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AUTHOR'S VIEW

RUNX3 defines disease behavior in pancreatic ductal adenocarcinoma

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ABSTRACT

Runt-related transcription factor 3 (*RUNX3*) functions downstream of transforming growth factor beta (TGFβ) and plays dual roles in pancreas cancer by both suppressing (by inhibiting proliferation) and promoting (by enhancing migratory and metastatic capacity) disease progression. Consideration of the contextual regulation of *RUNX3* together with its myriad downstream effects may help improve clinical outcomes for pancreas cancer patients.

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The last few decades of intense research have not improved the prognosis for patients with pancreatic ductal adenocarcinoma (PDA), which nearly always portends a rapid and painful death. PDA has an unusual proclivity for metastatic spread, with 53% of PDA patients presenting with clinically evident metastatic disease at the time of diagnosis.[1] For the remaining 47% of patients with locoregional disease, surgical resection can extend survival but provides little hope for cure.[2] Ultimately, these patients also succumb to metastatic or recurrent PDA, suggesting that microscopic dissemination is an early hallmark of the disease.[3]

Against this relentlessly challenging clinical backdrop, substantial progress has been made toward defining the genetic alterations that contribute to pancreatic cancer initiation and progression. Oncogenic *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) mutations are found in approximately 95% of pancreatic cancer patients and function as an initiating event that is further compounded by additional mutations or loss of tumor suppressor genes such as *tumor protein p53* (*TP53*) and *SMAD family member 4* (*SMAD4*).[4] Identification of these cardinal mutations has led to the development of robust preclinical models that faithfully recapitulate the hallmarks of PDA in mice, supplanting less predictive models (such as immortalized cell lines or xenografts) that only partially approximate the phenotypes of autochthonous PDA. As examples, oncogenic *Kras* and *Trp53* mutations have been engineered into their endogenous loci to allow pancreas-specific activation of these alleles using the Cre-Lox system. $Kras^{LSL\text{-}G12D/+}$;$Trp53^{LSL\text{-}R172H/+}$;$p48^{Cre/+}$ (*KPC*) mice develop autochthonous tumors of the pancreas that closely mimic the clinical syndrome, histologic features, and metastatic potential of human PDA.[5] More recently, a floxed *Dpc4*/*Smad4* allele that allows conditional deletion of this tumor suppressor gene was engineered into *KPC* mice to generate a $Kras^{LSL\text{-}G12D/+}$;$Trp53^{LSL\text{-}R172H/+}$;$Dpc4^{flox/+}$;$p48^{Cre/+}$ (*KPDC*) model of PDA.[6] The primary PDA from these *KPDC* mice progressed more rapidly than their *KPC* littermates, at the apparent expense of an attenuated metastatic burden. We identified the transcription factor *runt-related transcription factor 3* (*Runx3*), which is frequently overexpressed in *KPC* PDA but uncommon in *KPDC* PDA, as the key factor defining the distinct metastatic potentials of these 2 disease presentations. Runx3 enhanced the migratory potential of invasive *KPC* PDA cells and also stimulated the release of soluble factors that supported distant colonization of disseminated cells. We further showed an association of RUNX3 expression in the tumor epithelia with patient survival and defined the RUNX3 target osteopontin (SPP1) as a marker for distant relapse in PDA patients who underwent pancreatic resection.

Perhaps not surprisingly for such a potentially important metastatic switch, *Runx3* levels are regulated by several inputs operating at both the transcriptional and post-translational levels. In particular, the mutational status of *Trp53* and the gene dosage of *Dpc4* act cooperatively to define a *Runx3* set-point in 3 distinct genetic and phenotypic disease states: (1) highly metastatic and (comparatively) less locally aggressive disease in *KPC* mice; (2) less metastatic, more locally aggressive PDA in *KPDC* mice (i.e., loss of one allele of *Dpc4*); and (3) recovered metastatic potency in a highly proliferative local disease, generating an unusually lethal combination in *KPDDC* animals (i.e., complete loss in *Dpc4*/*Smad4*). Wild-type *Trp53* contributes to Runx3 degradation whereas point mutation of *Trp53* stabilizes it, leading to elevated levels of Runx3 with loss of heterozygosity (LOH) of *Trp53* in *KPC* animals. *Dpc4* gene dosage regulates *Runx3* in a biphasic manner: Runx3 levels are high when both copies of *Dpc4* are intact, decrease dramatically with loss of one *Dpc4* allele, and recover once again with LOH of the locus to generate functionally null *Dpc4* tumors. Although the myriad details of the contributors defining Runx3 levels remain to be elucidated, the combined assessment of Runx3 expression and *Dpc4* status in primary tumors can potentially be used to

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predict the most likely cause of patient demise, namely, local disease progression versus distant dissemination, and tailor therapies accordingly.

These findings also contribute to the sometimes vitriolic debate over whether *RUNX3* functions as an oncogene or tumor suppressor gene in human malignancies.[7,8] We believe it can be either or both, depending not only on the specific context, but also on whether one is considering primary tumor growth or metastatic spread (Fig. 1). In PDA, RUNX3 appears to suppress primary tumor growth through upregulation of key cell cycle inhibitors such as *cyclin-dependent kinase inhibitor 1A* (*CDKN1A* or *p21*), while promoting an invasive and metastatic phenotype by inducing secreted proteins like SPP1 and collagen type VI α1 (COL6A1) that stimulate motility and support distant colonization. This context-dependent classification of *RUNX3* as an oncogene or a tumor suppressor gene mirrors, and perhaps contributes to, the dual nature of TGFβ signaling, which lies upstream of *RUNX3*, in tumorigenesis.[9] The biphasic regulation of *RUNX3* expression as a function of *SMAD4* status further links *RUNX3* to the dichotomous TGFβ pathway and also provides a mechanism to promote PDA metastasis in the absence of canonical TGFβ signaling, in which the epithelial-to-mesenchymal transition (EMT) is surprisingly not observed.

Thus, *RUNX3* orchestrates a concerted program that tilts the balance from cell division to dissemination and targeting *RUNX3* and/or other downstream effectors may help to restrain PDA metastasis. The potential for increased local proliferation with inhibition of *RUNX3* can be counterbalanced with complimentary strategies specifically targeting cell cycle mediators such as cyclin-dependent kinases 4 and 6 (CDK4/6).[10] As a marker of metastatic potential, however, *RUNX3* can also potentially provide a tool to assess the proclivity of a patient's tumor for metastasis versus local growth. This, in turn, can inform the discourse on treatment options for a given patient, maximizing the value of existing modalities such as radiation and cytotoxic chemotherapy, even as we strive to develop more targeted approaches. For example, patients with low RUNX3 levels who are at lower risk for metastatic spread, might be spared the most aggressive systemic treatments (either in the neoadjuvant or adjuvant settings) and may instead benefit from a local therapy such as radiation. Conversely, patients with high RUNX3 in their tumor epithelial cells would be more likely to benefit from systemic chemotherapy, with the possibility of adding radiotherapy depending on the presence or absence of SMAD4. The sequence, scheduling, and duration of the various treatment modalities could likewise be informed by assessing RUNX3 levels and implications for tumor behavior.[6] Future studies will be required to validate or refute these hypotheses, but our deepening understanding of the metastatic drive in PDA through such integrated and iterative analyses of human specimens and novel genetically engineered mouse models will ultimately lead to more definitive treatments that significantly change the outlook for patients with this insidious disease.

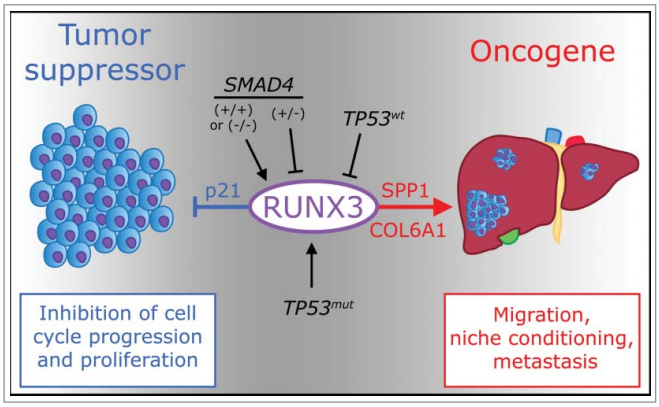

Figure 1. Dual function of *RUNX3* as oncosuppressor and oncogene. Expression of *runt-related transcription factor 3 (RUNX3)* in pancreatic cancer is influenced by genetic alterations of *tumor protein p53 (TP53)* and *SMAD family member 4 (SMAD4)*. *RUNX3* levels respond in a biphasic manner to *SMAD4* status: both wild-type and homozygous loss of *SMAD4* promote *RUNX3* expression, but heterozygous deletion of *SMAD4* inhibits it. *TP53* mutation and subsequent loss of heterozygosity stabilize *RUNX3* expression. *RUNX3*, in turn, induces expression of *cyclin-dependent kinase inhibitor 1A* (*CDKN1A* or *p21*), inhibiting cell cycle progression and proliferation, and also upregulates both osteopontin (SPP1) and collagen type VI α1 (COL6A1), which promote pancreas cancer cell migration and condition a metastatic niche favoring distant colonization.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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