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A self antigen reopens the games in pancreatic cancer

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Abbreviations: ENO1, α-enolase; GEM, genetically engineered mice; KC, *Kras*$^{G12D/+}$*Pdx1-Cre*$^{/+}$; KPC, *Kras*$^{G12D/+}$*Trp53*$^{R172H/+}$*Pdx 1-Cre*$^{/+}$; PDA, pancreatic ductal adenocarcinoma; TAA, tumor-associated antigen

We have recently demonstrated that the administration of a plasmid coding for α-enolase can elicit robust immune responses in genetically engineered mice that spontaneously develop pancreatic cancer, resulting in a significant improvement of their survival. This approach provides a springboard for the elaboration of new forms of immunotherapy for pancreatic cancer.

Pancreatic ductal adenocarcinoma (PDA) is one of the most chemoresistant—and hence lethal—cancers. Historically, neither chemotherapy nor radiotherapy has provided any significant survival benefit to PDA patients. In spite of intensive efforts, any attempt to improve the survival of PDA patients made in the past 15 years has indeed failed. This remained true even with the introduction of agents that would specifically target the signaling pathways considered of the utmost importance for the development and progression of PDA. The epidermal growth factor receptor (EGFR) inhibitor erlotinib combined with gemcitabine was shown slightly ameliorate patient survival as compared with conventional gemcitabine-based chemotherapy, but this was not the case for other EGFR-targeting agents as well as for inhibitors of matrix metalloproteases, farnesyltransferase and the vascular endothelial growth factor (VEGF).[1] Recently, the FOLFIRINOX (5-flourouracil, leucovorin, oxaliplatin and irinotecan) chemotherapeutic regimen has been associated with a limited survival advantage in patients affected by advanced PDA.[2] Still, therapeutic strategies that significantly prolong the survival of PDA patients are missing.

Immunotherapy has been considered as a potential approach to the management of pancreatic cancer. A few PDA-associated antigens, namely CEA, KRAS, MUC1 and gastrin, have been used to develop anticancer vaccines and have already been tested in clinical trials. These vaccines exerted little impact on the survival of PDA patients, yet some more recent approach hold some promises.[3] The failure of these vaccines to improve patient survival may be due—at least in part—to the fact that the immunogenicity of the corresponding tumor-associated antigens (TAAs) may not be sufficient to induce adaptive immune responses against malignant cells. Therefore, antigens that elicit both cytotoxic T lymphocyte (CTL) responses and humoral immunity may be more effective than agents that only activate a single arm of the immune system.

However, the therapeutic efficacy of immune responses involved in tumor rejection not only depends on proper antigen presentation by dendritic cells, but also on the magnitude of $CD4^+$ T-cell responses, which provide critical signals for the priming and maintenance of effector T cells. The selection of appropriate TAAs is therefore crucial for the design of efficient anticancer vaccines and an essential prelude to the induction of strong immune responses against cancers, especially very aggressive ones like PDA.

Serological approaches constitute useful tools for the identification of novel TAAs. By serological proteome analysis (SERPA), we have identified a dozen of PDA-associated antigens that are specifically recognized by circulating autoantibodies. Antibodies against one of these antigens, α-enolase (ENO1), can be detected in over 60% of PDA patients.[4] ENO1 localizes to the cytoplasm, where it functions as a glycolytic enzyme, as well as to the plasma membrane, where operates as a plasminogen receptor and plays an important role in cell migration.[5,6] ENO1-specific T-cell responses can be detected in PDA patients who bear ENO1-specific autoantibodies but not in those who do not.[5] Upon transfer into immunocompromised mice, ENO1-specific T cells inhibit the growth of xenotransplanted human pancreatic tumors. Despite the ubiquitous expression of ENO1, normal cells are spared by ENO1-specific CTLs, presumably because they express low levels of this enzyme.[5] These results have led us to develop a DNA vaccine targeting ENO1.

Improvements in genetic engineering have driven the establishment of ever more refined murine models of human cancer, allowing researchers to address important mechanistic and therapeutics questions. There are strains of genetically engineered

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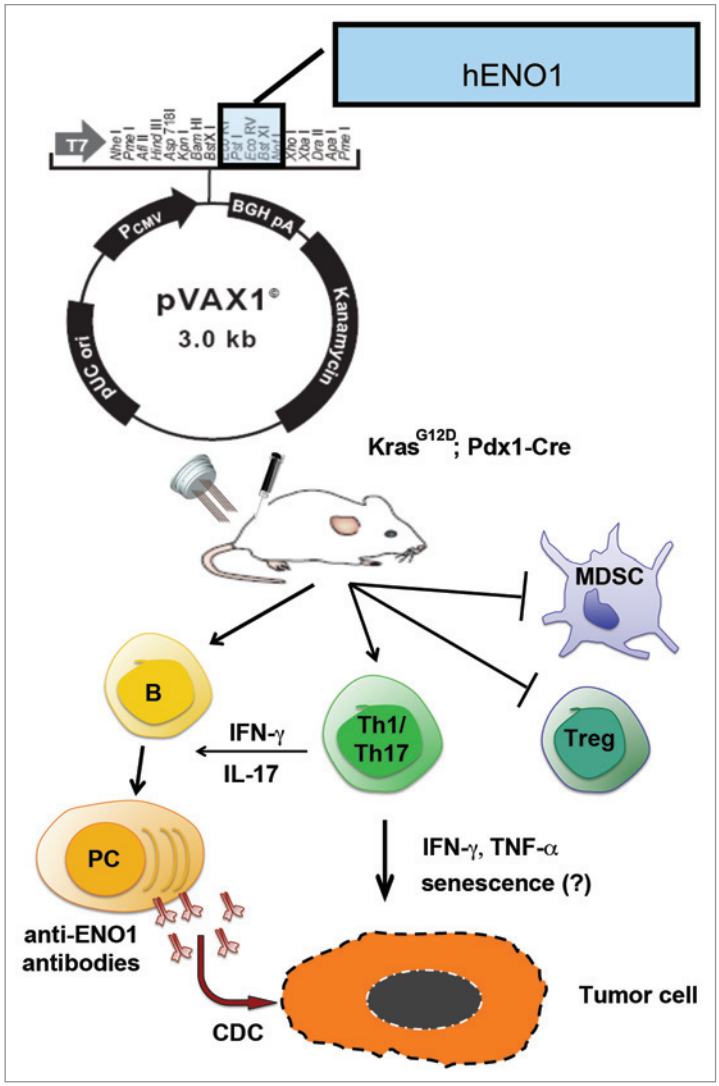

Figure 1. Effector mechanisms elicited by a DNA vaccine targeting α-enolase in murine models of spontaneous pancreatic cancer. The administration of an α-enolase (ENO1)-coding plasmid coupled to electroporation induces the production of IgG antibodies specific for ENO1 that are able to activate complement-dependent cytotoxicity (CDC) against pancreatic ductal adenocarcinoma (PDA) cells. The concomitant activation of ENO1-specific T_H1 and T_H17 cells and the release of interferon γ (IFNγ) and intereleukin-17 (IL-17) favor the isotypic switch of this humoral response toward cytotoxic IgG subclasses. IFNγ and tumor necrosis factor α (TNFα) released by T_H1 and T_H17 cells can also induce the senescence of tumor cells. Finally, a parallel reduction in the intratumoral abundance of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) allows for the elicitation of robust ENO1-specific T-cell responses.

mice (GEM) that spontaneously develop PDA, which are already being exploited for the development of novel diagnostic and therapeutic strategies.[7] We have used two of these strains to assess both the prophylactic and therapeutic potential of a ENO1-targeting DNA vaccine. Genetically engineered *Kras^{G12D}Pdx1-Cre* (KC) and *Kras^{G12D}Trp53^{R172H}Pdx1-Cre* (KPC) mice spontaneously develop lethal pancreatic carcinomas with different kinetics.[8] Both KC and KPC mice were vaccinated with a plasmid encoding human ENO1, which displays more than 95% identity (99% homology) with its mouse ortholog, resulting in the induction of a specific immune response that significantly prolonged survival: from 336–474 d for KC mice (representing the longest overall survival for these animals ever reported), and from 203–245 d for KPC mice. The ENO1-targeting DNA vaccine activated several immune effector mechanisms, including the production of high levels anti-ENO1 IgG antibodies, the activation of ENO1-specific T_H1 and T_H17 cells, as well as an intense recruitment of $CD3^+$ cells to the tumor bed (**Fig. 1**). Notably, anti-ENO1 IgGs were able to bind to murine PDA cells and induce their killing via complement-dependent cytotoxicity, while T_H1/T_H17 cytokines favored the switching to effector antibody subclasses. Furthermore the ENO1-targeting DNA vaccine significantly decreased the abundance of immunosuppressive cells in the tumor microenvironment, including myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) (**Fig. 1**). Of note, when these immunosuppressive cells rebounded to levels similar to those of control mice, tumor progression was no longer counteracted and animals died.[9] Still, the therapeutic efficacy of the ENO1-targeting DNA vaccine appeared to be very promising, especially when the administration protocol started at 8–9 mo of age.[9]

Altogether, our findings indicate that it may be possible to design adjuvant therapies to elicit anti-ENO1 responses to prevent tumor recurrence in resected PDA patients and to prolong survival of those patients that are not eligible to surgery. Appropriate immunochemotherapeutic regimens could transform our encouraging preclinical results into an effective clinical protocol. Accumulating evidence indicates indeed that multiple anticancer agents, including classic chemotherapeutics as well as targeted compounds, stimulate tumor-specific immune responses either by inducing immunogenic cell death or by engaging immune effector mechanisms.[10]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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