Mini-Review

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Advances in personalized neoantigen vaccines for cancer immunotherapy

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SUMMARY Immunotherapy, which targets T cell inhibitory receptors (immune checkpoints), is now being widely used to treat a variety of types of cancer combined with surgery, chemotherapy, or radiotherapy. However, immune checkpoint inhibitors are highly dependent on the ability to present diverse tumor antigens to T cells. Neoantigens, arising from somatic mutations and specifically targeting tumor cells, have the potential to stimulate a highly specific immune anti-tumor response. Technological advances such as genomic sequencing and bioinformatics algorithms for epitope prediction have directly facilitated the development of neoantigen vaccines for individual cancers. Currently, several preclinical studies and early clinical trials using neoantigen in combination with checkpoint inhibitors have resulted in robust T cell responses and antitumor action. In the future, efforts will be made to optimize effective personalized neoantigen vaccines targeting individual tumors and to elucidate the immune mechanisms underlying tumor evolution.

Keywords personalized neoantigen, immune response, cancer vaccine, immunotherapy

1. Introduction

Immune checkpoint therapy with antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4) or programmed death 1/ligand 1(PD1)/(PDL1) has overcome immune suppression and induced sustained regression of disease in a subset of patients with cancer. However, tumor cells are able to evade the immune system due to their weak immunogenicity, leading to reduced efficacy or immunotherapeutic failure in many patients (10 to 60% of treated patients respond, depending on the type of cancer) (*1*). A recent study has reported that immune checkpoint inhibitors are highly dependent on the ability to present diverse tumor antigens to T cells (*2*). Hence, the effective identification of antigens with strong immunogenicity in tumor cells has become a priority in immunotherapy, and better understanding of mechanisms has suggested that immunogenicity and tumorigenicity are synchronous processes resulting from mutagenesis.

Neoantigens are mainly generated from peptide fragments of mutant proteins that derive from mutated genes, which are commonly involved in carcinogenesis (Figure 1) (*3*). Neoantigens are expressed exclusively in tumor cells with individual specificity and provide the immune system with potential target antigens. Neoantigens can be presented to T cells by major histocompatibility complex (MHC) molecules and stimulate lymphocyte-mediated anti-cancer immunity to eradicate cancer cells. They are presumed to be more highly immunogenic than non-mutated self-antigens, due to the minimized influence from thymic selection, central and peripheral tolerance, and the risk of autoimmunity (*4*).

Technological advances such as high-throughput sequencing of whole cancer genomes and the improvement of prediction algorithms have facilitated the development of personalized neoantigen vaccines (*5*). Recent studies have demonstrated the potential role of neoantigens in cancer immunotherapy and cancer evolution (*6,7*). This mini-review briefly summarizes advances brought about by recent neoantigen-directed studies to provide a better understanding of their mechanisms in order to improve cancer immunotherapy.

2. Neoantigen identification and selection

Neoantigens are highly individual-specific and are derived from driver mutations or passenger mutations in cancer cells. Prioritizing cancer-specific neoantigens is crucial to successful tumor vaccine therapy (*8*). Theoretically, potential neoantigens are generated from tumor somatic mutations based on the assumption that a mutated sequence can be translated into a protein, which is then processed into a peptide with a binding affinity for an MHC molecule that results in a mutant

peptide-MHC complex that is recognized by T cell receptors (*9*). To create a personalized cancer vaccine, neoantigens must be computationally predicted based on matched tumor-normal sequencing data and then ranked (prioritized) according to their predicted capability to stimulate a T cell response. This process of predicting potential neoantigens involves multiple steps, including somatic mutation identification, human leukocyte antigen (HLA) typing, peptide processing, and peptide-MHC binding prediction. The general workflow is shown in Figure 2. Finally, the antigenicity of the synthesized neoantigens is determined using standard immunological assays (*10*).

Short peptides and long peptides comprise the sequence of neoantigens with different lengths. The former generally refers to peptides of 8-11 amino acids in length that are recognized directly by $CD8^+$ T cells as potential epitopes. Short peptides directly bind to MHC class I molecules expressed by all nucleated cells, most of which are not specialized for antigen presentation, leading to weak T-cell priming or immune tolerance (*11*). Long peptides, which are 15-

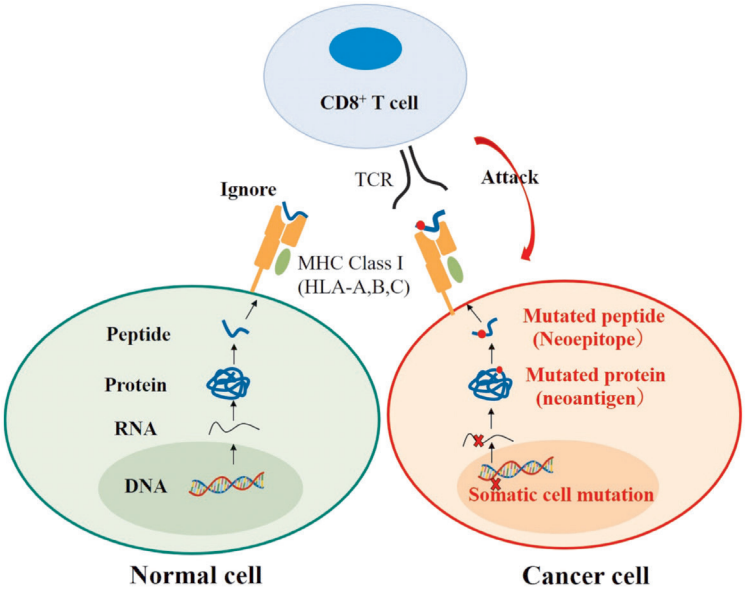

Figure 1. Peptide neoantigen. Variant peptides from mutated proteins (neoantigens) derived from somatic tumor-specific mutations can be presented as a mutant peptide-MHC complex on the cancer cell surface and can be recognized by T cell receptors (TCRs) to elicit an immune response.

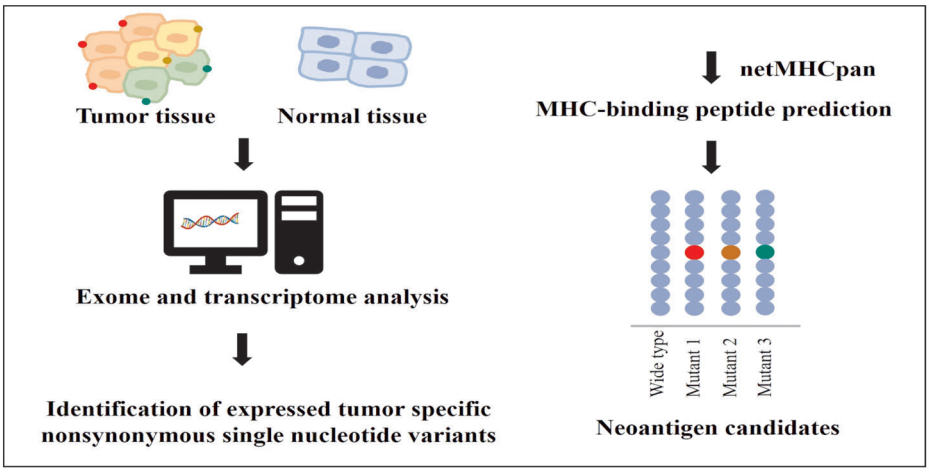

Figure 2. Diagram of the workflow for personalized neoantigen prediction. Clonal neoantigens can be expressed by intratumor heterogeneous mutations in tumor cells. Exome sequencing data from tumor tissue are compared with those from normal tissue to detect the full range of genomic alterations within a tumor. The expression of mutated antigens in the tumor is determined using transcriptome analysis. Then, the binding capacity to MHC molecules from mutations that encode a mutant protein is ranked using algorithms such as netMHCpan. The recognition of potential neoantigens is determined using standard immunological assays.

31 amino acids in length, are taken up and processed by professional antigen-presenting cells (APCs) for presentation and elicit MHC class I and MHC class II T cell activation (*12*). Studies have demonstrated that long peptides, which are superior to short peptides, can induce both $CD4^+$ and $CD8^+$ T cell responses (*6,7*). Clearly, both $CD8^+$ and $CD4^+$ T cells are critical to respectively recognizing antigens bound by MHC class I and II molecules on the cell surface. However, the challenge is to accurately identify optimal long peptides and to analyze MHC class II neoepitopes using current algorithms, as has been summarized elsewhere (*13,14*). Future developments may leverage artificial intelligence or machine learning with high-throughput sequencing and larger datasets of cancer-specific HLA ligands, T cell epitopes, and clinical responses to improve neoantigen prediction reliability (*13,15*).

In addition to the precise identification of highly expressed tumor-specific antigens, another step is to determine the therapeutic efficacy of neoantigens. That efficacy relies on a highly immunogenic environment including recruitment of professional APCs to the site of tumor antigen expression, uptake of the antigens by APCs, and maturation, activation, and trafficking of APCs to vaccine-draining lymph nodes where T cell activation occurs (*16*).

3. Clonal neoantigens and tumor evolution

The interplay of the adaptive immune system and evolving tumors is ongoing during the development and progression of tumors. On one hand, mutations provide fitness through the activation of key driver events or loss of tumor suppressor genes during evolution. On the other hand, a minority of mutations may result in neoantigens and provide targets for the immune system to inhibit the evolving tumor. Tumor cells undergo clonal selection pressure due to a variety of genetic and microenvironmental factors, which induce mutation frequencies that vary markedly within tumors (*17*).

Genomic heterogeneity including mutational burden and types, which might render tumors refractory to treatment, has also been found to correlate with heterogeneous immune cell infiltration. The interaction between an evolving cancer and a dynamic immune microenvironment was investigated by the TRACER-x consortium (*18*). Two hundred and fifty-eight regions from 88 early-stage, untreated non-small-cell lung cancers were analyzed and the immune cells, cancer mutations, and epigenetic marks were identified in these regions. The study found that sparsely infiltrated tumors exhibited a waning of neoantigen editing during tumor evolution, while immune-infiltrated tumor regions exhibited ongoing immunoediting, with either loss of heterozygosity in human leukocyte antigens or depletion of expressed neoantigens. That study revealed that local tumor-infiltrating lymphocytes influence the evolution of cancer through immunoediting of neoantigens.

Another study explored the relevance of the neoantigen burden, clonal neoantigen heterogeneity, and prognosis in patients with early-stage non-small-cell lung cancer included in the Cancer Genome Atlas project (*19*). In an immunotherapy-naïve setting, these patients were found to have significantly longer overall survival if their tumors contained a high number of clonal neoantigens and exhibited low levels of neoantigen heterogeneity. Gene-expression analysis revealed a subset of immune-related genes that were upregulated in the high clonal neoantigen group, indicating an inflammatory tumor microenvironment. That study demonstrated that the underlying mechanism of why the tumor overall mutation burden was not an optimal biomarker for checkpoint blockades in clinical settings since the clonal expression of neoantigens by tumor cells, rather than the overall mutational burden, determines the response to checkpoint blockade therapy (*20*).

The aforementioned study by the TRACER-x consortium found that immunogenicity could be lost through serial transplantation, while these tumors maintained their malignant potential according to different selective pressures (*18*). These fundamental findings have led to a basic understanding of the mechanism of neoantigens: due to the occurrence of T cell-mediated neoantigen immunoediting, a broad neoantigen-specific T cell response should be sought to avoid tumor resistance (*21*).

4. Neoantigen quality and quantity

Intratumor neoantigen heterogeneity, owing to the evolving tumor mutational landscape, poses a major problem to the management of early and advanced cancers. Neoantigen vaccines can only induce T cells to target a small number of tumor cells if the neoantigens are derived from mutated subclones, thus limiting the clinical efficacy of neoantigens (*22*). Because of their quantity and quality, clonal neoantigens are currently becoming a focus of immune-mediated control (*23*).

Previous research on cancer immunotherapy investigated the class I antigen processing pathway that elicits $CD8^+$ T cells to extensively kill cancer cells. However, there is mounting evidence of the promising efficacy of class II-specific neoantigens in cancer immunotherapies (*24,25*). In addition to CD8 T cells, the CD4 T cells are also required and may be crucial determinants of a successful response to immunotherapy (*26*). A recent study demonstrated that a successful immune response depends on the presence of neoantigens that trigger responses from both CD4 and CD8 T cells (*27*). Therefore, quality neoantigens should include both MHC class I and MHC class II epitopes to ensure CD8 cytotoxic T lymphocyte priming and CD4 T cell help for a robust immune response.

In the context of neoantigen-based cancer vaccines, mRNA/DNA or synthetic long peptides, encompassing both MHC class I and MHC class II epitopes, are typically used (*28*). Vaccination with a multi-epitope personalized neoantigen may be a promising strategy to induce intratumoral heterogeneous neoantigen-specific $CD4^+$ and $CD8^+$ T cell immune responses with a higher probability of antitumor efficacy (*29*). However, the challenge is to develop a general method for efficient stimulation of potent antitumor T cell responses (*30*). Direct injection of unformulated neoantigens has been tested in many studies (*7*). Nonetheless, the ultimate therapeutic efficacy of these peptide vaccines is limited by inefficient delivery to the desired lymphoid organs. Ex vivo-pulsed dendritic cell vaccines are promising but suffer from several limitations, including difficulties in preparation and expansion (*31*). In the future, engineered intelligent biomaterials, which can deliver several to several dozen neoantigens together with adjuvants to target APCs, are expected to achieve precise control of balanced MHC class I and II loading of antigens in order to elicit the most potent and broad T cell responses (*32*).

5. Neoantigen vaccine and checkpoint blockade therapy

If a neoantigen displayed on the surface of tumor cells bound to MHC molecules is recognized by a CD8 T cell, this cell can target and kill any tumor cells that express the same neoantigen. According to many studies, however, T cell priming neoantigen vaccines alone are not sufficient to trigger an effective immune response against the tumor because the cytotoxic response can be blocked by an immunosuppressive environment in the context of tumors (*33*).

Immune checkpoint therapy with antibodies targeting CTLA4 or PD1/PDL1 can overcome immune suppression across a variety of types of cancer (*34*). However, only a fraction of patients responds to immune checkpoint blockade with sustained regression. Given that the therapeutic benefit of an immune checkpoint blockade is currently limited to patients with pre-existing tumor-specific T cell responses, multifaceted approaches such as potent cancer vaccines specific to tumor neoantigens are anticipated to increase immune response in tumors treated with an immune checkpoint blockade (*35*). A study has demonstrated the nonsynonymous tumor mutation burden associated with the clinical benefit of anti–PD-1 therapy (*36*). Immune checkpoint blockades result in significant therapeutic responses to tumors with an increased mutation-associated neoantigen load. Importantly, studies on checkpoint blockades highlighted the positive correlation between the somatic mutation burden and the consequent emergence of clinically beneficial neoantigens (*37*). A recent study reported that acquired resistance to an immune checkpoint blockade can arise in association with the evolving landscape of mutations, some of which encode tumor neoantigens recognizable by T cells (*38*).

These findings imply that immune checkpoint blockades, which serve as vaccine adjuvants, are highly dependent on the ability to present diverse tumor antigens to T cells. Combining a blockade with neoantigen vaccines may improve antitumor efficacy or mitigate the development of acquired resistance. It is tempting to speculate that future studies involving the combination of T cell priming-neoantigen vaccines with T cell suppression-preventing checkpoint blockades may translate into a clinical benefit for patients with cold tumors (*39*).

6. Challenges for neoantigen vaccines

The broad range of neoantigens and their positive association with improved immune responses suggests their obvious advantages, including the possibility of mass production, easy monitoring of immune responses, and a tolerable safety profile. Nonetheless, the challenging aspects of anticancer vaccination are the identification of immunogenic neoantigens for vaccination and the difficulty of their intrinsic personalized nature: the bench-to-bedside timeframe. Therefore, the development of the accurate epitope-predicting algorithms and the optimization of efficient validation tools are currently top priorities for personalized neoantigen-based cancer immunotherapy. In addition, the development of an effective delivery strategy targeting multiple clonal neoantigens to elicit broad and potent T cell responses against tumor heterogenicity remains a challenge.

7. Conclusion

Personalized immunotherapy with neoantigens is one of the most promising approaches in cancer treatment. Precise identification of immunogenic neoantigens and an in-depth analysis of the immune-suppressive tumor microenvironment are required for an effective neoantigen-based cancer immunotherapy.

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