学 位 論 文 の 要 旨

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論 文 内 容 の 要 旨

INTRODUCTION

Numerous clinical studies on cancer immunotherapy have revealed that immune checkpoint blockade (ICB) therapy is effective against various types of cancers, including gastrointestinal cancer. Programmed cell death 1 (PD-1) blockade therapy has been approved for multiple malignancies, although its clinical effectiveness is limited. In addition, the overall response rate of advanced colon cancer to PD-1 blockade therapy is only 5–6 %. Therefore, novel treatment modalities are needed to improve the therapeutic efficacy of ICB therapy for colorectal cancer (CRC) patients.

In addition to direct cytotoxic effects, some anti-cancer chemotherapeutic drugs have the potential to modulate the immune system and augment antitumor responses in cancer-bearing hosts. Recent studies have revealed the underlying mechanisms, namely regulation of the immunogenicity of tumor cells, mitigation of regulatory T cell (Treg)-mediated immunosuppression, reduction of myeloid derived suppressor cells (MDSCs), induction of mature dendritic cells, and induction of the homeostatic proliferation of T cells. More specifically, cyclophosphamide (CP) decreases immunosuppression by Tregs when given at low/medium doses. Oxaliplatin (L-OHP) can induce immunogenic cancer cell death. 5-fluorouracil (5-FU) decrease MDSCs in cancer-bearing hosts. Theoretically, combining these chemotherapeutic drugs could mitigate immunosuppression by Tregs and MDSCs, and induce immunogenic cancer cell death, thereby promoting anti-cancer T cell immunity.

5-FU and L-OHP are standard chemotherapeutic drugs for colon cancer. In this study, we combined 5-FU/L-OHP with CP (triple combination chemotherapy). We demonstrated that the triple combination chemotherapy had superior antitumor effects to 5-FU/L-OHP therapy alone.

We also revealed that the antitumor effects of the triple combination therapy were mainly attributable to host T cells, and that therapeutic efficacy could be boosted by ICB therapy.

MATERIALS AND METHODS

Wild-type BALB/c, BALB/c nu/nu, and C57BL/6 female mice (6–7 weeks old) were kept under specific pathogen-free conditions. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University. (IZ30-104, IZ31-41, and IZ31-66). CT26 and MC38 are colon carcinomas originating in BALB/c and C57BL/6 mice, respectively. Wild-type BALB/c mice were injected subcutaneously (s.c.) with 5×10^5 CT26 cells into the flank. On day 10, the mice were randomly divided into four groups. On days 10 and 18, the mice were injected intraperitoneally (i.p.) with either one or all of CP (50 mg/kg), 5-FU (50 mg/kg), and L-OHP (6 mg/kg). In the case with BALB/c nude mice, the drugs were injected i.p. on days 8 and 16. The tumor volume (mm^3) and body weight were measured every 4 days. In the MC38 model, C57BL/6 mice were injected s.c. with 5×10^5 MC38 cells into the flank. On days 10 and 18, the mice were injected i.p. with the indicated doses of chemotherapeutic drugs. On days 11 and 19, the mice were injected i.p. with anti-PD-1 monoclonal antibody (200 μg/mouse). To assess protective immunity, naïve and cured mice after therapy were injected s.c. with 5.0×10^5 CT26 or 2.5×10^5 MC38 cells into the flank. To examine cytotoxicity against cancer cells, the spleen cells of naïve or cured mice were cultured with the indicated tumor peptide with interleukin (IL)-2 (20 U/mL) for 4 days. H-2L^d-binding AH1 peptide (SPSYVYHQF) and H-2K^b-binding p15E peptide (KSPWFTTL) were used as tumor antigenic peptides for CT26 and MC38, respectively. Cytotoxicity was measured using a 5-h ^{51}Cr-release assay. To analyze tumor-infiltrating immune cells, tumor tissues were resected from mice individually on day 4 after the second chemotherapy. Similarly, spleen cells were analyzed. Analysis was performed using the FACSCalibur.

RESULTS AND DISCUSSION

In the CT26 model, a significant difference was observed between the 5-FU/L-OHP and CP combination therapy and either therapy alone. The triple combination therapy cured two out of six mice. No body weight loss was observed in mice treated with any therapy. The antitumor effects were weakened in nude mice. Two out of three cured mice after the triple combination chemotherapy rejected re-challenged CT26, implying the induction of protective immunity. Higher levels of anti-CT26 cytotoxicity were generated from the spleen cells of cured mice compared to those from naïve mice after *in vitro* stimulation with the AH1 peptide.

We analyzed tumor-infiltrating immune cells and spleen cells 4 days after the second therapy. 5-FU/L-OHP therapy with or without CP increased the proportion of $CD8^+$ T cells in tumor sites. Although a similar tendency was observed in terms of spleen cells, increases in the proportions of $CD8^+$ T cells were more distinguishable in tumor sites. 5-FU/L-OHP therapy

decreased the proportion of granulocytic MDSCs (G-MDSCs), and increased that of monocytic MDSCs (M-MDSCs), whereas the additional CP treatment reversed these changes.

In the MC38 model, either or both of 5-FU/L-OHP and CP significantly suppressed the tumor growth, whereas no mouse was cured. However, additional combination with anti-PD-1 antibody cured three out of six mice. The triple combination chemotherapy transiently decreased the body weight in MC38-bearing mice. All three cured mice rejected re-challenged MC38. Higher levels of anti-MC38 cytotoxicity were generated from the spleen cells of cured mice compared to those from naïve mice after *in vitro* stimulation with the p15E peptide. We also examined tumor-infiltrating immune cells and spleen cells in treated MC38-bearing mice. The 5-FU/L-OHP therapy increased the proportions of $CD8^+$ T cells in tumor sites and the spleen, whereas the changes were not so apparent as those in the CT26 model. We finally examined the antitumor effects when anti-PD-1 antibody therapy was combined with either double (5-FU/L-OHP) or triple (5-FU/L-OHP/CP) combination therapy using the MC38 model. As a result, the triple combination chemotherapy was more effective than the double chemotherapy when combined with anti-PD-1 antibody therapy.

In terms of underlying mechanisms of antitumor effects induced by the triple combination chemotherapy, T cells, especially $CD8^+$ T cells, must be main effectors because the antitumor effects were apparently attenuated in nude mice. However, M-MDSCs and Tregs could affect the antitumor effects by the triple combination chemotherapy. In the CT26 model, the proportion of $CD11b^+$ cells among $CD45^+$ cells in tumor sites was decreased after the triple chemotherapy in CT26-bearing mice. In addition, the triple chemotherapy decreased the proportions of M-MDSCs and $CD4^+$ T cells in tumor sites. Given that M-MDSCs have a greater suppressive effect than G-MDSCs on antigen-stimulated $CD8^+$ T cells and that Tregs are $CD4^+$ T cells, the decrease in proportions of M-MDSCs and/or $CD4^+$ T cells at tumor sites could participate in the therapeutic efficacy of the triple combination therapy in CT26-bearing mice. On the other hand, different results were obtained in the MC38 model; the triple chemotherapy increased the proportions of $CD8^+$ T cells and M-MDSCs but showed no effect on that of $CD4^+$ T cells in tumor sites. An increase in the proportions of $CD8^+$ T cells was more apparent in CT26 tissues than in MC38 tissues. This difference may account why the MC38 model needed for additional anti-PD-1 antibody therapy.

CONCLUSION

We demonstrated that a combination of 5-FU/L-OHP and CP is a promising immunogenic chemotherapy. The antitumor effects were mainly due to host T cells. Also, the therapeutic efficacy of triple combination therapy was boosted by ICB therapy. These results could contribute to the development of more effective chemotherapy plus ICB combination therapy for CRC patients.