Pneumoconiosis Compensation Fund Board

Final Report

Project title:

Induction of Mesothelioma-Specific $CD8^+$ T Cell Response for Immunotherapy and Prevention

1. Introduction

Mesothelioma is an asbestos-associated malignant form of cancer, which often has very poor prognosis in humans after disease onset (1). Since the prevalence of this lethal cancer is increasing worldwide including in Hong Kong, we may be facing the peak incidence in recent years (2). The current standard of care for this life-threatening malignancy is pemetrexed and cisplatin combination chemotherapy approved decades ago, which only achieves suboptimal improvements in patients' survival (1, 3). Although the recent immunotherapy using immune checkpoint inhibitors targeting cytotoxic T lymphocyte associated protein 4 (CTLA4), PD1 and PD-L1 has improved therapeutic efficacy in certain cancers, their effects are unsatisfactory in patients with mesothelioma (3). The first randomized phase III trial involving immunotherapy in mesothelioma with anti-CTLA4 antibody failed to meet its primary end point of improved overall survival (4, 5). PD1 and PD-L1 checkpoint inhibitors have been shown some promising results in treating advanced mesothelioma in phase I/II trials, yet the overall responsive rate is limited to <30% (6, 7). Since conventional vaccine strategies are insufficient in eliciting specific anti-mesothelioma immune responses, new approaches need to be explored.

The overall objective of the proposed study is to induce mesothelioma-specific $CD8^+$ T cell immunity for immunotherapy and prevention. Using a model sPD1-$p24_{fc}$/electroporation (EP) vaccine, we have demonstrated that vaccine-elicited $CD8^+$ T cells confer complete prevention and therapeutic cure of AB1-GAG malignant mesothelioma(8). The efficacy was attributed to vaccine-elicited $CD8^+$ T cells with T-bet^+, $Eomes^+$ and IFN-γ^+TNF-α^+ phenotypes that could retain their effector functions once infiltrated into tumor(9), reduce myeloid-derived suppressive cells (MDSC) and $CD4^+CD25^+Foxp3^+$ regulatory T lymphocytes (Treg) cell populations(10, 11), and lead to the complete clearance of tumor cells(8, 9). Thus, we hypothesized that, if the vaccine is highly potent and specific, it is possible to use active vaccination to harness the immune system and to reinstate immune

surveillance by overcoming tumor-associated immune suppression (Aim 1). During the first year of funding, under this aim, we have found that mRNA of three tumor antigens, including prostatic acid phosphatase (PAP), Twist Family BHLH Transcription Factor (TWIST) and osteopontin (OPN), can be detected from both human and mouse malignant mesothelioma. Among them, PAP and TWIST could serve as potential vaccine antigens because of their elevated expression in various tumors and their immunogenicity in cancer patients (12, 13). These findings inspire us to further evaluate the anti-mesothelioma efficacy of PD1-based PAP-DNA/EP vaccine, as well as PD1-based TWIST-DNA/EP vaccine, during the year 2 study.

Considering that dying tumor cells are immunogenic for triggering immune responses against a broad range of epitopes (14, 15), we hypothesized that antitumor immune responses can be elicited against wild-type (WT)-AB1 mesothelioma during the effective elimination of AB1-GAG malignant mesothelioma achieved by sPD1-p24$_{fc}$/EP vaccination, a process so-called antigen spreading (Aim 2). During the first year of funding, under this aim, we have found that that antigen spreading generated by sPD1-p24$_{fc}$/EP vaccine-mediated eliminations of AB1-GAG mesothelioma indeed resulted in the induction of effective tumor-specific cytotoxic CD8^{+} T cells, which were capable of inhibiting PD1/Tim3 expression on their surface, reducing the number of MDSCs, and rejecting wild-type AB1 malignant mesothelioma(16). We have also found that high dose (10^{9} PFU) intratumoral (i.t) delivery of modified vaccinia virus Tian Tan strain (MVTT) could efficiently eliminate established large solid AB1 mesothelioma. Moreover, infection of AB1 cells with MVTT led to oncolytic lysis of tumor cells and exposure of calreticulin (CRT) protein, and release of high mobility group box 1 protein (HMGB1) and ATP, which are the three major biomarkers of antigen spreading occurring during immunogenic cancer cell death(17). Further investigation of the detailed mechanisms underlying these findings is the specific objective during the year 2 study.

After one year's investigation on this project, we have found that soluble PD-1-based vaccine enhanced immune responses of PAP42, a PAP-derived 42-mer epitope (18), in C57BL/6 mice and interestingly, human PAP42 was more immunogenic than mouse PAP42 and induced PAP-specific T cells cross-recognizing mouse PAP antigen. Since C57BL/6 mouse doesn't support the growth of BALB/c-originated AB1 mesothelioma cells, in order to examine the anti-tumor effect of PAP-DNA/EP vaccine constructs, we have established B16F10 melanoma model with overexpression of PAP antigen in C57BL/6 mouse. However, to our surprise we found that PAP42 DNA/EP vaccination, even in the fusion form with soluble PD-1, induced limited anti-tumor effect against PAP-expressing melanoma. In

addition to PAP antigen, we have also evaluated the immunogenicity of TWIST proteins, including TWIST1 and TWIST2, in our model. We found that only soluble PD-1-based TWIST1 DNA vaccine induced significantly enhanced TWIST1-specific T cell responses in BALB/c mice and importantly, in a prophylactic setting TWIST1 vaccination showed anti-mesothelioma activity by slowing AB1 mesothelioma growth and prolonging the survival of tumor-bearing mice. Furthermore, we have also evaluated the therapeutic efficacy of TWIST1 vaccination against established AB1 mesothelioma and found that, for the first time in mesothelioma immunotherapy, soluble PD-1-based TWIST1 vaccines was able to elicit anti-mesothelioma immunity and it worked synergistically with anti-CTLA4 antibody to enhance therapeutic efficacy against established mesothelioma (Aim 1). To develop a new method of oncolytic virus-induced antigen-spreading for mesothelioma elimination, we have found that combined use of oncolytic vaccinia MVTT with polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) depletion resulted in complete remission of AB1 mesothelioma in mice. Specifically, our findings demonstrated that intratumoral PMN-MDSCs are key DC suppressors in the mesothelioma tumor microenvironment (TME) that restrict the induction of antitumor cytotoxic T lymphocytes (CTLs), compromising the efficacy of MVTT-based virotherapy (Aim 2).