Nonviral Oncogenic Antigens and the Inflammatory Signals Driving Early Cancer Development as Targets for Cancer Immunoprevention

Nina J. Chu1,2, Todd D. Armstrong1, and Elizabeth M. Jaffee1,2

Abstract

Cancer immunoprevention is an emerging field that holds much promise. Within the past 20 years, prophylactic vaccines have been implemented on the population level for the immunoprevention of carcinomas induced by viruses, specifically hepatitis B virus (HBV) and human papillomavirus (HPV) infection. Armed with the success of prophylactic vaccines that prevent viral-induced tumors, the field must overcome its next hurdle: to develop robust prophylactic vaccines that prevent the remaining >80% of human cancers not induced by viral infection. In this review, we discuss some of the most promising non–virus-associated prophylactic vaccines that target endogenous neoantigens, including the earliest oncogene products, altered mucin 1 (MUC1) and α-enolase (ENO1), all of which produce new targets in the earliest stages of nonviral-induced tumorigenesis. We also highlight a novel attenuated Listeria monocytogenes–based vaccine expressing mutant oncogene Kras12D (LM-Kras) effective in a pancreatic cancer model. A novel chimeric human/rat HER-2 plasmid vaccine (HuRT-DNA vaccine) effective in a breast cancer model is also discussed. In addition to prophylactic vaccine developments, this review highlights the potential use of classic drugs, such as aspirin and metformin, as chemopreventive agents that can potentially be used as adjuvants to enhance the anticancer immunogenicity and efficacy of noninfectious prophylactic vaccines by modulating the inflammatory pathways within the early tumor microenvironment (TME) that propels tumorigenesis. Finally, timing of prophylactic vaccine administration is critical to its immunopreventive efficacy, providing a necessary role of current and emerging biomarkers for cancer screening and early cancer detection. Clin Cancer Res; 21(7): 1549–57. ©2015 AACR.

Introduction

In the past two decades, significant progress has occurred in the field of cancer immunoprevention as evidenced by the success of prophylactic vaccines in preventing cancers caused by viral infection, so-called "infectious tumors," which account for 10% to 20% of all human tumors (1, 2). The most successful prophylactic vaccines targeting infectious tumors are effective against hepatitis B virus (HBV) and human papillomavirus (HPV; refs. 1–3). The HBV-specific prophylactic vaccine, initially developed to prevent chronic acute hepatitis, had the unintentional favorable effect of dramatically reducing the incidence of post-hepatitis hepatocellular carcinoma (HCC; refs. 1, 2). Conversely, the original intention for developing the prophylactic HPV vaccine was to prevent cervical cancer caused by persistent HPV infection. Hence, the HPV vaccine was the first "pure" implementation of human cancer immunoprevention (1). The recent population-based data indicating the efficacy of the HPV vaccine in Australia following the implementation of Australia’s National HPV Vaccination Program in 2007 is very promising, and represents the first fully government-funded HPV vaccination program using the prophylactic quadrivalent HPV vaccine Gardasil (Merck; 4–6). In 2011, the prevalence of vaccine-targeted HPV genotypes in women of ages 18 to 24 years was significantly lower in postvaccinated women than in prevaccinated women (6.7% vs. 28.7%; ref. 4), while in 2014, the prevalence of HPV genotypes in postvaccinated women further declined to 1.6% (5). Such significant reductions in HPV prevalence should be indicative of an eventual decrease in HPV-related cervical cancer incidence. Although the long-term efficacy of the HPV vaccine in preventing cervical cancer has not yet been determined because of its recent worldwide administration, the results of clinical trials predict a near complete prevention of cancer occurrence (1). Because most human cancers are not induced by viral infection, and are therefore termed "noninfectious tumors," the current goal of cancer immunoprevention is to translate the successes of prophylactic vaccine development from infectious tumor models to noninfectious tumors (2).

There are two reasons for the success of prophylactic vaccines that combat infectious tumors. First, these vaccines target known viral antigens involved in the tumorigenesis process (1). Second, they work when given to those at risk before exposure (1). Also
Select driver antigens and their antigenic classifying methods to identify a small at-risk population who would benefit most (6). In contrast, a number of additional challenges must be addressed to successfully develop prophylactic vaccines that target noninfectious tumors. The first is the need to identify the optimal tumor antigens that can serve as vaccine targets. Currently, most antigens that have been identified are not unique to the cancer, but are instead variations of or differentially expressed endogenous self-antigens (1, 2, 7, 8). Although these antigens may serve as targets for primary cancer prevention, they may also increase the risk of autoimmune responses to similar antigens expressed on normal tissue (1). Second, emerging data in animal models suggest that immune escape in the form of immune editing and immune tolerance mechanisms begins early in cancer development, at the time of the first genetic alteration that initiates the cascade of genetic and inflammatory changes that eventually lead to cancer development (9–11). Thus, it is likely that any vaccine that targets a noninfectious tumor-promoting antigen will likely need to be given with other agents that modulate the early inflammatory tumor microenvironment (TME) and precancerous mechanisms of immune evasion (9).

This challenge raises the need for biomarkers that can identify populations at risk for a given cancer type and who will benefit most from early immune-based interventions. However, with careful selection of oncogenic antigen targets that are specific “driver” gene products that arise early in a particular cancer’s development, adjuvant regulation of the inflammatory TME, and robust diagnostic and prognostic screening for the early detection of cancer, there is great potential for the successful development of prophylactic vaccines that prevent nonvirally induced tumorigenesis.

### Antigen Targets for Prophylactic Vaccine Development

The first step toward the design of effective vaccines for cancer prevention is the selection of the antigen target. An optimal antigen target should be an early alteration in a normal gene that is likely an initiator (driver) or facilitator of tumor development to ensure that the immune response is directed toward a “causative” signal without which the developing tumor cannot survive. Vaccination of such a target is likely to produce an immunopreventive response that is both efficacious and the least toxic, mounting a focused and concerted immune response to early neoplasms (1, 8). The difficulty lies in predicting the earliest antigenic profile of developing tumors and discovering such antigens that are dysregulated in the earliest stages of tumorigenesis, along with their differential expression among other cell types. However, the rapidly progressing field of cancer genetics is uncovering the earliest cancer driver genes and their expressed neoantigens associated with the development of different cancers. The various methods used to discover potential cancer driver genes and their expressed antigenic targets are discussed in detail elsewhere (12–14). Currently, there are sets of known driver gene antigens for some cancers that are classified according to their tumor/normal tissue exclusivity and cellular localization (Table 1). Tumor-specific antigens (TSA) are antigens that are exclusively expressed on tumor cells and not on any other cells (8). TSAs are often mutated proteins, such as Kras (3) and p53 (3). Tumor-associated antigens (TAA) are endogenous antigens present on both tumor and normal cells but are dysregulated in their expression or cellular localization. The HER-2/neu receptor is one example of a TAA (3, 8). As progress in cancer immunotherapy and immunoprevention has greatly accelerated in the past two decades, a novel class of antigens, oncoantigens, has been defined.

### Table 1. Select driver antigens and their antigenic classifications

<table>
<thead>
<tr>
<th>Driver antigen</th>
<th>Oncogenic state</th>
<th>Cellular location</th>
<th>Type of antigen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kras</td>
<td>Mutated</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>34,63</td>
</tr>
<tr>
<td>Nras</td>
<td>Mutated</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>63</td>
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<tr>
<td>HER-2/neu</td>
<td>Mutated, overexpressed</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>26,64</td>
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<tr>
<td>EGFR</td>
<td>Mutated</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>65</td>
</tr>
<tr>
<td>MUC1</td>
<td>Overexpressed</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>3,17</td>
</tr>
<tr>
<td>EphA2</td>
<td>Overexpressed</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>17,66</td>
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<tr>
<td>ALK</td>
<td>Activated fusion protein</td>
<td>Plasma membrane</td>
<td>Oncoantigen</td>
<td>64,65</td>
</tr>
<tr>
<td>BRAF</td>
<td>Mutated</td>
<td>Cytoplasm</td>
<td>TSA</td>
<td>64</td>
</tr>
<tr>
<td>MEK1</td>
<td>Mutated</td>
<td>Cytoplasm</td>
<td>TSA</td>
<td>64</td>
</tr>
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<td>Mutated</td>
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<td>TSA</td>
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<td>Cytoplasm</td>
<td>TSA</td>
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<td>bcr-abl</td>
<td>Activated fusion protein</td>
<td>Cytoplasm</td>
<td>TSA</td>
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</tr>
<tr>
<td>ERG</td>
<td>Activated fusion protein</td>
<td>Cytoplasm</td>
<td>TSA</td>
<td>17,68</td>
</tr>
<tr>
<td>WT1</td>
<td>Mutated, activated fusion protein</td>
<td>Nucleus</td>
<td>TSA</td>
<td>17,69</td>
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<tr>
<td>p53</td>
<td>Mutated</td>
<td>Nucleus</td>
<td>TSA</td>
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<td>BRCA1</td>
<td>Mutated</td>
<td>Nucleus</td>
<td>TSA</td>
<td>70</td>
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<tr>
<td>PTEN</td>
<td>Mutated</td>
<td>Cytoplasm</td>
<td>TSA</td>
<td>70</td>
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<td>Nucleus</td>
<td>TAA</td>
<td>17,71</td>
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<td>Survivin</td>
<td>Overexpressed</td>
<td>Nucleus, cytoplasm</td>
<td>TAA</td>
<td>17,72</td>
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<td>TAA</td>
<td>17,73</td>
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<td>RhoC</td>
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<td></td>
<td>TAA</td>
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<td>MYCN</td>
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<td>Nucleus</td>
<td>TAA</td>
<td>17,75</td>
</tr>
<tr>
<td>Cyclin B1</td>
<td>Overexpressed</td>
<td>Nucleus, cytoplasm</td>
<td>TAA</td>
<td>17</td>
</tr>
</tbody>
</table>

**NOTE:** Driver antigens are expressed products of driver genes that have undergone early alterations or dysregulated expression to initiate or facilitate tumorigenesis. Driver antigens are key to the neoplastic process and must support tumor growth. The classifications of driver antigens are determined by cell-type expression and subcellular localization. TSAs are exclusively expressed on tumor cells and not on any other cells. TAs are endogenous antigens present on both tumor and normal cells. Candidate TAs that are optimal targets for prophylactic vaccine development are overexpressed on tumor cells. Oncoantigens are specifically expressed extracellularly or on the cell surface. Select driver antigens that are discussed in this review along with other known driver antigens are used as examples for each class.
in the context of vaccine therapy. Oncoantigens are antigens that must support tumor growth and are specifically expressed extracellularly or on the cell surface (1, 15). Oncoantigens are thought to be the most suitable targets for immunopreventive vaccines due to their requisite roles in driving tumorigenesis and their accessible location to elicit immune responses (15).

Cancer-testis (CT) antigens are a class of promising TAAs that are currently being investigated for their potential to be candidate novel targets used for therapeutic and prophylactic vaccine development (16). CT antigens are predominantly expressed in the testis but are also found in the ovary and trophoblast, having cellular functions in transcriptional regulation, recombination, apoptosis, adherence, and cell mobility (16). These antigens are overexpressed in a variety of different tumors, while minimally expressed in normal tissues. Hence, this emerging class of antigens represents attractive targets that can be used for cancer vaccine development (16). Two CT antigens, MAGE-A3 and NY-ESO-1, have already been classified by NCI as being among the top 10 antigens in the Project for the Prioritization of Cancer Antigens and are currently being tested in clinical trials for their immunotherapeutic efficacy in combination with adjuvants (16, 17). Much work needs to be done to determine which CT antigens are specifically oncoantigens. For many CT antigens, their fundamental roles in driving tumorigenesis along with their cellular localization have yet to be determined. These antigen characteristics will influence the type and strength of immunologic responses produced against them when used as immune targets (16).

**Vaccines: Emerging and Currently under Development**

Several promising noninfectious prophylactic vaccines are under development and are targeting TSAs, TAAs, and oncoantigens. Specific targets include KrasG12D, HER-2/neu, MUC1, and ENO1. In addition to the type of antigen, there are a number of antigen-delivery approaches being tested that take advantage of the immune system’s best mechanisms for activating potent adaptive and innate immune responses. It is not yet clear which of these delivery systems offers the best immunity and safety profiles. Different delivery systems have been extensively discussed in other reviews (18–20). We highlight only a few that target the categories of antigens that hold promise for primary prevention with emphasis on studies conducted in individuals at risk for a particular cancer.

**Listeria monocytogenes vector engineered to target mutated Kras (KrasG12D, LM-Kras)**

Keenan and colleagues developed a vaccine composed of recombinant attenuated intracellular bacterium Listeria monocytogenes (LM) engineered to express the most common mutation found in the oncogene Kras, KrasG12D, to test as prevention in a preclinical model of pancreatic ductal adenocarcinoma (PDA). The mutant KrasG12D was chosen as the antigenic target because it is the earliest and most prevalent mutant oncogene in PDA development (21). The KrasG12D+/−/Tpp53R172H+/−;Pdx-1-Cre (KPC) mice are genetically programmed to spontaneously develop tissue-specific PDA following the earliest mutation, which is KrasG12D, and exhibit a natural stepwise progression from premalignant pancreatic intraepithelial neoplasias (PanIN) to invasive PDA (21). L. monocytogenes was used as a vehicle to express the driver KrasG12D gene due to its ability to induce robust CD4+ and CD8+ T-cell immunity via its selective infection of antigen-presenting cells (APC) over phagocytic cells, thereby maintaining immunogenicity while significantly decreasing toxicity (21–23). The LM-Kras vaccine was administered either alone or with regulatory T cell (Treg) depletion because Tregs were shown to be an early inhibitor of immune infiltration into PanINs in this model of PDA development (11). Mice received treatment either at the time of the earliest genetic change (PanIN 1) or at the time of late-stage PanIN development. Mice that received LM-Kras in combination with Treg depletion at the earliest time point had significantly prolonged survival accompanied by decelerated PanIN progression compared with KPC mice that were unvaccinated, given LM-Kras alone, or given combination treatment at the time of later-stage PanIN development (21). These results demonstrate that after the first genetic alteration occurs in neoplastic cells, prophylactic immune-based vaccination requires combination therapy to alter the already inflammation-prone early TME. This study also highlights the importance of identifying the optimal time for administering a prophylactic vaccine, which will require biomarkers to identify the population at risk for early premalignant changes. A drawback to this model is that the KrasG12D mutation is present from birth. Further research using inducible KrasG12D gene products will allow for a more realistic test of a preventative Kras-specific vaccine (24).

**Chimeric human/rat HER-2 plasmid**

Despite the widespread clinical use of HER-2 targeted small-molecule inhibitors and monoclonal antibodies, trastuzumab and pertuzumab, there remains a lack of HER-2/neu prophylactic and therapeutic vaccines implemented in the clinic (25, 26). In a study conducted by De Giovanni and colleagues (25), FVB-huHER2 transgenic mice were vaccinated with tumor cells expressing human HER-2 with recombinant IL12 adjuvant (HER-2 cell vaccine) or chimeric human/rat HER-2 DNA plasmid (HuRT-DNA vaccine). The FVB-huHER2 mice overexpress the human HER-2 gene and spontaneously develop mammary tumors within the first 6 months of life, making FVB-huHER2 mice a suitable model to study immunoprevention of tumorigenesis through huHER2 targeting prophylactic vaccines (25). Mice given the HuRT-DNA vaccine had the longest mean tumor-free survival, compared with mice given either HER-2 cell vaccine or mock vaccination (25). The HuRT-DNA–vaccinated mice also had a significant reduction of mammary tumors per mouse and produced a high level of anti-huHER-2 antibodies compared with HER-2 cell–vaccinated mice (25). The serum isolated from HuRT-DNA vaccinated mice was able to inhibit the growth of huHER-2–positive tumors in immunodeficient xenograft-carrying mice (25). The results of this study show that chimeric human/rat HER-2 plasmid vaccine is a promising immunopreventive treatment based on its ability to break immunologic tolerance to huHER-2 in a huHER-2–tolerant host (25, 26). The chimeric human/rat HER-2 plasmid vaccine can also generate robust memory via production of high levels of specific anti-huHER-2 antibodies, an important component of any preventive vaccine (25).

**MUC1**

MUC1 is a mucin-like, glycosylated TAA that is overexpressed in several epithelial carcinomas, including breast, colon, lung, prostate, and ovarian carcinomas (3, 27, 28). The...
posttranslational modifications of MUC1 define its tumorigenicity (3, 27). For example, in normal colon epithelial cells, MUC1 is hyperglycosylated, whereas in neoplastic cells lacking luminal polarity, MUC1 is hypoglycosylated (3, 27). A human-MUC1 is hyperglycosylated, whereas in neoplastic cells lacking luminal polarity (3, 27). For example, in normal colon epithelial cells, MUC1 peptide vaccine did not elicit significant adverse effects, such as autoimmune disease postvaccination (27). Patients who did not respond to the vaccine, evidenced by a lack of increase in production of anti-MUC1 IgG antibody, also had high levels of circulating myeloid-derived suppressor cells (MDSC) before vaccination (27). High levels of this immunosuppressive cell population dampen antitumor adaptive immunity (29), thereby decreasing the immunogenicity of the MUC1 peptide vaccine (27). Consequently, this study provides evidence that the success of such a vaccine in primary prevention will require adjuvants/immune modulators that decrease immunosuppression (29). The results of this study demonstrate the ability of the MUC1 peptide vaccine in combination with the Poly-ICLC adjuvant to strongly activate the adaptive immune response without significant adverse effects, while generating immune memory to the oncogenic antigen. These mechanisms may help to decrease the prophylactic potential of MUC1 peptide vaccines in reducing recurrence of premalignant lesions, leading to the eventual prevention of colorectal cancer, which must be shown by prolonged monitoring of cancer occurrence in vaccinated patients. This study further highlights the need to consider ways to alter suppressive immune cell populations like MDSCs simultaneously at the time of preventative vaccination when the host has already been exposed to the initiating genetic alteration.

ENO1

ENO1, or α-enolase, is a TAA that has dual roles depending on its cellular location of expression. ENO1 functions as a glycolytic enzyme in the cytoplasm, while on the membrane, it functions as a plasminogen receptor affecting cell migration and metastasis (30–32). Overexpression of ENO1 is observed in a variety of cancers, including brain, breast, colon, gastric, head and neck, kidney, liver, lung, ovary, pancreas, prostate, and skin among others (31). Like MUC1, ENO1 is also subject to posttranslational modifications which, depending on type, determine its overexpression in specific cancer types (31). To assess the potential of using ENO1 as a TAA for prophylactic vaccine development, KPC mice were vaccinated with ENO1 plasmid at 4 weeks of age, when early PanINs are prevalent (30). ENO1 plasmid vaccine was able to significantly increase mean survival times by delaying onset of PDA (30). Vaccine immunogenicity was confirmed by high serum levels of anti-ENO1 IgG antibody, which induced complement-mediated cytotoxicity of antibody-bound cancer cells (30). Despite the low expression of endogenous ENO1 on normal cells, the humoral and cellular ENO1-specific CD8⁺ cytotoxic T lymphocytes (CTL) produced by the ENO1 plasmid vaccine spared normal cells and lysed only cancer cells overexpressing ENO1 (30, 32). The specific cytotoxicity achieved by this vaccine avoided the autoimmunity that is normally associated with TAA targeting vaccines. In addition, the ENO1 plasmid vaccine reduced the expansion of MDSCs and Tregs, while promoting T-helper 1 (Th1) and Th17 responses (30). This study exhibits the dual potential of ENO1 plasmid vaccines to concurrently prevent onset of PDA and downmodulate immunosuppressive, protumorigenic cell populations.

Early Genetic Changes Lead to Early Inflammation

Early tumorigenesis is the result of the initiation of early genetic alterations, namely activation of oncogenes and/or silencing of tumor suppressors that not only initiate carcinogenesis in the tumor cells themselves, but also induce a cascade of inflammatory events that create an early TME that supports and propagates tumor development (9, 10). This TME is created by inflammatory signals that recruit suppressive immune cell populations and nonneoplastic stromal cells that enhance tumor growth, while dampening immune activation, even in the earliest premalignant stages (9, 10). Studies have shown that the early activation of oncogenic drivers, such as mutant Kras in neoplastic cells, is sufficient to initiate the formation of the early TME in response to inflammatory signaling, and that in vivo silencing of mutant Kras in early premalignant lesions completely and rapidly reverses the transformed ductal epithelium and desmoplastic stroma in transgenic mouse models of PDA and lung adenocarcinoma (24). PDA is a cancer model that is characterized by extensive fibroinflammatory stroma that mediates its resistance to chemotherapies (24, 33). KrasG12D is the earliest and most persistent oncogenic mutation throughout PDA progression, as it is present in at least 92% of early-stage PanINs and remains expressed in late-stage PanINs (34). Early mutant Kras-driven neoplastic cells secrete cytokines, IL8, IL6, and granulocyte macrophage colony-stimulating factor (GM-CSF), which recruits Tregs and MDSCs to the TME (10, 24, 35, 36). These immunosuppressive cell populations maintain and propagate tumorogenesis by promoting immune tolerance and escape, while preventing the infiltration of activated CD4⁺ and CD8⁺ T cells to the early TME (11). Despite the presence of activated T cells in the periphery induced by treatment, the nonpermissive TME created by these immunosuppressive cell populations prevents the infiltration of activated T cells into the TME to perform antigen-specific killing of tumor cells (9). Therefore, in order for noninfectious prophylactic vaccines to achieve maximal immunogenicity and anticancer efficacy, the early targeting of activated oncogenic drivers needs to be coupled with modulation of the early inflammatory immunosuppressive TME that may otherwise hinder effective treatment.

Agents for Chemoprevention That Alter Early Inflammation in Premalignancies

The immune-modulating antibodies that alter signals like CTL-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1) receptor in the TME are successful treatment approaches for patients with existing cancer that is resistant to other therapeutic modalities (37). These agents act on previously induced T cells (37). Although they are providing durable treatment effects in patients with metastatic cancers, their utility for primary prevention is currently limited by their serious autoimmune
toxicity profiles (38). In addition, it is not clear that signals like CIL-4 and PD-1 are the most important signals for modulation in the early developing TME. Therefore, agents that have lower risk for severe toxicities and that alter the earliest inflammatory changes will be the most successful for evaluation in healthy patients at risk for cancers.

Some chemopreventive agents have exhibited immunopreventive potential in cancer models and may be used as adjuvants to create an anti-inflammatory, permissive TME for increased prophylactic vaccine efficacy. Two agents in particular, aspirin and metformin, which are widely used for the treatment of non-cancer-related inflammatory diseases, may prevent tumorigenesis via anti-inflammatory mechanisms when given chronically.

**Aspirin**

Aspirin belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) that irreversibly inactivate COX-1 and COX-2 to prevent the synthesis of prostanoids, including prostaglandins, prostacyclin, and thromboxane A (29, 39, 40). Aspirin’s inhibition of these metabolites produces its analgesic, antipyretic, anti-inflammatory, and cardiovascular effects (39, 40). Several clinical studies have shown that a safe low daily dose of aspirin, taken for the prevention of cardiovascular disease, also decreases the incidence and recurrence of colorectal cancer (39, 40). In addition, a meta-analysis of cardiovascular trials revealed that a low daily dose of aspirin may reduce cancer incidence and cancer mortality from all cancers combined (39, 40). Aspirin’s chemopreventive effects result from its modulation of inflammatory signaling pathways that regulate the TME. Aspirin suppresses the proinflammatory NF-kB and STAT3 pathways involved in cancer initiation (41, 42), while preventing PGE2-mediated differentiation of immature human monocytes into immunosuppressive MDSCs (29).

**Metformin**

Metformin, a widely used treatment for type II diabetes mellitus, prevents tumorigenesis via two primary mechanisms: alteration of tumor metabolism by inhibiting mTOR through AMPK modulation and inhibition of proinflammatory cancer-promoting NF-kB and STAT3 pathways (42–44). The NF-kB/STAT3 signaling pathway plays a critical role in inflammation-mediated carcinogenesis. Several studies have demonstrated that the NF-kB/STAT3 pathway is activated by oncogenic driver genes, such as Kras, and secretion of specific cytokines, IL6 and IL11, by early lesions and tumor-infiltrating immune cells in an autocrine and paracrine fashion (42). Hence, inhibition of neoplastic cell metabolism and early inflammation within the TME allow metformin to reduce the prevalence of a variety of cancers, including PDA, HCC, melanoma, and triple-negative breast cancer (42, 43, 45–47). These studies support metformin’s potential as an adjuvant chemopreventive drug to enhance the efficacy of non-infectious tumor targeting vaccines.

**Timing of Immunopreventive Vaccine Administration Requires Cancer Screening**

Synthesizing a potent prophylactic vaccine that selectively elicits an immune response to target oncoantigens to eliminate potential neoplastic cells, while using adjuvants in combination to modulate the inflammatory composition of the TME, is vital to the success of cancer immunoprevention. Tantamount to vaccine development is precise timing of vaccine administration. Studies have shown that early administration of prophylactic vaccines, before exposure to the causative agent (i.e., virus), can effectively prevent cancer occurrence (1, 2). More recently, vaccination of KPC mice that have an existing Kras mutation that initiates tumor development with an LM-Kras vaccine, slows progression to PDA if early inflammatory changes are also halted (21). This finding provides an explanation for why infectious vaccines that work well for preventing cancers before exposure to the virus do not as single agents prevent even early cancerous changes from progressing to cervical cancer and HPV+ oropharynx carcinoma (20).

Early vaccine administration aids in shifting immune control toward prolonged equilibrium or full elimination, reducing immune evasion and tumor escape (1, 7). Timing of administration is critical to achieve optimal prophylactic vaccine efficacy, hence identification of specific diagnostic and prognostic biomarkers for cancer screening is of significant importance to the success of cancer immunoprevention. These biomarkers will need to identify the early inflammatory changes and the regulators of the early genetic, epigenetic, and inflammatory changes that facilitate cancer development. Certain at-risk populations that are characterized by family history of cancer incidence as well as personal history of premalignant lesions are of particular interest as candidates for early prevention by vaccines.

In addition to oncogenic driver genes that initiate carcinogenesis via an inflammatory TME, epigenetic modifications of DNA and the associated proteins are mechanisms of immune evasion that not only can be used as biomarkers for cancer screening, but also can be targeted for adjuvant therapies that enhance the efficacy of prophylactic vaccines by regulating the polarity of inflammatory cells within the TME (48–50). Recent studies have elucidated the role of histone deacetylases (HDAC) in defining the phenotype of cancer-associated MDSCs and regulating the expression of OX40L by malignant cells, IL10 by Tregs, and antigen presentation by tumor cells (48, 50–54). Thus, modulation of the early immunosuppressive TME by epigenetic agents can increase the immunogenicity of tumor cells and augment the antitumor properties of immune cells. Additional efforts will be required to further elucidate the role of these epigenetic changes as biomarkers for identifying at-risk populations.

**Emerging Epigenetic Markers for Potential Screening of Early Cancers**

**microRNAs**

Recent studies have identified epigenetic signatures, specifically microRNA (miRNA) profiles and methylation patterns of a variety of cancers that have the potential to be used for novel noninvasive methods of cancer screening. miRNAs are short endogenous noncoding RNAs that are approximately 20 nucleotides in length that negatively regulate protein expression by inhibiting translation and/or by targeting mRNAs for degradation (55). Alterations in miRNA expression are important for cancer development, and cancer-specific miRNA profiles have been uncovered that propagate and are associated with certain cancer types (56). The noninvasive isolation of circulating miRNAs from a simple blood draw along with its stability in harsh conditions (e.g., freeze-thawing, RNase digestion, and wide pH range) confers feasibility to using circulating miRNAs for cancer screening in the clinic (57, 58).
DNA methylation

DNA methylation patterns are also promising diagnostic and prognostic biomarkers for cancer screening. DNA methylation is an epigenetic modification in which methylation occurs at cytosine residues of cytosine-phosphate-guanine dinucleotides (CpG islands), transcriptionally silencing genes (59–61). Hypermethylation of tumor-suppressor genes and hypomethylation of oncogenes are mechanisms of tumorigenesis, and specific DNA methylation patterns are characteristic of varying cancers (60, 61). DNA methylation signatures can be found in any biologic tissue sample or bodily fluid, which facilitates screening (59). DNA methylation is one of the most robust epigenetic markers and, like circulating miRNAs, is resistant to harsh conditions, withstanding most storage conditions (59). Therefore, DNA methylation’s wide biologic prevalence and stability also make it a potential biomarker for clinical cancer screening.

Challenges

Despite the advances made in prophylactic vaccine development, elucidation of inflammatory dynamics within the developing TME, chemoprevention, and biomarker discovery, there remain challenges that need to be overcome to advance the cancer immunoprevention field. A significant challenge that necessitates further investigation is precisely when or how early along the developmental timeline prophylactic vaccines for nonviral cancers should be administered to achieve optimal immunoprevention. The ideal time point, together with the composition of the procarcinogenic inflammatory response at that time point for the administration of nonviral tumor targeting prophylactic vaccines, needs to be established. A proposed time line for prophylactic vaccine administration within the context of a genetic and inflammatory cancer progression model is depicted in Fig. 1.

For noninfectious cancers, a major risk associated with using TAAs as targets for prophylactic vaccines in combination with adjuvants is autoimmune toxicity (1). Because TAAs are expressed on both normal cells and neoplastic cells, a robust vaccine-elicited immune response targeting self-antigens may induce autoimmunity (1, 8). Adjuvants, such as cytokine therapy, immune checkpoint blockade, and chemotherapeutic agents, enhance the immunopreventive efficacy of prophylactic vaccines by inhibiting immunosuppressive cell populations, creating a more permissive TME, and increasing immunosurveillance to reduce tumor evasion (3, 9, 21, 29). Particular advances has been made in the field of developing immune checkpoint inhibitors, such as antibodies against CTLA-4 and...
the receptor–ligand axis, PD-1/PD-L1, to reduce the inhibition that these receptor/ligand pairs exert on T-cell activation (37). Although immune checkpoint blockade has proven to be successful as a therapy, both alone and in combination, in various advanced cancers, particularly in melanoma, specific immune-related adverse events are induced that mainly include high-grade gastrointestinal, hepatic, and endocrine toxicities as a result of breaking immunologic tolerance upon administration of such antibodies (38). These high-grade adverse events will particularly be a problem if immune checkpoint inhibitors are given long-term in a preventative setting in combination with noninfectious prophylactic vaccines. The heightened immune activation triggered by such adjuvants to enhance vaccine immunogenicity may further contribute to autoimmunity (3, 29, 38). In addition to autoimmunity, toxicity is an issue with prolonged use of other antigen nonspecific adjuvants, specifically cytokine therapy (1, 20). Cytokine toxicity, like immune checkpoint blockade toxicities, is a challenge particularly for cancer immunoprevention because prophylactic treatment may necessitate chronic lifelong dosing (1, 20). Prophylactic vaccine efficacy can also be compromised by hypermutation of TSAs, low frequency of oncoantigen overexpression, and heterogeneous intratumoral oncoantigen expression (2).

A challenge in the area of circulating miRNA and DNA methylation biomarker discovery for early cancer screening is identifying signatures that will not only identify the presence of pre-cancer, but also be indicative of stage and/or progress of the pre-cancer, along with its tissue specificity. This level of detail and specificity has yet to be achieved. Epigenetic profiles need to be established in a cancer developmental context to aid in ascertaining the ideal time for vaccine administration in transgenic mice and human models. In addition, consistency needs to be established for circulating miRNA sample processing and quantification. Currently, there are inconsistencies with miRNA isolation from serum or plasma (56, 62). There is also debate about what constitutes the most suitable standard or endogenous control for qRT-PCR, which would affect accurate quantification (56, 62). Developing reference procedures to standardize processing and analysis will be necessary to facilitate accurate comparisons between independent laboratories.

Conclusions

The field of cancer immunoprevention has made great advances in developing successful prophylactic vaccines that elicit immune responses to prevent infectious tumors. The worldwide population-level administration of HBV vaccines has significantly reduced the occurrence of HCC, and clinical trials predict the near 100% prevention of cervical carcinogenesis in patients vaccinated with HPV vaccines. The success of prophylactic vaccines in preventing the occurrence of infectious tumors lies in the vaccine’s ability to induce specific immune recognition and attack of viral antigens. However, a current challenge facing the field of immunoprevention is developing effective, safe, and immunogenic prophylactic vaccines that target endogenous oncoantigens over-expressed by noninfectious tumors, which account for the majority of human cancers. The efficacy of infectious vaccines is also determined by the inflammatory composition of the early TME initiated and propagated by early oncogenic driver genes. Hence, aspirin and metformin may be promising chemopreventive adjuvants that aid prophylactic vaccine efficacy by downregulating early local inflammatory pathways while exhibiting a safer toxicity profile when compared with immune-modulating agents that are approved for cancer treatment. Potential exists to combine these chemopreventive anti-inflammatory agents with antigen-targeted vaccines as a means to delay or prevent the onset of tumorigenesis.

The early administration of prophylactic vaccines, before the onset of tumorigenesis, has proven to significantly affect the degree of anticancer protection. Therefore, cancer screening methods for detection of the earliest premalignant changes are needed to fully implement immune-based interventions for primary cancer prevention. Epigenetic signatures, particularly circulating miRNA subsets and DNA methylation status of gene promoters, are emerging as powerful diagnostic indicators of early cancers. Further studies are needed to determine when to vaccinate along the cancer developmental timeline for prophylactic vaccines to cause maximal immunoprevention. This knowledge, aided by more predictive screening methods, and an improved understanding of the procarcinogenic inflammatory changes that help shape the developing TME, will propel the field of immunoprevention into the mainstream.

Disclosure of Potential Conflicts of Interest

E.M. Jaffee has the potential to receive royalties, through her institution, for intellectual property related to Listeria mesothelin and GVAX vaccines that is owned by Johns Hopkins University and licensed to Aduro Biotech. No potential conflicts of interest were disclosed by the other authors.

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Nonviral Oncogenic Antigens and the Inflammatory Signals Driving Early Cancer Development as Targets for Cancer Immunoprevention

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