Pneumoconiosis Compensation Fund Board

Final Report

Project title:

Enhancing immunotherapeutic efficacy of mesothelioma by overcoming MDSC-mediated immunosuppression

Contents

1. Introduction

Malignant mesothelioma is a lethal type of cancer linked to historical exposure to airborne asbestos that typically arises from the pleura. The incidence and mortality of mesothelioma continue to rise in developing countries primarily (1). Treating malignant mesothelioma is challenging because the majority of patients (>75%) experienced relapse even after multimodality treatment (combined surgery, chemotherapy, and/or radiotherapy) (2). Chemotherapy with pemetrexed plus cisplatin has been the only approved regimen for more than a decade, but this approach only achieved modest benefits at best and many patients are unfit for such treatment (3). Although antibodies targeting immune checkpoint molecules, such as cytotoxic T lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD1) and programmed death-ligand 1 (PD-L1), have improved therapeutic efficacy in certain cancers, their effects are unsatisfactory in patients with mesothelioma (4). In particular, the first randomized phase III trial against mesothelioma using anti-CTLA-4 antibody failed to meet its primary end point of improved overall survival (5, 6). PD1 and PD-L1 checkpoint blockade antibodies have been shown some promising results in treating advanced mesothelioma in phase I/II trials, yet the overall responsive rate is below 30% (7, 8).

Recently, oncolytic virotherapy has emerged as a promising cancer immunotherapeutic strategy for the treatment of solid tumors including malignant mesothelioma, yet the mechanism underlying the limited virotherapeutic efficacy remain elusive(4, 9).

Direct virus-mediated oncolysis of cancer cells is one of the major mechanisms of oncolytic virotherapy. During oncolysis, danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) are released into the microenvironment, which can modulate the immunogenicity of released tumor antigens by creating an immune-activating environment and subsequently eliciting or reinforcing tumor-reactive T cell responses(10). The crucial role of adaptive T cell immunity in oncolytic virotherapy has been demonstrated in both preclinical and clinical studies(11, 12). However, the tumor microenvironment (TME) is often an immunosuppressive environment that inhibits the activation of tumor-reactive T cells by inducing tolerogenic dendritic cells (DCs) and $CD25^{+}Foxp3^{+}$ regulatory T lymphocytes (Tregs) (10, 13, 14). A number of studies indicated that bone marrow myeloid-derived suppressor cells (MDSCs) in the TME can dampen the responsiveness of cytotoxic T lymphocytes (CTLs)(15), leading to limited efficacy in patients, especially when the TME is highly immunosuppressive(16-19). Because T cell immunity is indispensable for the efficacy of oncolytic virotherapy, the better understanding of restrictive mechanisms in the TME is particularly important for improving the clinical outcomes of the virotherapeutic strategy.

MDSCs represent one of the major immunosuppressive populations in the TME and a major obstacle to the effectiveness of cancer immunotherapy(15). In malignant mesothelioma models, we have previously reported that MDSCs expand quickly with the development of tumor lesions and contribute to the inhibition of tumor-reactive CTL responses(20, 21). Consistently, decreased numbers of MDSCs in the TME are likely associated with the generation of antigen-specific CTL responses and therapeutic efficacy during oncolytic virotherapy in patients(16). MDSCs consist of monocytic (M) and polumophonuclear (PMN)-MDSCs. A recent study further indicated that targeting the COX-2-PGE_2 pathway during vaccinia virotherapy is capable of decreasing PMN-MDSC levels while increasing antitumor CTL responses(22). Moreover, an earlier study using the COX-2 inhibitor celecoxib improved DC-based immunotherapy against mesothelioma by reducing the PMN-MDSC frequency(23). We have recently shown that virotherapy using modified vaccinia Tiantan virus (MVTT) induced oncolysis of AB1 mesothelioma cells *in vitro* and resulted in regression of established AB1 mesothelioma dose-dependently *in vivo*(24). Importantly, we found that combined use of oncolytic vaccinia MVTT with PMN-MDSC depletion by anti-Gr-1 antibody induced potent and long-lasting antitumor cytotoxic T lymphocytes (CTLs), leading to clearance of mesothelioma in mice when MVTT was used even at less frequent and 10-fold lower doses. These findings provided evidence that depletion of tumor PMN-MDSCs by specific antibodies during oncolytic virotherapy is a new approach to treat malignant mesothelioma and may potentially improve therapeutic efficacy of mesothelioma patients. To this end, however, the absence of the human equivalent to the murine Ly6G (Gr-1) marker makes it difficult to design antibody targeting human MDSCs and importantly, the biomarker(s) of human PMN-MDSCs remain to be identified.

The overall objective of the proposed study is to specifically deplete PMN-MDSC through modulation of CCRK signaling during oncolytic virotherapy, aiming to enhance the immunotherapeutic efficacy of mesothelioma. This hypothesis is based on our very recent discovery that inhibiting cell cycle-related kinase (CCRK) signaling could diminish PMN-MDSC-mediated immunosuppression and inhibited

tumorigenicity of hepatocellular carcinoma (25). In this study, we found that combined use of oncolytic vaccinia MVTT with polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) depletion by either antibody or CXCR2 pepducin resulted in complete remission of AB1 mesothelioma in mice. Thus, these findings provided a proof-of-concept that CXCR2 inhibition can specifically and effectively counteracts PMN-MDSCs-mediated immunosuppression in the TME. For this purpose, the anti-mesothelioma efficacy of combined MVTT and CXCR2-specific inhibitors was further examined in our mouse model. We found that CXCR2 inhibitor SB265610 amplifies the antitumor efficacy of vaccinia virotherapy against mesothelioma, probably due to specifically and effectively depletion of PMN-MDSCs as well as inhibition of PMN-MDSC-mediated immunosuppression by SB265610 administration.

2. Results

2.1 PMN-MDSCs as critical suppressors of DCs for the blockade of vaccinia virotherapy-induced antitumor CTLs.

Antitumor cytotoxic T lymphocytes (CTLs) are essential for immune surveillance, yet the blockade of eliciting such CTLs during oncolytic virotherapy remains incompletely understood. In the proposed study, we hypothesized that targeting and depleting polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) would augment antitumor CTL responses for the improvement of the efficacy of oncolytic virotherapy. Therefore, we firstly sought to demonstrate the critical role of PMN-MDSCs in restricting T cell immunity and their underlying restrictive mechanisms in the tumor microenvironment (TME) during vaccinia virotherapy

2.1.1 Depletion of PMN-MDSCs but not of M-MDSCs during MVTT virotherapy unleashes tumor-reactive CTLs, leading to the therapeutic cure of established mesothelioma.

MDSCs represent one of the major immunosuppressive populations in the TME and a major obstacle to the effectiveness of cancer immunotherapy (15). MDSCs are a heterogeneous population which consists of monocytic (M) and polumophonuclear (PMN)-MDSCs. To investigate their roles in the vaccinia virotherapy, two MDSC-depleting agents, anti-Ly6G monoclonal antibody 1A8 and the specific depleting peptibody H6-pep, were explored in our mesothelioma model. 1A8 is routinely used to deplete $Ly6G^+$ cells, primarily PMN-MDSCs, whereas H6-pep is the peptibody for preferentially depletion of M-MDSCs (24, 26). To explore whether the depletion of MDSCs enhanced the therapeutic efficacy of modified vaccinia Tiantan (MVTT)-based oncolytic virotherapy, BALB/c mice bearing 7-day-old wild-type AB1 mesothelioma were simultaneously injected with low-dose MVTT (1×10^7 PFU) in combination with either 100 μg of 1A8 or Pep-H6 for the specific depletion of PMN-MDSCs and M-MDSCs, respectively (**Figure 1A**). We found that two MVTT treatments alone slowed tumor growth and resulted in tumor regression in 1/7 mice, whereas 1A8 alone did not impact tumor growth at all (**Figure 1B-C**). Strikingly, however, the 2 times combined low-dose MVTT and 1A8 treatment effectively controlled tumor growth and eventually led to complete elimination of established AB1 mesothelioma (**Figure 1B-C**). By contrast, the combined MVTT and Pep-H6 treatment did not show significant antitumor activity or synergistic effects in mesothelioma elimination (**Figure 1D-E**).

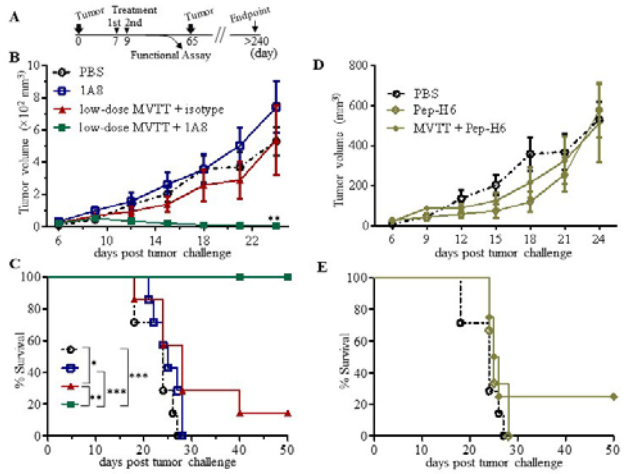

Figure 1. Depletion of PMN-MDSCs enhances MVTT virotherapy efficacy. (**A**) Schematic representation of treatment schedule. 5×10^5 AB1 cells were s.c inoculated into BALB/c mice and grown for 7 days, following i.t administration of MVTT, 1A8 antibody, Pep-H6 peptibody, combined MVTT and 1A8, combined MVTT and Pep-H6 or PBS control. An additional treatment was scheduled at day 9 in each group. Tumor growth (**P = 0.0069 compared to PBS-treated group) (**B, D**) and survival curves (**C, E**) of mice were calculated.