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COMMENTARY

Beyond Stiffness

Collagen Signaling in Pancreatic Cancer and Pancreas Regeneration

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Pancreatic ductal adenocarcinoma (PDA) is projected to be the second leading cause of cancer-associated deaths in the United States by 2030.[1] The dismal prognosis of PDA patients is due to early dissemination of the tumor, therapy resistance, and the fact that most patients present with locally advanced or metastatic disease at diagnosis.[2] Thus, a small percentage of patients are candidates for surgical resection, the current most effective therapy. PDA patients receive chemotherapy and radiotherapy as standard treatment, which often renders limited improvement of prognosis; as a result, the current overall 5-year survival rate after PDA diagnosis is approximately 10%.

PDA is characterized by a prominent stroma, composed of a wide variety of nonneoplastic cells, including cancer-associated fibroblasts, myeloid cells, lymphocytes, and vascular endothelial cells, as well as marked extracellular matrix (ECM) deposition.[3] This complex fibrotic network often constitutes up to 80% to 90% of the tumor volume and has important functions in PDA progression, including tumor growth, invasion, metastasis, immune evasion, and therapy resistance. For example, as a result of extensive ECM deposition, interstitial fluid pressure is elevated within the tumor, which impairs perfusion and drug delivery.[4] ECM is also considered a barrier for T-cell infiltration, resulting in a significant fraction of PDA being T-cell deficient.[5] In addition, the ECM also promotes tumor progression directly through ECM-activated signaling pathways on tumor cells.[6] Despite the observation that ECM signaling can support PDA progression, multiple studies have reported that high stromal content of PDA correlates with a more favorable outcome in PDA patients.[7,8] Furthermore, the depletion of ECM-producing cancer-associated fibroblasts in robust preclinical models resulted in a more aggressive PDA phenotype and reduced survival.[9] These contrasting data suggest that the stroma may also have tumor-restricting function and underline the complexity of ECM biology in PDA. Therefore, it is important to understand the function of specific ECM components and the signaling pathways they activate.

Collagens are the most abundant ECM protein in PDA stroma. Collagens function as major structural components of the ECM and interact with cells through cell surface receptors. There are four major classes of collagen receptors: integrins, discoidin domain receptors (DDRs), glycoprotein VI, and leukocyte-associated Ig-like receptor-1.[10] Among these collagen receptors, DDRs are unique in that they are receptor tyrosine kinases. DDRs contain an N-terminal extracellular discoidin domain containing a collagen binding site. There are two types of DDRs: DDR1 and DDR2. Expression of DDR1 and DDR2 can overlap, but generally DDR1 is expressed by epithelial cells, whereas DDR2 is expressed in cells of mesenchymal origin. DDRs are important for physiological development, as evidenced by the phenotype of *Ddr1*- or *Ddr2*-deficient animals, which includes smaller organ size, reduced bone growth, and defects in lactation and embryo implantation.[11,12] Yet, the tissue-specific functions of DDRs are not

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fully understood. More important, considering the abundance of collagens in the stroma of different cancer types, especially PDA, identifying the contribution of DDRs to tumor progression is critical and potentially an untapped therapeutic avenue.

In an effort to understand the contribution of DDR1 in pancreas remodeling and PDA progression, Ruggeri et al,[13] in this issue for the first time genetically ablated *Ddr1* in *KPC* mice (*Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Ptf1a*$^{Cre/+}$), which is a genetically engineered mouse model widely used to recapitulate the progression of PDA. Ruggeri et al[13] found that *Ddr1* was expressed highly in PDA, and strikingly, *Ddr1*-null *KPC* mice developed well-differentiated, less proliferative, and poorly metastatic tumors compared with control *Ddr1*−wild-type *KPC* mice. These data are consistent with previous studies from our group and others, suggesting that DDR1 functions to promote PDA progression. For example, collagen signaling through DDR1 can drive tumor cell plasticity.[14,15] Consistent with this, pharmacologic inhibition of DDR1 significantly reduces PDA tumor growth, reduces cancer cell invasiveness, and improves chemotherapy response *in vivo*.[16,17] DDR1 activity has also been implicated in driving tumor progression in other cancer types. For instance, genetic and pharmacologic inhibition of DDR1 reduced tumor initiation and tumor progression in *KRAS*-mutant lung adenocarcinoma, and the combinatory inhibition of DDR1 and Notch signaling induced the regression of *KRAS*-mutant patient-derived lung xenografts with a therapeutic efficacy that was favorable compared with standard chemotherapy.[18] However, in breast cancer, the function of DDR1 is more complex. An earlier study found that DDR1 signaling enables breast cancer cells to undergo metastatic reactivation in multiple target organs,[19] whereas a more recent study genetically ablated *Ddr1* in *MMTV-PyMT* mice and found that *Ddr1*-null mammary tumors grew faster, featured by a more basal phenotype with increased epithelial tension, matricellular fibrosis, and lung metastases.[20]

Therefore, the function of DDR1 appears to be cancer type or microenvironment dependent. This might be due to cell lineage differences of DDR1-expressing cells in different cancer types. How DDR1^{+} cells contribute to the progression of cancer might determine the outcome of DDR1 targeting. For instance, in the *MMTV-PyMT* model, *Ddr1* was found to be expressed by luminal cells but not myoepithelial cells, and deletion of *Ddr1* induced basal differentiation of CD90^{+}CD24^{+} cancer cells, resulting in the increase of mitotic basal cells.[20] Intriguingly, an unbiased bioinformatics study based on large human genomic and transcriptomic data sets reported that *DDR1* expression is significantly enriched in a small subset of drug-resistant aldehyde dehydrogenase-positive (ALDH^{+}) stem-like cancer cells in breast cancer, independent of cancer molecular subtype.[21] This report suggests that targeting DDR1 remains an attractive goal in breast cancer. In *KRAS*-mutant lung cancer, DDR1 was also found to be expressed by a subset of cancer cells exhibiting an aggressive transcriptional profile, although cell lineage was not specifically identified.[18] Although Ruggeri et al[13] found that *Ddr1* deletion strikingly inhibited *KPC* tumor progression, deletion of *Ddr1* did not improve survival because loss of *Ddr1* also induced severe pancreatic atrophy. As a result, the animals died from pancreatic insufficiency. This strongly suggests that DDR1 is expressed by a subset of cells that can regenerate exocrine pancreas function during tissue damage. To further investigate the function of *Ddr1* at an earlier stage of PDA tumorigenesis, Ruggeri et al[13] crossed *Ddr1*-null mice into *KC* (*Kras*$^{G12D/+}$; *Ptf1a*$^{Cre/+}$) animals and treated these mice with cerulean to induce experimental pancreatitis. Interestingly, *Ddr1* was expressed at a low level in normal pancreas but was markedly up-regulated during tissue damage caused by pancreatitis or neoplastic lesions. Compared with control mice, the pancreatic acinar cell population recovered more slowly in *Ddr1*-null mice, due to a delay in epithelial cell proliferation. As a result, *Ddr1*-null mice failed to resolve tissue damage at 6 weeks after cerulean treatment, resulting in a dramatic decrease of pancreatic acinar tissue mass.

Ruggeri et al[13] hypothesized that the delay in damage recovery was due to a disruption in the regeneration potential of acinar cells. They investigated a Stathmin1−positive acinar cell population, which is a recently identified functionally and molecularly distinct acinar subpopulation with progenitor properties.[22] Following chemical-induced pancreatitis, these cells proliferate to recover the injured acinar tissue. At baseline, control and *Ddr1*-null mice had similar numbers of Stathmin1-positive cells. After injury, control mice showed a large expansion of Stathmin1-positive, proliferating cell nuclear antigen−positive cells; however, the expansion was substantially delayed and quickly subsided in *Ddr1*-null mice. These data strongly suggest that DDR1 is critical for acinar progenitor expansion to regenerate the injured pancreas. This is relevant to the observation that DDR1 enhances PDA cancer cell plasticity and resistance to chemotherapy. It is important for future studies to determine the function of Stathmin1-positive cells during PDA progression and whether these cells have cancer stem cell properties; in addition, it is also important to determine whether DDR1 contributes to stemness and the expansion of this cancer cell population that leads to tumor aggressiveness and therapy resistance.

Another interesting phenotype of the *Ddr1*-null *KPC* mice is increased fibrosis, which was also observed in *Ddr1*-null *MMTV-PyMT* tumors.[20] This might be a compensatory response due to the failure of injured tissue regeneration. Consistent with these observations, the total number of fibroblasts increased in *Ddr1*-null pancreas during recovery following cerulean treatment. However, because *Ddr1* was deleted globally in this study, it is difficult to determine whether this fibrotic reaction was induced by paracrine signals indirectly or a direct effect of *Ddr1* ablation in fibroblast progenitors. A conditional *Ddr1* deletion model in a cell type−specific manner may help address this question. More important, DDR1 is a well-appreciated contributor to the fibrotic reaction and collagen remodeling in other fibrotic diseases, such as pulmonary fibrosis and renal fibrosis.[23,24] Recent advances in single-cell transcriptomic technologies will enable the identification of DDR1-expressing cells

in different organs and tissues, to better understand the cell-specific function of DDR1 involved in tumorigenesis and fibrosis in the future.

Taken together, this study from Ruggeri et al[13] illuminates the important function of DDR1 during pancreatic acinar tissue regeneration and PDA development. The extensive desmoplasia and high expression of DDR1 in PDA strongly suggest that it is a potential therapeutic target in desmoplastic tumors, such as PDA. Although orally available DDR1-specific inhibitors are available,[17,25] their chronic use should be recommended with the knowledge that genetic ablation of *DDR1* can cause acinar atrophy and pancreatic insufficiency. Pancreatic insufficiency can be mitigated, at least temporarily, by supplementation with dietary pancreatic enzymes.[26] In conclusion, data from Ruggeri et al[13] further validate collagen-induced DDR1 activation as a critical component of pancreas remodeling and PDA progression and highlight DDR1 as an attractive target for consideration in PDA therapy.

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