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Improving Clinical Efficacy of Cancer Vaccines Using Neoantigens Identified in Cancer Patients

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Review Article

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Abstract

At present, cancer is the most lethal disease in developed countries. Although many of cancer patients respond to conventional anticancer therapies, including surgery, chemotherapy, and radiotherapy, some cases relapse and even become resistant to therapy. Particularly, metastatic cancers are difficult to treat. Therefore, novel therapeutic approaches with better clinical efficacy are required for advanced cancers. In the past decades, our understanding of tumor immunology has been increased noticeably and immunotherapy has been investigated for treatment of cancer. However, the complex relationships between tumor cells and immune cells influence the outcome of immunotherapy. Cancer vaccines are being explored widely to either prevent cancer or to treat disease. Recently, two prophylactic cancer vaccines have been approved by the U.S. Food and Drug administration (FDA) for virus-induced cancers. Other cancer vaccines showed limited successes which is more likely due to inability in identification of tumor specific antigens. Tumor cells can harbor mutations in mismatch repair genes that prevent the repair of DNA errors that arise as cell division. These cells have high potential to create neoantigens. As immune responses can be efficiently produced against neoantigens, neoantigens can be used to develop cancer vaccine. However, some of antigens produced in mismatch repair-deficient tumors are also produced by normal cells, and cancer vaccines using these shared antigens may show unintentional side effects. But, a tumor specific neoantigen-based vaccine wouldn't induce such side effects. Furthermore, combining of tumor-specific neoantigen vaccine with other cancer therapies could result in high clinical efficacy. In this article, we discuss recent advancements in cancer vaccines, especially neoantigen-based cancer vaccines, and personalized cancer immunotherapy.

Keywords: Cancer; Immunotherapy; Vaccine, Immune Responses; Tumor Associated Antigens; Clinical Efficacy

Abbreviations: USFDA: United States Food and Drug Administration; Taas: Tumor Associated Antigens; IL2RA: Interleukin 2 Receptor Alpha; Foxp3: Fork Head Box P3; Tsas: Tumor Specific Antigens; NGS: Next-Generation DNA Sequencing; PD1: Programmed Cell Death 1; PD-L1: Programmed Death-Ligand 1; CTLA4: Cytotoxic T-Lymphocyte Associated Antigen-4; NSCLC: Non-Small Cell Lung Cancer.

Introduction

In the recent years, immunotherapy with the aim of augmenting body's immune system to recognize and destroy tumor cells has been widely investigated as a novel cancer therapy modality. Immune responses can be naturally generated against cancer cells. On the other hand, cancer cells can inhibit anticancer immune responses. In the advanced stages of some malignancies $CD4^+$ T cells and $CD8^+$ T cells were found to be increased in the tumor microenvironment. But not all T cells act as antitumor effector immune cells. $CD4^+$ Th1 cells, cytotoxic $CD8^+$ T cells, type 1 macrophages, and NK cells function as the major antitumor immune cells. Some other tumor-associated immune cells, especially regulatory T cells and myeloid derived suppressor cells (MDSCs), are generally known as suppressors of antitumor immune responses [1]. Cancer vaccines should induce potent antitumor immune responses and overcome the immunosuppressive effects of the tumor microenvironment.

In the 1960s, tumor associated antigens (TAAs) were discovered and it was suggested that these antigens are involved in the rejection of tumors [2]. In the past decades, vaccination with tumor specific/associated antigens and immunogenic vectors has been successfully used to increase antitumor immune responses. In general, cancer vaccines did not induce noticeable toxicity. Recently, two prophylactic cancer vaccines have been approved by the U.S. Food and Drug administration (FDA). One of these vaccines is for human papillomavirus involved in most cervical cancers and other vaccine is against the hepatitis B virus which is associated with hepatic cancer [3]. These vaccines are against virally-induced cancers which are immunogenic due to expression of viral antigens at the tumor cell surface. Other cancer vaccines showed limited successes in cancer patients and their efficacy was lower than 5% [4]. The limited success in those cancer vaccines, in contrast to virally-induced cancer cells, may be due to this fact that these types of cancers are not immunogenic or appropriate tumor specific antigens were not used in these cancer vaccines and/or due to potent suppression of antitumor immune responses in cancer patients.

Antigen-specific stimulation of T cells can enhance the number of tumor-specific T cells. Adoptive immunotherapy with *ex vivo* stimulated and expanded T cells showed beneficial efficacy in some cancer patients, especially melanoma patients, but not in other types of cancer [5,6]. Tumor antigen-pulsed dendritic cells can induce antigen-specific T cells, both *in vitro* and *in vivo*. In several murine tumor models, prophylactic dendritic cell vaccines showed proper efficacy in prevention of tumor growth. However, most therapeutic dendritic cell vaccines were unable to reject established tumors or to induce long-lasting delay in tumor growth [7]. These findings emphasize that for improving the antitumor efficacy of tumor antigen-based vaccines, *ex vivo* expanded lymphocytes, and dendritic cell vaccines are necessary.

Vaccines should affect the balanced immune responses in malignancies by disturbing immunosuppressive and immunostimulatory pathways. Inhibition of immunosuppression together with stimulation of antitumor immune responses favors tumor eradication or tumor growth suppression. Regulatory T cells are a subpopulation of $CD4^+$ T cells which express high levels of the cell surface molecule CD25 (IL2RA) and the transcription factor Foxp3 and play an important role in the maintenance of immune tolerance and induction of immunosuppression. These cells can hamper antitumor immune responses. Accordingly, a high frequency of regulatory T cells within tumor tissues has been proposed to predict a worse prognosis in some types of cancer. Furthermore, effectiveness of anticancer immunotherapeutic approaches may be restricted by increased number of regulatory T cells as well as myeloid derived suppressor cells [1]. Vaccines that impede the peripheral induction of regulatory T cells and myeloid derived suppressor cells and/or counteract the suppressive function of these cells can elicit potent antitumor immunity.

Clinical Efficacy of Cancer Vaccines

The choice of target antigen is imperative in designing a cancer vaccine. Non-targeted vaccines such as tumor lysate vaccines have been used in preclinical studies. However, these vaccines can generate T cell responses against shared antigens expressed both by malignant cells and by non-malignant cells in the tumor tissue [8]. The majority of antigens provided by tumor lysate vaccines are non-mutated self-proteins, and high avidity T cells

recognizing these antigens are likely deleted during T cell development within thymus [9]. Consequently, a limited repertoire of T cells available to respond to these vaccines.

The clinical efficacy of several peptide-based cancer vaccines was explored in phase I/II studies. So far, peptide-based vaccines did not show satisfactory results, and no peptide vaccines had been reached the market yet. However, several preliminary studies have demonstrated the potential success of peptide vaccines in overcoming obstacles of cancer immunotherapy. Multiple peptide epitopes can be incorporated in a single vaccine to increase the chance of activating multiple T cells. Such vaccines also have a high potential to avoid tumor escape induced by loss or changes of epitopes during tumor growth [10]. In a phase II clinical trial, multiple peptides vaccination resulted in induction of immune responses and increased overall survival in patients with advance head and neck cancer [11] while vaccination with the melanoma peptide antigen gp100 induced weak gp100-reactive T cell responses [12]. *Ex vivo* generated dendritic cells, the most potent antigen presenting cells, are used to present tumor antigens to T cells [7]. In patients with metastatic melanoma, vaccination with monocyte ($CD34^+$ progenitor)-derived dendritic cells pulsed with peptides derived from four melanoma antigens (Melan A/MART-1, tyrosinase, MAGE-3, and gp100) generated detectable T cell responses to some of these antigens [13]. Vaccination with melanoma peptide-pulsed $CD34^+$ progenitor-derived dendritic cells resulted in expansion of melanoma-specific interferon-gamma-producing $CD8^+$ T cells in the peripheral blood of patients with metastatic melanoma [14]. Monocyte-derived dendritic cells loaded with killed allogeneic melanoma cells also induced MART-1 specific $CD8^+$ T cell immunity and objective clinical responses in some vaccinated patients with stage IV melanoma [15]. Vaccination with dendritic cells loaded with allogeneic tumor cell lysate was well-tolerated in advanced melanoma patients and produced clinical responses in some patients [16]. In 2010, FDA approved *ex vivo* generated dendritic cell vaccine, Sipuleucel-T, for the treatment of prostate cancer which was the first FDA-approved therapeutic cancer vaccine [17].

Tumor Associated Antigen-Based Cancer Vaccines

In most peptide-based cancer vaccines TAAs were used which are overexpressed in cancerous cells but they are also often expressed in low levels in healthy cells. MAGE-A3, gp100, Melan-A/Mart1, Her2/Neu, and NY-ESO-1 are examples of self-antigens expressed on tumor cells that can be recognized by T cells [18]. These antigens are non-mutated self-antigens and genes encoding TAAs are present in normal genome [19]. Importantly, mechanisms involved in the maintenance of immunological tolerance to self-antigens can restrict induction of immune responses against TAAs-based vaccines [1]. Also, generation of potent immune responses against these antigens results in autoimmunity [20]. This issue of TAAs can be responsible for poor efficacy and autoimmunity concerns observed with TAA-based vaccines in clinical trials.

Tumor Specific Antigen (Neoantigen)-Based Cancer Vaccines

Tumor specific antigens (TSAs) are not expressed in normal cells. TSAs are suitable choices for cancer vaccines. Tumor cells harbor mutations in genes controlling cell growth as well as other genes. Mutations that prevent the repair of DNA errors arise as cell divide, this defect called mismatch repair deficiency which have the potential to create neoantigens. These mutations can be contributed to the expression of neoantigens or TSAs. The immune system recognizes TSAs as foreign antigens and immunization with such antigens can induce potent antitumor immune responses. Identification of such TSAs is fundamental for successful cancer immunotherapy. However, identification of TSAs has been difficult, and even impossible, in most cancers due to technical limitations. Recently, technological advances in high-throughput DNA sequencing provided evidences indicating that tumor cells are vastly different from normal cells at the genetic level [21,22]. These changes lead to expression of unique antigens (neoantigens) in tumor cells. Neoantigens are newly recognized antigens by the immune system. Thus, the immune system can distinguish tumor cells from normal cells and destroy them by recognition of these neoantigens. As neoantigens are only expressed in the tumor cells, mechanisms involved in the induction of immunological tolerance do not hamper antitumor efficacy of neoantigen-based vaccines and such vaccines have lower risk of generating autoimmunity. Development of next-generation DNA sequencing (NGS) technologies facilitates the detection of mutations in genes encoding cell surface neoantigens. The number of genes encoding neoantigens in a tumor genome can also be measured by NGS [23]. In addition, algorithms can be used to predict which of the mutations would create neoantigens [23,24]. NGS and neoantigen prediction algorithms are beneficial to identify which neoantigens will likely induce an immune response in

cancer patients and help to prepare novel vaccines producing T cell responses to neoantigens.

Recently, identification of neoantigen-reactive tumor infiltrating lymphocytes has been reported from several types of cancers [25]. It is suggested that neoantigens have been responsible for the clinical efficacy of dendritic cell-based vaccines and adoptive T cell immunotherapies observed in some cancer patients. Noticeable tumor regressions and the onset of autoimmune melanocyte destruction have been reported in a significant proportion (50-70%) of patients with metastatic melanoma receiving adoptive transfer of tumor-reactive T cells and high dose IL-2 therapy after a nonmyeloablative lymphodepleting regimen [5,6]. Tumor exome analysis revealed a neoantigen-specific T cell reactivity in melanoma [26]. High-throughput epitope discovery also revealed frequent recognition of neoantigens by $CD4^+$ T cells in melanoma [27]. Using screening approaches to identify the antigens recognized by clinically effective tumor infiltrating lymphocytes in metastatic cancer patients, it has been found that tumor infiltrating lymphocytes isolated from metastatic cancer patients recognize mutated antigens derived from genes essential for carcinogenesis [28].

In patients with colorectal, pancreatic, bile duct, and lung cancer, vaccination with a neoantigen peptide, mutated Ras peptide, in combination with IL-2 and/or GM-CSF resulted in induction of immune responses to mutant Ras peptides in 20/37 patients with overall survival of 16.9 months [29]. Adoptive transfer of TCR-transduced T cells specific for a mutation-derived neoantigen significantly suppressed the progression of glioma xenografts in mice [30]. Recently, tumor-infiltrating lymphocytes were expanded from human resected ovarian cancer metastases. Autologous somatic mutations were identified with analyses by whole-exome and transcriptome sequencing. All mutated neoepitopes were expressed in antigen presenting cells and then cocultured with TIL fragment cultures. Results showed that mutation-reactive T cells infiltrated ovarian cancer metastases [31]. Vaccination of patients with melanoma with a personal neoantigen vaccine targeting 20 predicted personal tumor neoantigens induced polyfunctional $CD4^+$ and $CD8^+$ T cells which were able to target 60% and 16% of the 97 unique neoantigens used across patients, respectively. Four of six vaccinated patients had no recurrence at 25 months after vaccination. Two vaccinated patients showed recurrent disease. Subsequent treatment of these two patients with anti-PD-1 therapy resulted in expansion of neoantigen-specific T cells and complete tumor regression [32].

Improving Clinical Efficacy of Neoantigen-Based Cancer Vaccines by Immune Checkpoint Blocking

Vaccines can alter expression of several cell surface molecules in immune cells. Some immune cell surface molecules are highly involved in cellular immune responses. Programmed cell death 1 (PD1), also known as CD279, is expressed at the surface of various immune cells, including T cells, and functions as an immune checkpoint [4]. PD1 may promote apoptosis in antigen specific T cells and reduces apoptosis in regulatory T cells. Programmed death-ligand 1 (PD-L1), a member of the B7 superfamily, is a critical coinhibitory molecule that regulates T cell responses. The interaction between PD1 and PD-L1 attenuates T cell activation and promotes the development and function of regulatory T cells. Expression of PD1 and PD-L1 was found on several tumor cells [33-35]. It has been shown that immune checkpoint inhibitors targeting PD1 or PD-L1 will reduce the tumor growth.

Elevated frequencies of T cells expressing immune checkpoint molecules, such as PD-1, CTLA-4, and Tim-3, have been reported within tumors from different cancer patients [36-38]. Immune checkpoint inhibitors have been successfully used in combinational cancer therapies [4]. Immune checkpoint blockade using monoclonal antibodies enables the immune system to recognize and destroy tumor cells. In some cancer patients, administration of agents targeting PD1 and CTLA4 has been resulted improvement of antitumor immune responses and, in some cases, durable remissions [4]. Neoantigen-specific T cells can be the functional targets of immune checkpoint inhibitors [39-41]. $CD8^+$ tumor infiltrating lymphocytes reactive to clonal neoantigens (present in all tumor cells) were identified in early-stage of non-small cell lung cancer (NSCLC) and expressed high levels of PD1 [42]. In addition, sensitivity to PD1 and CTLA4 blockade in patients with advanced NSCLC and melanoma was increased in tumors enriched for clonal antigens [42]. Recently, resistance to immune checkpoint therapy has been reported in patients that initially respond to immune checkpoint blockade [43]. Upregulation of alternate immune checkpoint has been associated with adaptive resistance to immune checkpoint blockade [44]. It has also been shown that acquired resistance to immune checkpoint blockade can

arise in association with the evolving landscape of mutations [45]. Data obtained from genomic analysis reveals the genetic heterogeneity within single tumors. Furthermore, a relationship between clonal neoantigens burden and overall survival is found in adenocarcinomas. Intratumor neoantigen heterogeneity can influence antitumor immunity produced by cancer vaccine. Accelerate advancement in neoantigens discovery will help developing personalized cancer vaccines with high clinical efficacy.

Conclusion

In the past decades, cancer vaccines have been designed to target tumor antigens common to multiple patients. TAAs are generally overexpressed in malignant cells and expressed at lower levels in healthy tissue or normally expressed during embryogenesis or in testes. Vaccination with TAAs resulted in poor clinical efficacy due to pre-existence of immunological tolerance to self-antigens. On the other hand, breaking tolerance to TAAs by anticancer vaccination results in generation of strong immune responses against tumor cells but also against healthy tissues expressing the self-antigens. Exploiting TSA-based vaccines mitigates immunological tolerance and autoimmunity concerns associated with TAAs. However, TSAs have not been identified in most types of cancer. Due to difficulty of identifying antigens that are expressed exclusively on tumor cells of multiple patients (shared TSAs), identification of tumor-specific mutated antigens specific to a single patient (personalized TSAs) may be helpful in achieving a successful immunotherapy. Based on our experiences and available data, neoantigen-based vaccines represent a potential new class of cancer immunotherapy. These antigens are usually specific to the tumor of individual patient and provide tumor specific targets for personalized cancer vaccines. In recent clinical trials, neoantigen-targeting vaccines in combination with checkpoint blockade monoclonal antibodies produced potent tumor-specific T cell responses and satisfactory clinical outcome in cancer patients. Such personalized cancer immunotherapy needs accelerate advancement in neoantigens discovery.

Future Perspectives

Based on our experiences and available data, neoantigen-based vaccines represent a potential new class of cancer immunotherapy. These antigens are generally specific to tumor of the individual patient and provide tumor specific target for personalized cancer immunotherapy which seems to be more efficient therapeutic procedure in compare to the current immunotherapy practices.

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