**Tumor Microenvironment**

**Major finding:** Stromal cancer-associated fibroblasts (CAF) shape PDAC tumor heterogeneity and behavior.  
**Concept:** Stromal CAFs induce MAPK and STAT3 signaling associated with increased PDAC proliferation and metastasis.  
**Impact:** The impact of stromal cells on tumor heterogeneity can influence tumor response to therapy.

**CANCER-ASSOCIATED FIBROBLASTS CONTRIBUTE TO PDAC HETEROGENEITY**

The tumor microenvironment comprises a heterogeneous collection of normal and tumor cells, including cancer-associated fibroblasts (CAF). In pancreatic ductal adenocarcinoma (PDAC), this heterogeneity has made it difficult to discern the precise contribution of stromal CAFs to tumor progression and aggressiveness. To better define the role of CAFs in regulating PDAC heterogeneity, Ligorio, Sil, and colleagues performed single-cell RNA sequencing (scRNA-seq) and proteomics in mouse models and patient samples of PDAC. scRNA-seq of patient-derived PDAC and CAF cells cocultured at various ratios revealed two gene signatures contributing to proliferation (PRO) and epithelial-mesenchymal transition (EMT) within PDAC cells, a subpopulation of which coexpressed both signatures (DP) when cultured at the highest CAF:PDAC ratio. Coculturing patient-derived PDAC cell lines with CAFs caused a positive shift toward both PRO and EMT phenotypes with increased proliferation and invasion in vitro and accelerated tumor growth and metastatic tumor burden in vivo. Further, PDAC cell lines exposed to CAF-conditioned media underwent a shift toward the DP phenotype and increased activation of MAPK and STAT3 signaling pathways. Similarly, enrichment of MAPK and STAT3 signaling in the DP population was also observed in primary and metastatic patient PDAC tumors; combined pharmacologic inhibition of these pathways abrogated the DP phenotype and suppressed PDAC cell invasion in vitro. CAF-derived TGFβ1 was a critical factor for PDAC cell proliferation, as treatment with a neutralizing antibody against TGFβ1 impaired proliferation; subsequent introduction of recombinant TGFβ1 restored proliferation and induced the DP phenotype in PDAC cells. Analysis of PDAC patient tumor glands revealed a relationship between stromal content and PDAC cell type and identified 8 distinct gland types defined by their composition of DP, PRO, EMT, and double-negative cells and correlated with response to different therapies. These results demonstrate the importance of stromal CAFs in influencing PDAC tumor composition, signaling, biology, and response to therapy.


**Targeted Therapy**

**Major finding:** Sequential inhibition of PARP and WEE1 limits tumor growth and reduces toxicity in multiple cancers.  
**Mechanism:** Elevated basal replication stress in tumor cells renders them susceptible to sequential inhibition.  
**Impact:** A sequential PARPi and WEE1i therapeutic regimen should be assessed in humans.

**SEQUENTIAL INHIBITION OF PARP AND WEE1 MINIMIZES TOXICITY**

Although multiple potent inhibitors of S and G2 checkpoint proteins, such as the WEE1 inhibitor (WEE1i) adavosertib, have been shown to limit tumor cell growth, their clinical efficacy has been limited due to high toxicity. Fang and colleagues evaluated the therapeutic potential of combined inhibition of poly(ADP-ribose) polymerase (PARP) and WEE1 in various cancer types. Multiple cell lines subjected to olaparib (a PARPi) or adavosertib exhibited increased activity of S and G2 DNA-damage checkpoint proteins, and treatment with the PARPi talazoparib induced a G2-M arrest. Concurrent PARPi and WEE1i treatment abrogated PARPi-induced G2 arrest and resulted in increased mitotic catastrophe and subsequent apoptosis. In a xenograft model of ovarian cancer, concurrent treatment with PARPi and WEE1i inhibited tumor growth, yet prolonged therapy induced significant weight loss which required cessation of treatment, resulting in tumor relapse. PARPi-associated proteomic signatures and DNA damage induced by PARPi treatment persisted for days following removal of PARPi in vitro and in vivo, suggesting that sequential therapy could maintain efficacy. Consistent with these findings, subsequent sequential treatment in vitro with PARPi and WEE1i was as effective as concurrent treatment in inducing apoptosis and limiting tumor cell growth, regardless of the order of treatment. In normal cells, which have less basal replication stress (RS) than cancer cells, sequential treatment with PARPi and WEE1i reduced RS induction compared with concurrent treatment, mitigating the toxicity associated with concurrent treatment; consistent with these findings, pretreatment with hydroxyurea or cyclin E overexpression increased basal RS in normal cells, which sensitized them to sequential treatment with PARPi and WEE1i. In vivo, sequential treatment with PARPi and WEE1i preserved the antitumor efficacy of monotherapy or concurrently combined therapy without the associated toxicities of the latter regimens. These results indicate that sequential treatment with PARPi and WEE1i significantly reduces toxicity in vivo and may be an effective therapeutic strategy in patients.

Sequential Inhibition of PARP and WEE1 Minimizes Toxicity


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