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Immunotherapy in preneoplastic disease: targeting early procarcinogenic inflammatory changes that lead to immune suppression and tumor tolerance

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Abstract

Recent advances in immunotherapy have demonstrated that single agent vaccines can be effective when given as primary prevention before exposure to the causative agent, and partially effective in some patients with existing cancer. However, as tumors develop and progress, tumor-induced immune suppression and tolerance present the greatest barrier to therapeutic success. Preneoplastic disease represents an important opportunity to intervene with tumor antigen–targeted vaccines before these mechanisms of immune evasion outpace efforts by the immune system to destroy precancerous cells. However, as we discuss in this review, emerging evidence suggests that procarcinogenic inflammatory changes occur early in cancer development, in both patients and mouse models of cancer progression. Defining early inhibitory signals within tumor microenvironments will yield insights that can eventually be used in the clinic to target these events and deliver treatments that can be used in addition to cancer vaccines to prevent premalignant and early invasive cancers.

Keywords

cancer vaccines; preneoplasia; immunotherapy; pancreatic cancer

Introduction

The recent FDA-approval of two immunotherapies for cancer treatment has established immunotherapy as a legitimate therapeutic modality as well as an emerging and exciting area of clinical research with a rich pipeline that may yield many new cancer drugs. Yet, despite this progress, immune-based therapies so far have demonstrated only limited success in the clinic. One reason for this failure is that clinical trials testing immune-based therapies

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Conflicts of interest

E.M.J. receives commercial consulting fees from Bristol-Meyers-Squibb (Pancreatic Advisory Board Consultant), has the potential to receive royalties, milestone payments, and travel reimbursement from BioSante and Aduro Biotech (compliance governed by Johns Hopkins University Conflict of Interest Committee).

typically study patient populations with advanced disease who have failed many prior therapies, indicating that their cancer is already resistant to multiple treatment modalities. A second explanation is based on the recent discovery that mechanisms of immune evasion and suppression develop earlier than previously thought, at the time of the earliest stages of cancer development. These findings from preclinical models and advanced cancer patients imply that multiple mechanisms of immune tolerance have been established by the time of cancer diagnosis. With improved screening techniques and the discovery of new biomarkers and genetic signatures defining different cancer biologies, there is hope for detection and treatment of cancer in its premalignant or early invasive stages. Immunotherapy aimed at this population should be based on knowledge of early suppressive signaling events present in preneoplasia; thus, continued investigation into these early networks may be essential for successful development of early immunologic interventions.

Lessons learned from virally associated cancers

Cancer vaccines are inherently different than vaccines for infectious disease. Most vaccines for infectious disease are used in a primary prevention setting and given in childhood before disease exposure. They induce neutralizing antibody responses to clear infections before they can cause harm. However, much can be learned from the use of immunotherapy to treat persistent viral infections and the cancers that can arise as a result. Vaccines for established or chronic infection have a much lower success rate than those administered for primary prevention, as with the example of human papilloma virus (HPV) vaccination. The two FDA-approved vaccines for HPV are aimed at young adolescents with the goal of decreasing sexually transmitted disease and ultimately, HPV-associated cancers. In populations with no exposure to the HPV strains targeted by the vaccine, there is greater than 90% efficacy for prevention of infection and cervical intraepithelial neoplasia (CIN).[1] However, in studies of all women who were vaccinated regardless of HPV infection status, this rate dropped to 50% or lower, due to ineffectiveness in previously or currently infected individuals.[1] Although this has likely contributed to the design of the preventive HPV vaccine, which elicits neutralizing antibodies, other HPV vaccines attempting to enhance the cellular immune response as therapeutic interventions have had similarly low rates of success. In one trial administering a DNA vaccine targeting mutated E7 to women with $HPV16^+$ CIN stage two and three (CIN2/3), the rates of regression following vaccination with the highest dose were 33%, slightly higher than the rate of spontaneous regression in historical controls (25%).[2]

Foreign antigens are more likely to elicit a powerful immune response than self-antigens from spontaneously occurring cancers, making virally related cancers an ideal candidate for the use of cancer vaccines. However, even early on in persistent infections, before the development of cancer, there is downregulation of $CD8^+$ T cell responses to viral antigens, resulting in viral immune escape. Hepatitis C infection often becomes chronic, leading to liver fibrosis, and eventually in some cases, hepatocellular carcinoma. The standard of treatment for hepatitis C infection has been, for many years, pegylated interferon-alpha, a potent immunostimulator, and ribavirin, an antiviral; this combination results in viral clearance and recovery in 43–80% of infected individuals, depending on viral genotype.[3] As in other persistent infections such as HIV, T cells in hepatitis C–infected patients demonstrate markers of exhaustion and decreased activation, such as impaired cytokine secretion and upregulated immune checkpoint markers, including PD-1, CTLA-4, and TIM-3.[3] Despite the role of the immune system in eliminating these viral infections and preventing virally associated cancers, there are early events that prevent the maximum function of the immune response, creating barriers to treatment by immunotherapy.

Targeting early oncogene expression in cancer development

With the advent of technically superior sequencing techniques, there has been rapid progress in the characterization of genetic mutations in many tumor types. This provides a framework of initiating genetic alterations, including oncogene activation or tumor suppressor loss, which can be targeted with immunotherapy earlier in disease. Interventions made within this window of opportunity could prevent preneoplastic lesions from progressing to cancer, a strategy that could be particularly useful for populations known to be at increased risk for particular types of cancer. However, results from the few attempts at vaccination against precancer, both in preclinical models and clinical trials, have overall been ineffective, but also provide valuable insights into how these vaccination strategies can be improved.

In addition to clinical trials for CIN2/3, a precursor to cervical cancer, cancer vaccines have been used in a clinical setting for ductal carcinoma *in situ* (DCIS), a noninvasive breast cancer that has the potential to become invasive. In a trial of a dendritic cell vaccine targeting HER-2/*neu*, 18.5% of patients had a complete response following vaccination, with lower rates in patients who had estrogen receptor–positive DCIS.[4] HER-2/*neu* expression was down-regulated in 50% of patients who had residual DCIS, suggesting vaccination could induce immunoediting in cancers that were not eradicated by the immune system.[4] In preclinical treatment models, there have been similar findings on the partial efficacy of cancer vaccines in the course of cancer progression. In a mouse model of spontaneous colon cancer, intervention with a dendritic cell vaccine prevented progression to colon cancer by reducing the total number of intestinal polyps.[5] However, the vaccine, which targets an overexpressed protein in colon cancer and induced $CD4^+$ and $CD8^+$ T cell responses, did not result in a statistically significant increase in survival.[5] Thus, there is increasing evidence that mechanisms of treatment resistance and immune evasion that occur in advanced cancers are also present to some degree in preneoplastic conditions. As the use of immunotherapy in preneoplasia moves forward, it will become increasingly important to define what these suppressive signals are and identify druggable targets for use in combination with vaccination.

Barriers to prevention of cancer progression by cancer vaccines

Pancreatic ductal adenocarcinoma (PDA) is characterized by an intense desmoplastic response that occurs in the stroma, presenting unique challenges for drug delivery and effective treatment. Mouse models of spontaneous developing pancreatic cancer have been genetically engineered and are based on expression of dominant active K-Ras and loss of p53 expression, two common genetic alterations in human PDA.[6,7] These mouse models recapitulate the progression to PDA via pancreatic intraepithelial neoplasia (PanIN) stages one, two, and three, accompanied by increasing genetic and cellular abnormalities, as well as the characteristic stromal reaction.[7] This model, therefore, provides an opportunity to define the sequential events that contribute to a suppressive tumor microenvironment, which is poorly vascularized and highly treatment resistant. As observed in many other cancers, $CD4^+Foxp3^+$ T regulatory (T_{reg}) cells and $CD11b^+Gr\text{-}1^+$ cells are two dominant populations infiltrating cancers, but they are also present in PanINs.[7] Studies in this mouse model of PDA have revealed that granulocyte macrophage colony-stimulating factor (GM-CSF) is secreted by tumors, which in turn, recruits $CD11b^+Gr\text{-}1^+$ cells responsible for impairing $CD8^+$ T cell function and barring their infiltration into tumors.[8]

In addition, the PDA mouse model recapitulates the early expression of the $Kras^{G12D}$ oncogene mutation, known to occur early in human PDA development. Mutated *Kras* is present in approximately 40% of early stage, and 87% of late stage, PanINs in humans, and likely contributes to GM-CSF secretion, early infiltration of the $CD11b^+Gr\text{-}1^+$ cells, and T

cell suppression.[6] K-Ras has also been shown to induce production of IL-8, a transcriptional target of this oncogene,[9] and a known attractant of granulocytes and macrophages, providing another mechanism for recruiting suppressive immune cells to the developing tumor.[9] Work is ongoing in our laboratory and others to more completely characterize these cells infiltrating the site of pancreatic tumor development and the signals that recruit them to PanINs, so as to build on genetic and histopathological models of cancer progression with an additional inflammatory progression model (Fig. 1).

In addition to recruiting suppressive immune cell populations, developing tumors alter the microenvironment in other ways to evade the protective antitumor response. CIN2/3 patients who had spontaneous regression of their cervical lesions had weak T cell responses to E6 and E7 antigens in their blood, yet were found to harbor extensive $CD8^+$ T cell infiltrates in cervical epithelium.[10] Lesions that persisted were characterized by the absence of $CD8^+$ T cells and downregulation of MAdCAM-1, the receptor for $\alpha_4\beta_7$ integrin, which is expressed by $CD8^+$ T cells infiltrating cervical tissue.[10] Thus, in these early lesions that do not regress, the dysplastic epithelium has already developed mechanisms for excluding $CD8^+$ T cells and evading the immune system.

Additional barriers to T cell activation, both systemically and within tumor microenvironment, include immune checkpoint molecules, such as CTLA-4, PD-1, and LAG-3, which dampen T cell signaling following antigen recognition. Tumors up-regulate inhibitory ligands for these T cell checkpoint molecules, representing an adaptive strategy to inhibit T cell activation. With the U.S. Food and Drug Administration (FDA) approval of anti-CTLA-4 for metastatic melanoma and trials investigating drugs to target other immune checkpoints, monoclonal antibodies against immune checkpoints represent a novel class of cancer therapeutics. In the future, it is likely that we will see the combined use of cancer vaccines to stimulate an antitumor T cell response and the blockade of immune checkpoints to overcome inhibitory signaling encountered within the tumor microenvironment. As research continues in this field, we may find that immune checkpoints are also expressed in preneoplasia and warrant targeting in combination with vaccination, even in early disease.

Conclusions

Over the past few years, the opportunities for the use of immunotherapy in cancer have expanded. However, challenges to effective treatment remain in the form of suppressive tumor microenvironments, and infiltrating cell populations induced and recruited by tumor signaling. In addition, we are discovering that these suppressive signaling networks begin early in the progression of cancer. This window of time before cancer has progressed provides an ideal setting for the use of immunotherapy while there is a role for the protective antitumor immune response. Combination immunotherapy to induce potent $CD8^+$ T cell responses and to target suppressive signaling will be necessary, even at the earliest stages, to switch off the progression to cancer and immune evasion, and turn on mechanisms that eliminate preneoplasia. As the ability to detect and treat early stage cancers increases, interventions used in combination with traditional cancer therapies or vaccines should include agents that modulate the early suppressive events occurring in cancer development. Continuing to define what these signals are is critical for building a model of inflammatory progression.

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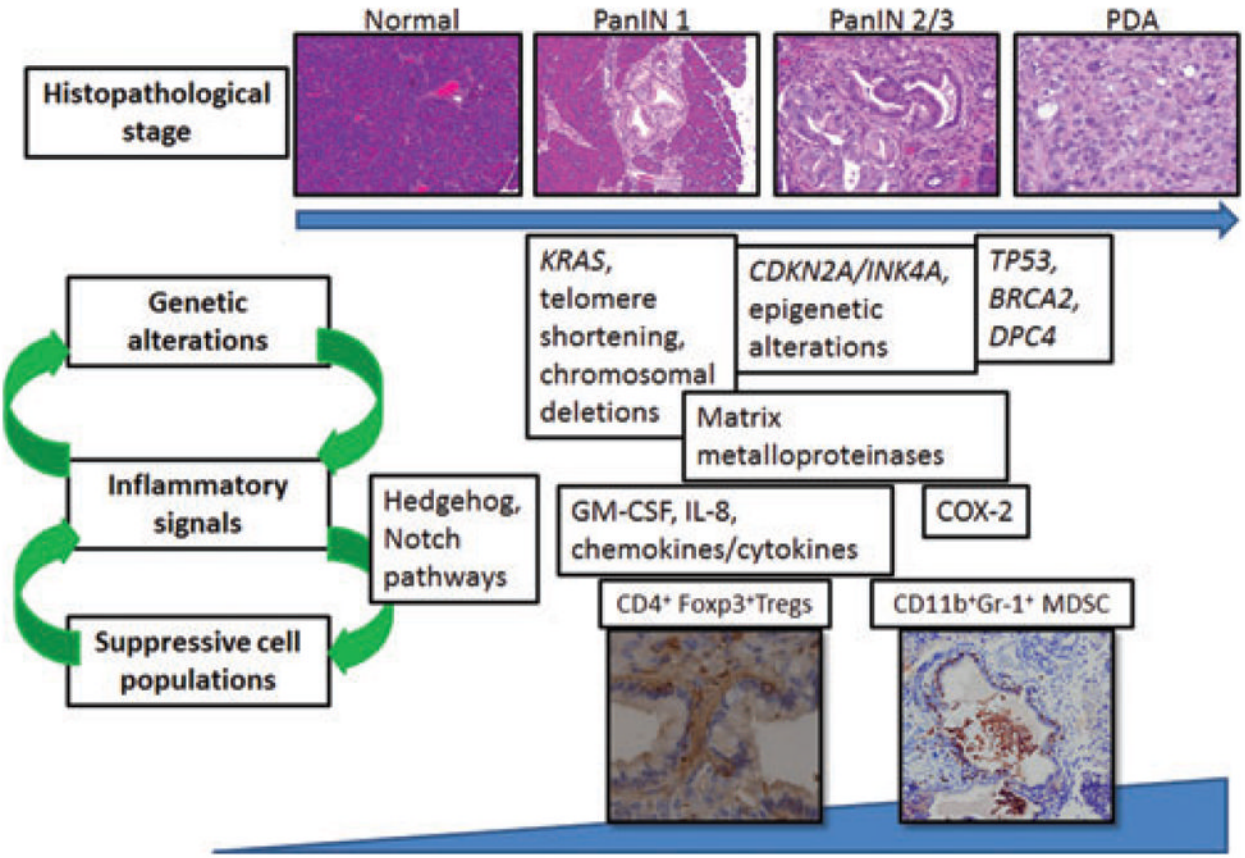

Figure 1.

Inflammatory progression model for pancreatic ductal adenocarcinoma (PDA). Previous models have described the timing of genetic alterations in relation to different PanIN stages, which represent increasing degrees of cellular atypia, loss of normal tissue structure, and genetic abnormality. We propose a new model incorporating the relationship between genetic mutations and gene expression changes with inflammatory cytokines and signaling present in the tumor microenvironment, as well as with cell populations recruited to the tumor microenvironment. Using PDA as an example, we show that genetic mutations, such as *Kras*, can induce secretion of inflammatory cytokines, such as GM-CSF and IL-8, which induce and recruit immature myeloid and granulocytic cells, as well as suppressive T_{reg} cells, that then drive immune tolerance and escape. These procarcinogenic immune cells can contribute to an inflammatory milieu, that is capable of suppressing effector T cell responses, recruiting additional suppressive cells, modifying tumor vasculature, and contributing to further DNA damage and mutations, all of which results in cancer progression.