Runx3 Directs Cellular Traffic in Pancreatic Cancer

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Deaths from pancreatic cancer are primarily attributable to metastasis, but a small fraction of patients die from local disease. Pancreatic ductal adenocarcinoma (PDA) is particularly aggressive, metastasizing early and to a high degree. However, a small fraction of PDA patients die from aggressive primary disease, and the reasons for these distinct disease outcomes are not clear. PDA generally begins as lesions known as pancreatic intraepithelial neoplasms (PanIN), which typically carry activating mutations in KRAS and inactivating mutations in CDKN2A/INK4A and TP53, with later event in PanIN-to-PDA progression being loss of DPC4/SMAD4. Previous studies of these mutations in mice have yielded some insights into the pathogenesis of PDA. Mice expressing pancreas-restricted Kras and Trp53 mutations (KPC) die from a combination of local and metastatic disease.
disease, similarly to the majority of human patients. In contrast, loss of Dpc4/Smad4 in the context
of an activating Kras mutation reduces metastatic potential. To further understand the molecular
factors underlying the metastatic program in PDA, postdoctoral fellow Dr. Martin Whittle and
colleagues in the laboratory of Dr. Sunil Hingorani (Clinical Research and Public Health Sciences
Divisions) generated new combinations of PDA mutations in mice and charted disease
progression. They found that Dpc4 gene dosage affects levels of the Runx3 transcription factor, and
in turn, the levels of Runx3 can predict and explain possible disease courses in patients.

The authors first generated mice carrying a heterozygous activating Kras mutation, a heterozygous
inactivating Trp53 mutation, and a heterozygous deletion of Dpc4 (KPDC), all targeted to tissue
progenitor cells of the developing pancreas. KPDC developed pancreatic tumors similar to those
found in KPC mice, but displayed shortened lifespan in comparison to mice without the Dpc4
deletion. Strikingly, KPDC mice displayed a large decrease in metastatic disease, contrary to
expectations from their shortened lifespan. Thus, the course of PDA development in KPDC mice is
shifted to a higher primary tumor burden versus metastatic disease. Analysis of a program of
morphological and molecular changes known as the epithelial-to-mesenchymal transition (EMT),
which is associated with metastasis, revealed a surprising insight: while both KPC and KPDC cells
underwent EMT, KPDC cells were impaired in migration and invasion.

To gain further insight into the molecular bases of the distinct PDA behaviors in KPC and KPDC
mice, the authors performed gene expression profiling in carcinoma cells from each mouse
strain. This analysis identified 15 genes expressed at least 2-fold higher in KPC than KPDC cells,
potentially representing genes that promote metastasis. Of these, the most highly expressed was
the transcription factor Runx3, at a level of 36-fold higher in KPC than KPDC.

Runx3 function in PDA was investigated using primary KPC and KPDC cells. Overexpression of
Runx3 in KPDC cells increased migration, while silencing Runx3 inhibited KPC migration. Growth of
cells in soft agar, testing anchorage-independent growth, was similarly affected. Further analysis
revealed direct regulation of the Osteopontin (Spp1) gene, which is upregulated in human PDA and
is a marker of poor prognosis, by Runx3. Spp1 is secreted and promotes cell migration, and it was
found that mice with high metastatic burden had high levels of circulating Spp1. Another gene
upregulated by Runx3, Col6a1, was found to increase cell migration and increase the ability of KPC
cells to seed lung metastases.

The authors next sought to determine if the Runx3 protein also performed similar functions in human
PDA cells, using two of the most highly and one of the lowest RUNX3-expressing lines. As in mice,
RUNX3 levels were predictive of migratory potential and were associated with SPP1 and COL6A1.
expression levels. They next analyzed the potential of RUNX3 gene expression levels to predict disease course, survival after surgery, and therapeutic response. In a cohort of PDA patients who underwent resection, RUNX3 levels were correlated with survival, with patients with low RUNX3 having a greater rate of survival than those with high RUNX3. Patients with low also RUNX3 levels also benefitted most from local radiation therapy, while those with high RUNX3 responded well to systematic radiotherapy, consistent with the study’s finding that high RUNX3 levels promote metastasis.

"The work helps us understand key aspects of the metastatic drive in pancreas cancer and potential ways to exploit that knowledge in the clinic. It also opens up important new areas of investigation including, for example, the existence of EMT-associated and EMT-independent modes of metastasis," said Dr. Hingorani.