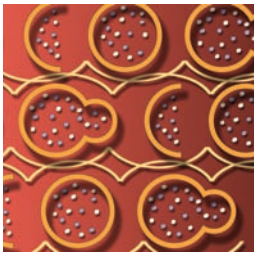
Autophagy Has Cell Intrinsic and Extrinsic Roles In Pancreatic Cancer

- A mouse model of PDAC with inducible autophagy inhibition allows evaluation of the effects on tumorigenesis.
- Blocking autophagy reduces PDAC cell growth, increases apoptosis, and elevates intratumoral macrophages.
- Autophagy inhibition suppress PDAC growth via both cell-autonomous and nonautonomous mechanisms.



Autophagy is increased in pancreatic ductal adenocarcinoma (PDAC) and supports tumor growth. However, the mechanisms by which autophagy promotes tumorigenesis have not been fully elucidated, and mouse models to investigate these mechanisms are lacking. Further, it is not clear if therapeutic inhibition of autophagy will be feasible. To determine the effects of targeting autophagy on PDACs, Yang and colleagues developed a mouse model that allowed reversible inhibition of autophagy in mouse models of autochthonous PDAC by inducible expression of a dominant negative mutant of ATG4B, a cysteine protease required to form the autophagosome membrane. Using this system, inhibiting autophagy reduced the growth of established PDAC tumors, and even intermittent autophagy inhibition was sufficient to suppress tumor growth and extend survival. Autophagy inhibition directly affected PDAC tumor cell growth, increasing apoptosis and reducing cell proliferation, but also increased intratumoral macrophages for an indirect effect on tumor growth. Accordingly, macrophage depletion reduced the antitumor effects of autophagy inhibition. Further, autophagy inhibition in the tumor stroma suppressed tumor seeding. In addition to delineating tumor cell intrinsic and extrinsic roles for autophagy in supporting PDAC maintenance, these results suggest that autophagy inhibitors warrant further investigation for the potential treatment of patients with PDAC. ■

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A Circulating Tumor Cell Signature May Guide Prostate Cancer Treatment

- A digital RNA-based circulating tumor cell (CTC) signature may serve as a prostate cancer biomarker.
- In prospective cohorts, CTC signatures predicted localized disease dissemination or drug response.
- Quantifying CTC-specific transcripts may enable noninvasive monitoring in patients with prostate cancer.

Blood-based biomarkers are needed to guide risk stratification and treatment in patients with prostate cancer, especially patients with advanced prostate cancer where metastatic lesions cannot be easily sampled. To identify potential predictive biomarkers of clinical outcomes, Miyamoto, Lee, and colleagues analyzed RNA from circulating tumor cells (CTC) from men with prostate cancer using microfluidic enrichment followed by digital quantitation of prostate-derived transcripts in a prospective trial of men with metastatic castration-resistant prostate cancer (mCRPC) treated with abiraterone. This sensitive, high-throughput approach allowed for the development of CTC digital signature based on expression of 8 prostate-specific transcripts differentially expressed in prostate cancer (*KLK3, KLK2, TMPRSS2, ARGR2, FOLH1, HOXB13, FAT1,* and *STEAP2*). In 27 men with mCRPC enrolled in a prospective study of first-line abiraterone, an elevated pretreatment metastatic signature (CTC_M) score was associated with more rapid disease progression and poor survival. Further, the expression of HOXB13 in CTCs was linked to shortened overall survival, as was presence of the androgen receptor splice variant transcript *ARV7*. A CTC score designed for more sensitive detection in localized prostate cancer (CTC_L score) predicted microscopic dissemination to seminal vesicles and/or lymph nodes in 34 men with clinically localized prostate cancer. These findings suggest that digital quantification of CTC-specific transcripts may allow noninvasive monitoring to guide treatment and predict outcomes in patients with metastatic or localized prostate cancer. ■

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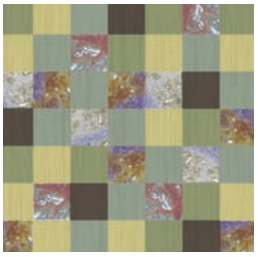
A Tumor Microenvironment Signature Is Predictive of Metastasis

- A stromal gene–enriched matrix index is predictive of the metastatic potential of human cancer.
- ECM changes were assessed by integrated molecular, cellular, and biomechanical analyses of ovarian cancers.
- Targeting matrix index molecules may enhance the efficacy of various therapeutic strategies.



The role of the tumor microenvironment (TME), which is created by the interactions of tumor cells and adjacent normal cells, has been well-characterized for tumor initiation but not as well for tumor metastasis. To obtain more in-depth insights into the evolution of the relationship between metastatic ovarian cancer and the TME during metastasis, Pearce, Delaine-Smith, Maniati, and colleagues analyzed the TME parameters of samples of metastatic ovarian cancer biopsies from 36 patients with high-grade serous ovarian cancer (HGSOC) representing different stages of metastasis. Analyses of six TME parameters—extent of disease (disease score), nonmalignant cell density, tissue mechanics, cytokines, and the matrisome RNA and protein profiles—revealed that the disease score was correlated with decreased densities of adipocytes and major remodeling of the extracellular matrix as well as increases in leukocyte density, cytokines that regulate leukocyte density and proliferation, proteoglycans, collagen bundles, and tissue stiffness. Disease score and tissue stiffness were both defined by a set of 22 matrisome molecules termed the matrix index, which was also correlated with immune-suppressive signatures. Evaluation of public gene expression datasets revealed that the matrix index was correlated with shorter survival in patients with HGSOC as well as patients with other epithelial or mesenchymal tumors. These results provide insights into the mechanism underlying the evolution of metastatic cancer cells within their metastatic microenvironment. ■

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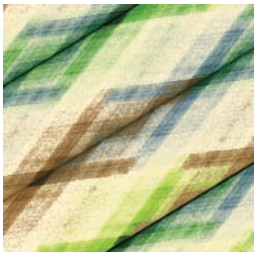
Aberrant MYC Expression Can Drive Neuroblastomagenesis *In Vivo*

- A transgenic zebrafish model shows that MYC expression is sufficient to transform neuroblasts.
- MYC upregulation via enhancer amplification or enhancer hijacking drives a subset of neuroblastomas.
- MYC is a potent oncogene in neuroblastoma and produces similar outcomes as *MYCN* amplification.



Amplification of *MYCN* drives approximately 20% of high-risk pediatric neuroblastomas and is associated with a poor prognosis, and an additional 10% of patients lacking *MYCN* amplification exhibit upregulation of MYC, suggesting that MYC may be an oncogenic driver in these tumors. To determine the role of MYC activation in neuroblastomagenesis, Zimmerman, Liu, and colleagues generated a transgenic zebrafish model of MYC overexpression driven by the dopamine beta-hydroxylase (*dβh*) promoter. MYC expression was sufficient to transform neuroblasts and promote neuroblastoma tumorigenesis in the zebrafish model, inducing tumorigenesis even more efficiently than MYCN. *MYCN* amplification and MYC upregulation were mutually exclusive in neuroblastoma cell lines, indicating that *MYC* may serve as a driver oncogene in tumors lacking *MYCN* amplification. However, *MYC* is not commonly amplified in neuroblastoma, suggesting that it may be upregulated by alternative mechanisms. Focal amplifications of enhancer regions downstream of *MYC* were observed in primary neuroblastomas and were associated with increased *MYC* expression. In another subset of neuroblastomas, *MYC* was upregulated by chromosomal translocations that resulted in enhancer hijacking. Further, clinical outcomes were similar between patients with MYCN-amplified and MYC-driven neuroblastoma, supporting a role for MYC as a driver oncogene. Collectively, these findings indicate that MYC is a potent oncogene in a subset of high-risk neuroblastomas, and demonstrate that MYC can be upregulated via enhancer amplification or enhancer hijacking. ■

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Gene Fusions May Be Actionable Drivers in HR$^+$ Breast Cancer

- Targeted next-generation sequencing identifies fusion genes that drive HR$^+$ breast cancer.
- Fusions involving *PIK3CA*, *AKT1*, *RAF1*, or *ESR1* were identified in 14% of advanced HR$^+$ breast cancers.
- Gene fusions were linked to drug resistance and may be potential targets in HR$^+$ breast cancer.

Hormone receptor–positive (HR$^+$) breast cancer, expressing the estrogen receptor (ER) or progesterone receptor (PR), represents the majority of cases and generally has a favorable prognosis. However, some patients with HR$^+$ breast cancer develop resistance to hormonal therapy and progress to metastatic disease. Mutations in *PIK3CA*, *TP53*, or *ERBB2* occur frequently in patients with HR$^+$ breast cancer, but driver mutations have not been identified in a subset of tumors. To identify additional driver mutations in HR$^+$ breast cancer, Matissek, Onozato, Sun, and colleagues used anchored multiplex PCR (AMP) to identify gene rearrangements in 173 patients with advanced HR$^+$ breast cancer. Intergenic fusions were discovered in 14% (24 of 173) of patients, including *ESR1*-associated fusions in 8 patients, and 2 fusions each involving *AKT3*, *NOTCH1*, *PRKCA*, and *BRAF*. The presence of these fusions was confirmed using FISH. Overexpression of identified PIK3CA, RAF1, or AKT3 kinase fusions was sufficient to activate mTORC1 signaling and induce oncogenic deregulation of breast epithelial cells in three-dimensional cultures. Further, AKT3 and RAF1 fusions conferred resistance to AKT and MEK1/2 inhibitors, respectively. *In vivo*, the *RPS6KC1–AKT3* fusion accelerated tumor growth. These tumors were resistant to estrogen withdrawal, but could be resensitized by CDK4/6 inhibition. In patients with HR$^+$ breast cancer, fusion-positive tumors were associated with shorter overall survival. Altogether, these findings suggest that intergenic fusions commonly drive tumorigenesis and drug resistance in HR$^+$ breast cancer, suggesting potential therapeutic targets. ■

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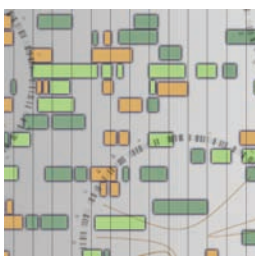
Diverse Genetic Alterations Converge on MAPK and PI3K Signaling in TNBC

- RNA and whole-exome sequencing identify potential oncogenic drivers in mouse models of TNBC.
- Heterogeneous alterations that activate MAPK or PI3K signaling occur in approximately half of TNBCs.
- Combined whole-exome and RNA sequencing may guide selection of therapeutic targets in TNBC.



No specific targeted therapies are available for patients with triple-negative breast cancer (TNBC). *TP53* mutations occur frequently in TNBC, but the majority of identified oncogenic genomic alterations are not widely recurrent, limiting their potential as therapeutic targets. To identify driver mutations in TNBC, Liu, Murphy, and colleagues performed whole-exome sequencing (WES) and RNA sequencing (RNA-seq) on genetically engineered mouse models of TNBC with breast-specific deletion of *Trp53* with and without loss of *Brca1*. WES revealed a low mutation rate, with an average of 30 somatic mutations in coding exons, and few of these alterations occurred in multiple tumors. Combined WES and RNA-seq in 72 tumors identified spontaneous amplifications (*Met*, *Yap1*, *Egfr*, and *Fgfr2*), deletions (*Pten*), oncogenic mutations (*Kras* and *Hras*), and fusion genes (*Fgfr2–Dnm3*, *Fgfr2–Tns1*, *Fgfr2–Zmydn8*, *Dhx9–Raf1*, *Rpl32–Raf1*, and *Dlg1-Braf*) in distinct tumors. Tumors with FGFR2 fusion proteins responded to FGFR2 inhibition, and tumors with RAF1 fusion proteins or MET overexpression responded to MEK inhibition. Overall, a potential driver event was identified in approximately 50% of the evaluated tumors, and, although the alterations were diverse, the majority increased activation of MAPK or PI3K signaling. Similarly, MAPK- or PI3K-activating mutations occurred in approximately 90% of human TNBC samples from The Cancer Genome Atlas. These data indicate that combined WES and RNA-seq may be beneficial in identifying targets for precision medicine, and the identification of diverse MAPK and PI3K activating mutations nominates these pathways as potential targets. ■

See article, p. 354.

In This Issue is written by *Cancer Discovery* editorial staff. Readers are encouraged to consult the original articles for full details.