

Enhancing the Anti-Tumor Effects of Cancer Peptide Vaccine Therapy

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Abstract

The clinical efficacy of cancer peptide vaccines has been considered inadequate. To enhance the anti-tumor effects of peptide vaccines, we have studied effective enhancement methods for peptide vaccine therapies such as intratumoral peptide injection and combination therapies with anti-PD-1 blocking antibody or anti-CD4 depletion antibody. We aim to present effective clinical applications of peptide vaccines.

Keywords: Peptide vaccine; Glypican-3; Intratumoral peptide injection; Anti-PD-1 blocking antibody; Anti-CD4 depletion antibody

Introduction

Cancer peptide vaccine therapies can be used to prolong survival while maintaining the quality of life (QOL) in patients and are expected to prevent or reduce recurrences. We have previously reported that glypican-3 (GPC3) is a cancer specific antigen [1-3]. Studies have identified GPC3-derived peptides that are capable of inducing peptide-specific cytotoxic T lymphocytes (CTLs) [4-6]. Several clinical trials for the GPC3 peptide vaccine therapy have been performed in hepatocellular carcinoma (HCC) [7-9]. Through previous studies, we have confirmed the safety and immunological efficacy of the vaccine and demonstrated its potential in inducing clinical effects in some patients [8-10]. However, the clinical efficacy of cancer peptide vaccine therapies is still considered inadequate. Consequently, we have attempted to develop effective enhancement methods for peptide vaccine therapies.

Intratumoral peptide injection enhances tumor cell antigenicity

Antigen-specific cancer immunotherapy involves antigen-specific CTLs, which recognize the antigen-derived peptides bound to major histocompatibility complex (MHC) class I molecules on the tumor cell surface and destroy the tumor cells. The low density of presented antigen bound to MHC class I molecules is one of the reasons why antigen-specific cancer immunotherapy has been ineffective in a clinical setting. We confirmed that most of the tumors exhibited enhanced expression of the human leukocyte antigen (HLA) class I molecules and its expression within the tumor area was higher than that outside it. Therefore, to induce additional peptide loading onto MHC class I molecules present on the tumor cells, we performed the intratumoral peptide injection for effectively enhancing the anti-tumor effect of peptide vaccines.

Intratumoral peptide injection was effective in inhibiting tumor growth and prolonging survival time. Furthermore, an antigen-spreading effect was detected after the peptide injection, which enhances tumor cell antigenicity and may be a valuable option in

improving the anti-tumor effects of antigen-specific cancer immunotherapy against solid tumors [11].

Programmed death-1 (PD-1) blockade enhances the antitumor effects of peptide vaccines

PD-1 is expressed on activated T and B cells, and it induces inhibitory signals [12]. Several studies have shown that the PD-1/PD-L1 pathway plays a critical role in compromised tumor immunity [13,14].

We have used peptide emulsified with incomplete Freund's adjuvant (IFA) for peptide vaccines in both animal models and clinical models. However, the peptide/IFA vaccination increased antigen-driven expression of the inhibitory receptors PD-1, LAG-3, CTLA-4, and Tim-3 in CTLs [15]. PD-1 blockade could partially rescue CTLs in a state of exhaustion. Therefore we employed the combination therapy by using the peptide vaccine and PD-1 blocking antibody. We demonstrated that PD-1/PD-L1 blockade enhanced the anti-tumor effects of peptide vaccines by increasing the immune response of vaccine-induced CTLs [16].

Anti-tumor effects of peptide vaccines were enhanced in combination with anti-CD4 antibody

Several studies have suggested that the depletion of CD4⁺ cells results in strong anti-tumor effects in tumor-bearing mice models due to the enhancement of CTL responses [17-19].

To enhance the anti-tumor effects of peptide vaccines, we included an anti-CD4 monoclonal antibody (mAb) (clone: GK1.5) in a mouse model. Using the IFN- γ ELISPOT assay, we determined that the number of ovalbumin (OVA)-specific CTLs induced by OVA peptide vaccine in combination with anti-CD4 mAb was higher than that induced by OVA peptide vaccine alone. Additionally, after the combined treatment with OVA peptide vaccine and anti-CD4 mAb, perforin and granzyme secretion from CD107a⁺ cells increased and the production of IL-2 and TNF from these CTLs increased as analyzed by the CD107a assay and cytokine assay, respectively

Finally, we observed that metastasis was remarkably suppressed by the peptide vaccine in combination with anti-CD4 mAb in a murine model of liver metastasis. The mouse model of liver metastasis was

developed by injecting tumor cells into the spleen. The evaluation of liver metastasis was performed using the weight of the murine liver, because the number of metastases in the liver could not be counted too many. The liver weight of the combination group treated with the OVA peptide vaccine and anti-CD4 mAb was significantly lower than those of the untreated group and the group treated with OVA peptide vaccine alone. However, the liver weight of the combination group showed no significant difference from that of the group treated with anti-CD4 mAb alone [20]. However, we believe that the enhanced inhibitory effects on metastasis in the combination therapy was derived from the enhancement in the multi-functionality of peptide-specific CTLs as determined by the IFN- γ ELISPOT assay, CD107a upregulation assay and cytokine assay. Further investigations must be conducted to evaluate liver metastasis based on the number of liver metastases.

Conclusion

Although peptide vaccines exhibit disadvantages such as weak anti-tumor effects, they also have several advantages such as systemic effects similar to chemotherapies, with fewer side effects. Our therapeutic strategies such as intratumoral peptide injection or the combination therapies with antibody drugs can enhance the anti-tumor effect of cancer peptide vaccine therapy.

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