Runx3 Directs Cellular Traffic in Pancreatic Cancer

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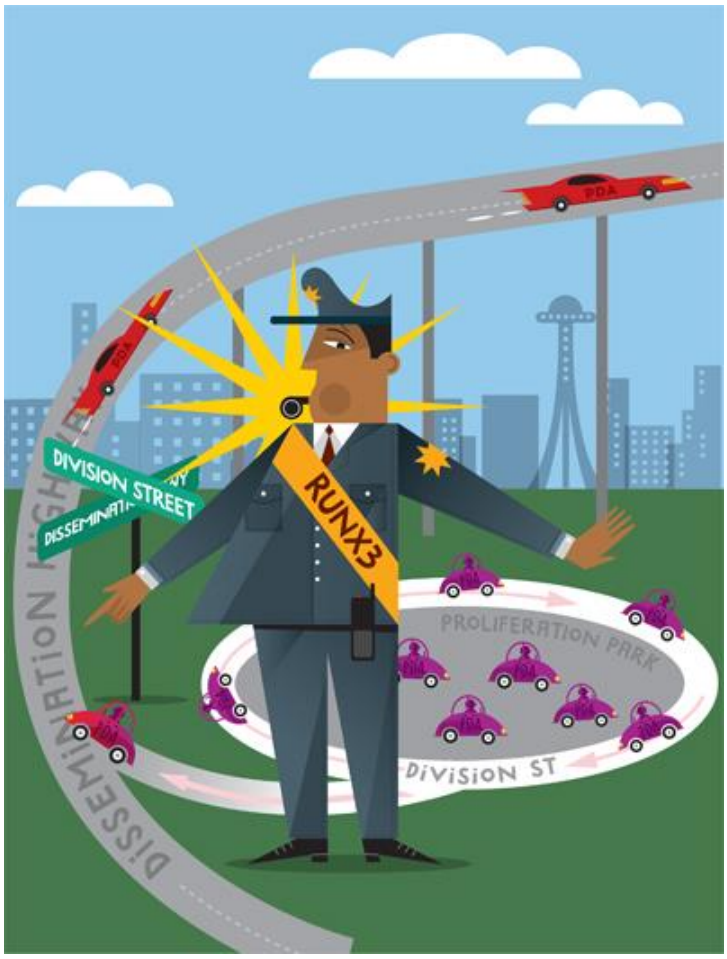

Runx3 acts as a traffic cop, deciding whether PDA cells will continue to circle Proliferation Park and stay on Division Street, or whether they will take the on-ramp to Dissemination Highway, leading to metastatic disease.
Image provided by Kimberly Carney, Fred Hutch News Service

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, 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.

Whittle MC, Izeradjene K, Rani PG, Feng L, Carlson MA, DelGiorno KE, Wood LD, Goggins M, Hruban RH, Chang AE, Calses P, Thorsen SM, Hingorani SR. 2015. RUNX3 Controls a Metastatic Switch in Pancreatic Ductal Adenocarcinoma. *Cell* 161(4):1345-1360.