas prior to normal tissue damage, suggesting that autophagy inhibition may be therapeutically beneficial. ■

See article, p. 914.

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NSD3–NUT Fusions Are a Therapeutic Target in NUT Midline Carcinoma

- *NSD3* is a recurrent *NUT*-fusion partner in NUT-variant NUT midline carcinoma (NMC).
- Enhanced proliferation and impaired differentiation in NSD3–NUT$^+$ NMC requires functional BRD4.
- Association of NSD3 with BRD4 promotes proliferation and blocks differentiation in BRD4–NUT$^+$ NMC.

NUT midline carcinoma (NMC) is an epithelial carcinoma caused by rearrangement of the *NUT* gene, usually via fusion to the BET family genes *BRD4* or *BRD3*. BRD–NUT oncoproteins promote proliferation and block differentiation, leading to an aggressive cancer that is difficult to treat. However, the mechanism by which BRD–NUT inhibits differentiation is unknown. Using a patient-derived NMC cell line, French and colleagues identified a novel *NUT* rearrangement that resulted in fusion with nuclear SET domain-containing protein 3 (*NSD3*, also known as *WHSC1L1*), which encodes a histone methyltransferase known to bind the extraterminal (ET) domain of BRD4. Analysis of additional NMC cases showed that *NSD3* is a recurrent *NUT*-fusion partner, found in four out of eight cases of NUT-variant NMC. The enhanced proliferation and impaired differentiation of these NMC cells were dependent on the presence of NSD3–NUT and its interaction with BRD4, and NSD3–NUT was sufficient to functionally substitute for BRD4–NUT, consistent with an oncogenic role for this fusion. Association of the NSD3 N-terminus with the BRD4 ET domain was also necessary to suppress differentiation in BRD4–NUT-expressing NMC cells and to induce formation of BRD4–NUT foci. Furthermore, BET inhibitor treatment induced differentiation and suppressed the proliferation of NSD3–NUT-expressing NMC cells, suggesting that this may be a beneficial therapeutic strategy. These findings identify *NSD3* as an oncogenic fusion partner and support further development of NSD3-targeted inhibitors for the treatment of NMC. ■

See article, p. 928.

AKT2 Has an Exclusive Role in Maintaining *PTEN*-Deficient Solid Tumors

- Depletion of AKT2 induces rapid and robust apoptosis in established *PTEN*-deficient spheroids.
- Silencing of AKT2 in *PTEN*-null cells upregulates p21, a critical mediator of AKT2-induced apoptosis.
- Whereas AKT1 knockdown is cytostatic, AKT2 depletion causes prostate cancer xenograft regression.

PTEN is frequently inactivated in many solid tumors, including prostate cancer, which depends on downstream hyperactivation of AKT for survival. However, the role of individual AKT isoforms in the maintenance of *PTEN*-deficient tumors is unclear. Chin and colleagues determined that whereas depletion of AKT1 or AKT2 prevented initial tumor spheroid formation in three-dimensional (3D) culture, knockdown or pharmacologic inhibition of AKT2 caused disintegration of established tumor spheroids derived from *PTEN*-deficient prostate, breast, and glioblastoma cell lines. Knockdown of AKT2 but not AKT1 or AKT3 induced apoptosis in tumor spheroids, indicating that this isoform is essential for *PTEN*-deficient tumor maintenance. Interestingly, depletion of AKT2 in two-dimensional culture reduced proliferation similar to depletion of AKT1 but led to a delayed and more modest apoptotic response in prostate cancer cell lines compared with 3D culture. Depletion of AKT2 resulted in upregulation of p21 and BAX and downregulation of the receptor tyrosine kinase insulin-like growth factor 1 receptor (IGF1R). Knockdown of p21 but not BAX rescued *AKT2* shRNA–induced apoptosis, suggesting that p21 is a downstream target of AKT2. In addition, pharmacologic IGF1R inhibition synergized with AKT2 depletion in promoting apoptosis. Importantly, although AKT1 silencing in established prostate cancer xenografts exhibited a largely cytostatic effect, AKT2 depletion increased active caspase 3 and p21 levels and led to tumor regression. These results indicate that AKT2 is required for the maintenance of *PTEN*-deficient tumors and highlight the need for preclinical development of AKT2-selective inhibitors. ■

See article, p. 942.

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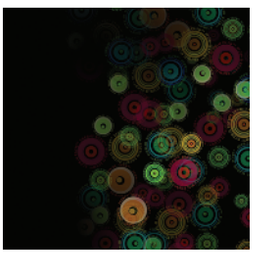
Single-Nucleus Sequencing Reveals Multiple *EGFR* Variants in Glioblastoma

- Single-cell sequencing provides the resolution necessary to identify subclonal oncogenic variants.
- Multiple *EGFR* variants exist in distinct, nonoverlapping tumor-cell subpopulations.
- The EGFRvII variant is oncogenic and may be therapeutically targeted using EGFR inhibitors.



Glioblastoma is a highly heterogeneous brain tumor characterized by mosaic amplification of genes encoding receptor tyrosine kinases (RTK), including the *EGFR* locus. Large-scale efforts to characterize bulk tumor genomes have identified multiple *EGFR* variants and missense mutations within a single tumor, but lack the resolution required to determine whether variants coexist and functionally cooperate within an individual cell. To assess tumor heterogeneity at the single-cell level, Francis, Zhang, Maire, and colleagues utilized a population-based framework method of single-nucleus sequencing in two *EGFR*-amplified primary glioblastomas and identified nonoverlapping *EGFR* variants generated by multiple somatic alterations in distinct tumor subclones. In addition, *EGFR* copy number and levels of EGFR truncation and C-terminal variants, including a novel deletion variant, were highly variable among individual cells. Clonal lineage evolution was reconstructed using a combination of coexisting clonal and subclonal events for reference, including *CDKN2A/CDKN2B* inactivation, *TERT* promoter mutation, and LOH, and was successfully used to identify subpopulations of cells defined by specific chromosomal alterations such as chromothriptic rearrangements. Notably, similar to EGFRvIII, expression of the EGFRvII variant was also oncogenic, as it promoted ligand-independent growth and tumor formation and was sensitive to EGFR inhibition *in vitro*, suggesting that this variant may be a therapeutic target. Together, these findings reinforce the notion that subclonal diversity and multiple alterations in a single RTK cooperatively contribute to tumorigenesis and treatment resistance in glioblastoma. ■

See article, p. 956.

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