Pneumoconiosis Compensation Fund Board

Final Report

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Project title:

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

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1. Introduction

Malignant mesothelioma is a rather aggressive cancer that develops from mesothelial cells lining the pleura, peritoneum or pericardium. The major cause of mesothelioma is likely the exposure of mesothelial cells to airborne asbestos(1). The prognosis for malignant mesothelioma remains rather disappointing. Although treatment of malignant mesothelioma at earlier stages has a better prognosis, cure is exceedingly rare. Due to the lack of effective methods for early detection and treatment, the median survival of malignant mesothelioma patients is commonly less than one year from its diagnosis(2). Tse *et al.* indicated that Hong Kong might be facing the peak incidence of malignant mesothelioma in recent years(3).

Malignant mesothelioma has received much attention worldwide because of asbestos control and huge financial compensation. The current mesothelioma prevention largely relies on asbestos control by avoiding environmental and occupational exposures. Because of the limitation of survival benefit and side effect from chemotherapy, immunotherapy approaches are highly desirable. Till now,

however, no effective vaccines have been developed to prevent or treat mesothelioma. In recent years, novel therapeutic and preventive approaches have been studied at pre-clinical and clinical stages including immunotherapy, which indicates the importance of this research area(1). Since conventional vaccine strategies are insufficient in eliciting specific anti-mesothelioma immune responses, new approaches need to be explored.

We have reported that programmed death-1 (PD-1) based DNA vaccine with electroporation (EP) generally achieves high level of T cell response especially $CD8^+$ T cells, which offer cytotoxic activities against tumor cells through the delivery of PD-1 (or its soluble form) binds its ligands, PD-L1 and PD-L2, which are expressed on antigen presenting cells and can be used as target for antigen delivery(4). We take advantage of these findings and further investigated the efficacy of a model sPD1-$p24_{fc}$/EP vaccine against mesothelioma, through which we have demonstrated that vaccine-elicited $CD8^+$ T cells confer complete prevention and therapeutic cure of AB1 malignant mesothelioma that express a model antigen, HIV-1 GAG(5). 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(6), reduce myeloid-derived suppressive cells (MDSC) and $CD4^+CD25^+Foxp3^+$ regulatory T lymphocytes (Treg) cell populations(7, 8), and lead to the complete clearance of tumor cells(6, 9). Thus, 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.

In order to take a step further, we hypothesized that it would be critical to identify novel tumor-specific antigens for constructing PD-1 based DNA vaccines to induce protective $CD8^+$ T cells for mesothelioma immunotherapy (Aim 1) and to develop a new method of antigen-spreading induced by vaccinia virus for mesothelioma elimination (Aim 2).

After one-year's investigation on this project, we have firstly described 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, so called antigen-spreading, and $CD8^+$ T cells elicited in this process confer protection against the lethal challenge of wild-type malignant mesothelioma by eliminating MDSCs (Aim 1)(10). The rejection of mesothelioma cells here relied on tumor-specific $CD8^+$ T cells capable of eliminating

both tumor cells and MDSCs, demonstrating the existence of undefined potential tumor specific/associated antigens in mesothelioma that would serve as ideal vaccine candidates. By semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR), we have shown 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 (11, 12). To confirm and extend our observations, we have also verified the expression of TWIST protein in AB1 mesothelioma by western blotting and obtained full-length TWIST and PAP genes from AB1 cDNA (Aim 1). By vaccination of mice with DNA vaccines encoding a PAP-derived 42-mer epitope (PAP42), we have found that PD-1-based PAP-DNA vaccine enhanced PAP specific immune responses (Aim 1). To develop a new method of oncolytic virus-induced antigen-spreading for mesothelioma elimination, we show in this report that intratumoral (i.t) delivery of vaccinia virus Tian Tan strain (VTT) could efficiently eliminate established large solid AB1 mesothelioma (Aim 2). In order to understand the underlying mechanism, we have found that infection of AB1 cells with VTT lead 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 hallmarks of antigen spreading occurring during immunogenic cancer cell death (13). We reasoned that combination of oncolysis and PD-1/PD-L pathway blockade has the potential to improve the antitumor activity of oncolytic VTT virus. However, soluble PD-1 armed VTT virus did not enhance antitumor efficacy against mesothelioma in our model. Importantly, we also found that oncolysis of mesothelioma by VTT virus failed to induce antigen-spreading, probably due to the accumulation of MDSCs in tumor which dampens the induction of antigen-spreading. These findings provide us with new directions to overcome localized immunosuppression by combination of oncolysis and MDSC depletion.

2. Research highlights

i. Antigen spreading-induced $CD8^+$ T cells confer protection against the lethal challenge of wild-type malignant mesothelioma by eliminating MDSCs(10).

This work has been published in *Oncotarget* and we acknowledged PCFB for financial support.

ii. PAP and TWIST could serve as potential tumor antigens to design vaccine candidates.

iii. PAP42 induced strong PAP specific cellular immune responses in C57BL/6 mice. PD-1-based PAP42 DNA/EP vaccine enhanced antigen specific immune responses in mice.

iv. i.t delivery of VTT led to therapeutic cure of established AB1 malignant mesothelioma in mouse model.

v. Although VTT virus can efficiently infect AB1 malignant mesothelioma to produce viral particles and lead to the surface exposure of CRT, release of HMGB1 and ATP, it fails to induce antigen-spreading.

vi. Accumulation of MDSCs in tumor might directly suppress the immune activation after VTT treatment.