Turning Up the Heat on the Pancreatic Tumor Microenvironment by Epigenetic Priming

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Abstract

The study by Gonda and colleagues, in this issue of *Cancer Research*, represents the first combinatorial approach based on epigenetic therapy priming to overcome resistance to immunotherapy in pancreatic cancer. The authors show that treatment with a DNA hypomethylating agent causes profound changes in the pancreatic cancer microenvironment, including increased numbers of tumor-infiltrating T cells, elevated IFN signaling, and immune checkpoint expression, as well as increased antigen presentation in tumor cells. Accordingly, they show that the combination of decitabine plus immune checkpoint blockade effectively restores antitumor immunity and results in a significant survival benefit in a widely accepted mouse model of pancreatic cancer. The study provides evidence for a new therapeutic approach for pancreatic cancer having antitumor efficacy through modulation of the immune suppressive microenvironment, leading to an increased response to immune checkpoint inhibitors. As the incidence of pancreatic cancer continues to increase, new treatment strategies for this devastating disease are urgently needed. Gonda and colleagues provide preclinical proof of concept for a new therapeutic strategy and address an unmet need for this difficult to treat disease.

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Pancreatic cancer often has a poor prognosis and is projected to become the second leading cause of cancer-related death in the United States behind lung cancer by 2030. The NCI's Surveillance, Epidemiology, and End Results (SEER) Program estimates that there will be 57,600 people diagnosed with pancreatic cancer in the United States and some 47,050 deaths from these cancers in 2020. The 5-year survival rate for pancreatic cancer is only 10.0% and has not changed in decades.

Immunotherapy has emerged as a promising treatment modality in a variety of malignancies and the development of immune checkpoint blockade drugs has transformed the cancer therapy landscape. The recent success of immunotherapy for some solid cancers has increased interest that this approach might also be effective in pancreatic cancer. Immunotherapy-based approaches are currently being pursued in the laboratory and in clinical trials for pancreatic cancer, including checkpoint inhibitors targeting CTLA-4 and PD-1/PD-L1, dendritic cell–based vaccines, and chimeric antigen receptor T cells (1), but to date these have been met with limited success and whether immunotherapy is an option in pancreatic cancer as monotherapy remains to be established.

The unique characteristics of the pancreatic cancer microenvironment itself represent a treatment barrier (2) and immune-suppressive properties of the tumor stroma contribute to the lack of benefit of immunotherapy in the disease. These include immunosuppressive cells [myeloid-derived suppressor cells, regulatory T cells (Tregs), and tumor-associated macrophages], high expression of immune checkpoint receptors, and lack of infiltrating cytotoxic T cells or antigen-presenting cells (1). Potential strategies to overcome microenvironmental barriers in preclinical cancer models of pancreatic cancer and other solid tumors as well as in clinical trials include combining immune modulators with epigenetic drugs (3, 4). The immunomodulatory activity of hypomethylating agents (HMA), in particular, suggests that they may improve the effectiveness of cancer immunotherapies, and HMAs have been used as priming agents for augmenting the clinical effectiveness of cancer immunotherapies (5), however, this combination approach has not been previously examined in pancreatic cancer.

Epigenetic alterations are prominent in pancreatic ductal adenocarcinomas (PDAC; ref. 6). Building on a previous study by the authors demonstrating that single-agent HMA inhibits tumor growth and prolongs survival in a rapid-onset PDAC mouse model (7), Gonda and colleagues used low doses of a HMA in different treatment protocols to reinvigorate antitumor immunity in a slower onset PDAC model (8), which is more commonly used and genetically similar to most human PDACs. They took an "epigenetic priming" approach using decitabine (5aza-dC; DAC) followed by immune checkpoint inhibitors (ICI) anti-PD1 or anti-VISTA (V-domain Ig suppressor of T-cell activation or PD-1H, an abundant immune checkpoint mediator in PDAC). While modest activity with single-agent DAC or ICI alone on tumor growth and mouse survival was observed, DAC followed by ICI therapy markedly inhibited tumor growth and prolonged mouse survival, with the most striking results obtained using DAC followed by anti-PD-1. The mechanistic discoveries and translational implications of these novel findings have significance toward moving their approach forward.

In addition to the significant effect of the combined DAC-ICI approach on survival, the authors observed important effects of single-agent DAC. The HMA increased tumor necrosis, decreased cell proliferation, and increased the number of tumor cells expressing two key cell-cycle progression inhibitors cyclin-dependent kinase inhibitors, *p21* and *p16*, which are often downregulated or silenced in human pancreatic cancers. These mechanistic data reveal why there is a loss of cell proliferation. In addition to the observed direct effect on the epithelial tumor cells, the authors demonstrated non-cell–autonomous effects of the HMA to recruit or expand a unique subset of immune cells. DAC alone increased the number of tumor-infiltrating lymphocytes (TIL) in the tumor microenvironment. Furthermore, DAC treatment increased recruitment and number of T cells expressing two checkpoint molecules, PD1 and VISTA, providing mechanistic explanation for the robust response to ICI and also suggesting a dual targeting approach of both ICI inhibitor molecules in combination with HMA to further improve efficacy.

The increase in tumor-infiltrating effector T cells after DAC treatment, which contributed to boosting the response to ICI, was accompanied by an increase in a subpopulation of "alternatively activated" or "M2-

polarized" macrophages. This was an unexpected finding, as M2-polarized macrophages are widely associated with an immune-suppressed microenvironment. The significance of this observation remains to be established, but the presence of the unique subset M2 macrophages may have contributed to the fact that although the DAC plus ICI was highly efficacious, the combination treatment was not curative. Targeting the M2-polarized macrophages or preventing their influx or expansion in combination with DAC-ICI may provide additional benefit. Therefore, a new approach to improve combining epigenetic therapy and immunotherapy may include targeting this subset of putatively immunosuppressive M2-macrophages.

Gonda and colleagues (8) demonstrated that DAC induced multiple immune pathways in pancreatic tumor cells, including upregulation of IFN-responsive genes, and that induction of endogenous retroviruses (ERV) and double-stranded RNA contributed to these pathways. These changes in cytokine expression and ERVs provide mechanistic explanation for why there is more immune cell infiltration and why DAC seems to be making the tumor "hotter" and more responsive to ICI. Furthermore, the findings are in agreement with other reports regarding the emerging concept that HMAs may act, at least in part, by inducing viral-like responses (viral mimicry) to ERVs and enhanced activation of the innate immune system, including the type I IFN system (9). In patients with various hematologic malignancies and independent of disease classification, Ohtani and colleagues reported that HMA induced ERVs and the type I IFN pathway in patients that responded to HMA (10), the first report of activation of the innate immune system and clinical response in patients with cancer treated with a DNMT inhibitor. Although correlating viral mimicry and response in patients with solid tumors has not yet been validated, it is encouraging that results from the study by Ohtani and colleagues were obtained from diverse disease entities (10).

Finally, it is now well-accepted that epigenetic therapies will require combinatorial approaches for most cancers, and the translational potential of the DAC-ICI combination for pancreatic cancer is supported by a number of key findings. First, no systemic toxicity was observed with the HMA, indicating that this

therapeutic combination will be well-tolerated in patients with pancreatic cancer. On the basis of this, Gonda and colleagues (8) advocate more frequent DAC and anti-PD-1 dosing and longer treatment, as well as HMA combined with dual ICI. Second, it is also thought that early epigenetic therapy administration will be essential, particularly because epigenetic therapies require long exposure in patients to be effective. Observations in paradigm III of this study (8) assessed the effects of DAC when the drug was started earlier, before the formation of tumors. The authors observed tropism of TILs to preinvasive pancreatic situ neoplasia lesions and although the number of TILs was not increased by DAC treatment, the presence of TILs at the early stage may be significant. Administering DAC early in disease progression or at initial diagnosis has the potential to block DNA methylation programming of the tumor microenvironment epigenome, has a greater impact on boosting the response to immunotherapy, and provides potential clinical benefit to patients with pancreatic cancer. The recent approval of oral DAC (INQOVI, Astex Pharmaceuticals, Inc.) by the FDA for myelodysplastic syndromes provides yet another epigenetic tool for use in pancreatic cancer and other solid tumors in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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