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Review

Significance of humanized mouse models for evaluating humoral immune response against cancer vaccines

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ABSTRACT

Because accumulation of mutations produces unique neoantigen patterns in cancer cells, it has been hypothesized that these patterns are recognized by the patients' immune system. Therefore, the development of cancer vaccines has been challenging owing to the possibility of an anti-cancer effect induced by the immune system against such neoantigens. However, it is difficult to develop effective vaccines because of the variety of mutations induced in cancer cells and human leukocyte antigen (HLA), which is predicted to present the neoantigen-derived peptides. Moreover, the activation of cancer-specific cytotoxic cells is inhibited by cancer cell immunoediting in each patient. Although cellular immunity can be analyzed *ex vivo*, there are no definite methods to evaluate humoral immunity. A humanized mouse model has been developed and used for evaluating the multipotency of hematopoietic stem cells. Presently, significant improvements in the humanized mouse model approach have partially recapitulated human humoral immunity *in vivo*, and human antibody production can be induced in the mouse model. These mice can be used to produce new cancer vaccines and to establish polypharmacy protocols in a preclinical model. In this review, we discuss the preclinical evaluation of the cancer vaccines using humanized mice transferred with patient-derived peripheral blood mononuclear cells (PBMCs) to advance a personalized medicine approach.

1. Introduction

It is well-known that a high rate of mutations leads to the accumulation of neoantigens in cancer cells. Therefore, enhancement of the immune function against such neoantigens is believed to induce anti-cancer effects. Massive effort has been made towards the development of anti-cancer vaccines [1]. However, the mutations observed in cancer cells are highly varied, which make the development of pan-specific vaccines difficult. Moreover, human leukocyte antigen (HLA) molecules also vary greatly and do not easily predict the efficacy and adverse effects of vaccines. Presentation of a specific peptide on the HLA molecules has been predicted by several algorithms, but their accuracy is inadequate [2,3]. Because neoantigens tend to have limited and unique sequences, the specific peptides also tend to be of a limited length. Therefore, HLA molecules, which are predicted to present neoantigens, are also very strictly determined. This is one of the most difficult issues to address for advancing personalized medicine for patients with cancer. To verify the overall vaccine effect of such peptides, humanized mice equipped with the human immune system have gradually attracted considerable interest.

Humanized mice have been developed to measure the multipotency of hematopoietic stem cells (HSCs). Until now, various immunodeficient mouse strains have been developed and used for examining HSC multipotency [4,5]. However, the development and differentiation of human T and B cells that enable the induction of

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complete humoral immunity have not been achieved in these mouse strains. Therefore, the humanized mouse models have not adequately analyzed human B cell functions. Two well-known immunodeficient mouse strains, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic (NOG) and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG), have been used for the development of human T and B cells via HSC transplantation [6,7]. However, even in mice, humoral immunity is severely diminished. Because these mice harbor a limited number of human myeloid cells, it is considered that the malfunction of humoral immunity is due to the developmental delay in human myeloid cells. Thus, transgenic mouse strains expressing human cytokine genes that enhance myeloid cell development were created, based on the NOG and NSG mice strains, and the production of antigen-specific IgG antibodies was observed along with other lymphoid tissues [8]. However, these mouse strains must mate with several other mice strains, and the selection of mice with three independent human genes must be continued. Despite the effort to maintain these strains, few antibodies are produced in these mice. Moreover, humanized mice use human HSCs derived from cord blood, indicating that the mice cannot mimic the immune environment of the patient, although such mimicry is essential for personalized diagnosis and medicine. If we use mobilized HSCs, it is highly invasive for the patients and is impractical. Following the first report by Williams, human peripheral blood mononuclear cells (PBMCs) have been transplanted in immunodeficient mouse strains [9]. However, because these immunodeficient recipient mice developed graft versus host disease (GVHD), the mouse model was not a suitable model for the analysis of human immune reactions during a long duration of vaccine treatment. Improvements of the recipient mouse strains using various transgenes related to the immune system are still continuing. These mouse strains are expected to develop normal human immune cells without the development of GVHD, in addition to antigen-specific IgG production. If such mouse strains are established, they will become promising tools for development of new cancer vaccines and protocols of vaccination and polypharmacy and verification of large volume of data needed to apply personalized medicine. Simultaneously, if the mouse strains can develop normal human cellular immunity, they may have a larger impact in this field. In this review, we will discuss the usefulness of current humanized mouse models to evaluate personalized peptide vaccination protocols.

2. Limitation of vaccine selection after neoantigen screening

As mentioned above, the high rate of mutations accumulates neoantigens in cancer cells [1,10]. Generally, serological analysis of recombinant cDNA expression libraries (SEREX) is performed forwide-scale screening and detection of cancer antigens in the sera of the patients [11]. The immune reaction, which targets the antigens selected by SEREX, may induce highly effective anti-cancer effects if the reaction functions effectively. In fact, several mouse models have been reported to successfully produce such characteristics.

However, it is also well-known that cellular immunity is significantly suppressed in tumor-bearing individuals. There are many reports to correlate malfunction and cancer immunoediting, involving Treg differentiation, expression of the ligands of immune checkpoint molecules that induce T cell exhaustion, and HLA expression downregulation [12,13]. However, several successful antigens, such as MAGE-A1, NY-ESO-1, and SSX-2, have been identified [14–17]. If these peptides are presented on class I HLA, they may be effective in invoking an immune response in the patient [18]. Some peptide vaccines are not effective in tumor-bearing individuals but are effective in inhibiting recurrence. For example, E75 and GP2 are breast cancer vaccines, the effects of which have not been identified in patients with cancer. However, the inhibition of recurrence has been observed in patients treated with peptides and granulocyte-macrophage colony-stimulating factor (GM-CSF) adjuvant [19,20]. Although the mechanism of action has not been determined, their efficacy is recognized in human erbB-2 receptor 2 (Her2) 1+ and Her2 2+ patients compared to that in Her2 3+ patients. Moreover, as previously described for general neoantigens, these peptide vaccines have limited HLA alleles for effective presentation.

Generally, peptide vaccines also need class I and class II HLA epitopes to activate both cytotoxic T cells (Tc) and helper T cells (Th). The reason is because Th cells supply cytokines essential for the expansion and survival of Tc cells [21]. Therefore, if we aim to establish a peptide vaccine with optimal effectiveness, the peptide sequence and HLA type of the patients become more restricted. However, prediction of the presentation ability of class I and class II HLAs using various algorithms is not always effective. Because most of the self-reactive T cells are deleted in the thymus by negative selection, neoantigen-specific T cells may be deleted in the thymus if the neoantigens are the only modified molecules and possess highly limited unique sequences.

We have been developing a candidate peptide, named CH401MAP, as a breast cancer vaccine [22]. CH401MAP is composed of 20 amino acid peptides of the HER2 molecule. CH401MAP peptides involve the epitope of a monoclonal antibody (mAb), CH401, which was developed by Ishida et al. at Sapporo Medical University; this mAb induced apoptosis of HER2-positive cancer cells [23]. The epitope is identified to be different from that of Trastuzumab, a well-known and effective molecularly targeted mAb drug. Compared with the epitopes of other already available HER2 peptide vaccines, the CH401MAP epitope has the highest affinity for HLA-A*24:02, an HLA class I allele present at the highest ratios in Japanese individuals. The peptide was predicted to be presented by other HLA isotypes. Eventually, the peptide has been predicted by several algorithms to be presented by most of the class I and class II HLA molecules of Japanese breast cancer patients. We also showed that PBMCs from the breast cancer patients can be stimulated by the peptides and secrete interleukin-2 (IL-2) [24]. These results suggest that CH401MAP also has the potential to activate cellular immunity in breast cancer patients.

Contrary to cellular immunity, there have been no successful reports on the induction apoptosis in cancer cells in response to a humoral immune reaction after cancer vaccination. Although active immunization with vaccines, which is simple, convenient, and yields high quality of life (QOL), has been effective for many infectious diseases, it is intriguing that humoral immunity in cancer patients does not induce apoptosis in cancer cells. In any event, evidence suggests suppression of humoral immunity in cancer patients.

To evaluate the effectiveness of the vaccines, it must be confirmed whether B cell clones expressing IgG molecules specifically reactive to the neoantigens are present among the B cells of the patient. Till date, however, methods have not been developed, other than directly injecting the antigen into the patients, to examine whether a humoral response is elicited after vaccination. No protocols exist for detecting the effector function related to humoral immunity, such as the activity of Th cells and production of antibodies against the peptide vaccine. It is one of the most serious problems to overcome.

Moreover, adverse effects due to vaccination are another serious problem. It is difficult to predict the adverse effects induced by the treatment in individual patients. Human papilloma virus vaccine, which has been developed for the prevention of cervical cancer and has been shown to be effective, occasionally leads to the development of severe adverse effects in the recipient [25]. The mechanism

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