

RESEARCH WATCH

Pancreatic Cancer

Major finding: TGF β induces a SMAD4-dependent EMT in the pancreas that promotes apoptosis.

Mechanism: EMT-dependent KLF5 inhibition by SNAIL switches SOX4 into a proapoptotic factor.

Impact: In certain contexts, TGF β -induced EMT can be tumor suppressive instead of oncogenic.

TGF β MEDIATES TUMOR SUPPRESSION VIA INDUCTION OF EMT-LINKED APOPTOSIS

Depending on the cellular context, transforming growth factor β (TGF β) has been observed to exert protumorigenic functions, such as inducing an epithelial–mesenchymal transition (EMT), or inhibit tumorigenesis by activating apoptosis. The proapoptotic functions of TGF β have been linked with the transcription factor SMAD4, which acts downstream of TGF β and is frequently deleted in pancreatic ductal carcinoma (PDAC). To elucidate the mechanism by which TGF β promotes apoptosis, David and colleagues used a *Kras*-mutant/*Smad4*-deleted PDAC murine model and found that reintroduction of SMAD4 sensitized cells to TGF β treatment and promoted changes in cell morphology and loss of E-cadherin consistent with EMT. Moreover, SNAIL was shown to be upregulated following TGF β treatment, and genetic depletion of SNAIL inhibited TGF β -induced EMT, apoptosis, and accelerated pancreatic carcinogenesis in SMAD4–wild-type cells, raising the unexpected possibility that EMT precedes apoptosis and is required for TGF β -mediated tumor suppression in the pancreas. An RNAi screen of TGF β -responsive genes to identify those necessary for *Smad4*-mutant PDAC growth identified the transcription factor gene *Sox4*, and chromatin immunoprecipitation sequencing and motif anal-

ysis revealed that SOX4-occupied loci were enriched for binding sites of the lineage-specific transcription factor KLF5. KLF5 co-occupied the vast majority of SOX4 binding sites and suppressed TGF β -induced apoptosis in *Smad4*-mutant PDAC cells, suggesting that KLF5 may determine SOX4 function in this context. Indeed, upon TGF β -driven EMT, KLF5 expression was suppressed by EMT-associated transcription factors including SNAIL, which served as a molecular trigger to switch SOX4 function from protumorigenic to proapoptotic and led to SOX4-dependent transcription of the proapoptotic genes *Bim* and *Bmf*. Together, these data suggest that TGF β -mediated EMT induces transcriptional reprogramming that alters the cellular KLF5:SOX4 ratio and allows for SOX4-mediated induction of apoptosis. These findings also provide an explanation for the frequent inactivation of SMAD4, but not TGF β receptors, in pancreatic cancer, and elucidate a mechanism by which TGF β -induced EMT is tumor suppressive in certain contexts. ■

David CJ, Huang Y-H, Chen M, Su J, Zou Y, Bardeesy N, et al. TGF- β tumor suppression through a lethal EMT. *Cell* 2016;164:1015–30.

Metabolism

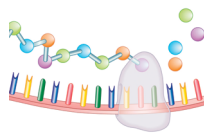
Major finding: Proline is restrictive in kidney cancer, and deletion of a proline production enzyme inhibits growth.

Approach: Differential ribosome codon reading identifies restrictive amino acids required for tumor growth.

Impact: Ribosome profiling can identify tumor-specific amino acid vulnerabilities that may be targeted.

DIFFERENTIAL RIBOSOME CODON READING REVEALS LIMITING AMINO ACIDS

The shortage of certain amino acids can restrict tumor growth, with different tumor types depending on specific amino acids for growth and survival. Asparagine deprivation therapy with L-asparaginase has been utilized to treat patients with acute lymphoblastic leukemia, serving as a proof of concept for therapies targeting amino acid vulnerabilities. To determine which amino acids are limiting in specific tumors, Loayza-Puch, Rooijers, and colleagues developed a differential ribosome codon reading method (termed diricore) using ribosome profiling measurements to detect the availability of amino acids for protein synthesis. This approach used global ribosome occupancy to identify limiting amino acids. In cancer cells, L-asparaginase treatment resulted in a specific diricore signal at asparagine codons, an increase in the enzyme responsible for synthesizing asparagine, and a reduction in aminoacylated asparagine-tRNAs. Diricore identified robust signals at methionine and proline codons in clear cell renal cell carcinoma (ccRCC) compared to normal tissues. The methionine signal was due to ribosomes at the ATG start codon, indicating a global increase in the rate of translation initiation, whereas the proline



signal suggested a limited availability of proline in the tumors. Consistent with this idea, ccRCCs had high levels of non-aminoacylated proline-tRNAs and high expression of pyrroline-5-carboxylate reductase 1 (PYCR1), an enzyme involved in proline synthesis. PYCR1 upregulation serves as a compensatory mechanism to maintain tumor growth. Knockdown of PYCR1 increased the level of non-aminoacylated proline-tRNAs and reduced ccRCC cell proliferation under conditions of limited proline availability, which were reversed by the addition of proline. Further, in xenografts of breast cancer cells with high PYCR1 expression, diricore signals were strong at proline codons, and PYCR1 knockdown suppressed cell growth *in vivo*. Taken together, these findings indicate that diricore is able to identify limiting amino acids that are required for tumor growth, and suggest that this method may detect amino acid vulnerabilities that may be rationally targeted in cancer therapy. ■

Loayza-Puch F, Rooijers K, Buil LCM, Zijlstra J, Oude Vrielink JF, Lopes R, et al. Tumour-specific proline vulnerability uncovered by differential ribosome codon reading. *Nature* 2016;530:490–4.

CANCER DISCOVERY

TGF β Mediates Tumor Suppression via Induction of EMT-Linked Apoptosis

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