主論文の要約

Oncolytic herpes simplex virus HF10 (canerpaturev, C-REV) promotes accumulation of $CD8^+PD\text{-}1^-$ tumor-infiltrating T cells in PD-L1-enriched tumor microenvironment

腫瘍溶解性単純ヘルペスウイルス HF10 (canerpaturev、C-REV) は、PD-L1 発現が高い腫瘍微小環境下において $CD8^+PD\text{-}1^-$ T 細胞の腫瘍への浸潤を促進する

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【Introduction】

Cancer therapy against a broad range of cancers has been improved using immune checkpoint inhibitors (ICIs). Although some patients with T cell inflamed malignancies respond to ICIs, most patients with non-T cell inflamed tumors do not respond to such immune therapy. Consequently, it is important to develop strategies that convert non-T cell-inflamed tumor (cold tumor) to T cell-inflamed tumor (hot tumor). Canerpaturev (C-REV) is an oncolytic virus (OVs) and has been known formerly as HF10. OVs are infected and replicated selectively in tumors that induce tumor cell lysis and activate an antitumor immune response. Tumor lysis caused by OV evokes inflammation after recruiting the immune cells, converting cold tumor into hot tumor. At the same time, the inflammation caused by OVs induces PD-L1 expression on APCs and tumors. Some immune cells, especially $CD8^+$ T cells, are important for the antitumor effect of OV; however, $CD8^+$ T cell activity is impaired by PD-1 / PD-L1 interactions. It has been reported that PD-L1 blockade by ICIs on APCs rather than tumor cells plays a crucial role in $CD8^+$ T cell activity. Although OV is an excellent treatment for converting cold tumor into hot tumor, PD-L1 induction by the inflammation may attenuate the effects of ICIs. In this study, we investigated the antitumor activity of C-REV in combination with anti-PD-L1 antibody using a poor immunogenic squamous cell carcinoma model (SCC-VII) and evaluated the immunological mechanisms of the antitumor effect.

【Materials and methods】

C-REV cytotoxic effect and viral replication assays in murine cell lines (SCC-VII and Pan02) were determined. The PD-L1 expression on APCs or tumors was investigated after C-REV treatment *in vitro* and *in vivo* by FACS. We investigated the combination effect of C-REV and anti-PD-L1 antibody in a bilateral SCC-VII tumor model. Immune cell profiling analyses were assessed to characterize the innate and adaptive immune responses after C-REV treatment in the tumor, spleen, and lymph nodes. The tumor-infiltrated lymphocytes (TILs) were collected using a gentle MACS dissociator and stained with conjugated antibodies. PD-1 expression was characterized *in vitro* co-culture of tumor cells treated with or without C-REV, $CD8^+$ T cells or bone marrow-derived dendritic cells (BMDCs).

【Results】

The PD-L1 expression on cells *in vitro* and *in vivo* after C-REV treatment: We found that the PD-L1 expression was significantly increased upon interferons (IFNs) stimulation *in vitro* in SCC-VII and Pan02 cell lines. We also observed that PD-L1 expression on SCC-VII and Pan02 was increased after C-REV infection compared to IFNs stimulations *in vitro*. C-REV treatment induced a significant PD-L1 expression in the tumor cell lines *in vivo*.

PD-L1 expression was highly persistent on macrophages ($CD11b^+F4/80^+$) and DCs ($CD11b^+CD11c^+$) in both control and C-REV–treated mice.

The rational combination of C-REV with two different doses of anti-PD-L1 antibody *in vivo*: We investigated the efficacy of combination between C-REV and anti-PD-L1 antibody by using a bilateral SCC-VII tumor model. C-REV monotherapy significantly suppressed tumor growth on both the injected and contralateral sides. Systemic anti–PD-L1 antibody exerted dose-dependent antitumor activity. The monotherapy groups significantly suppressed the tumor growth in injected side. In addition, the combination therapy of C-REV and anti-PD-L1 antibody showed a significant antitumor effect.

The remodeling of tumor immune responses after C-REV treatment: We found a significant increase in the percentage of NK cells ($NKp46^+CD3^-$), macrophages ($CD11b^+F4/80^+$), dendritic cells (DCs) ($CD11c^+MHCII^+$), and conventional DCs (cDCs: $CD11c^+$ $CD8^+$) in C-REV–treated tumor. In contrast, we observed no significant difference between control and C-REV group in myeloid immune cells, including macrophages and cDCs. These results suggest that C-REV remodels the tumor microenvironment and activates DC populations in the tumor.

The accumulation of $CD8^+PD\text{-}1^-$ tumor-infiltrating T cells after C-REV treatment: C-REV induced a significant increase in total T cells and $CD8^+$ T cells on both injected and contralateral sides on days 12 and 17. Most $CD8^+$ T cells expressed lower levels of PD-1 after C-REV treatment. Moreover, high levels of IFNγ from $CD8^+$ TILs were observed after C-REV treatment on the injected and contralateral sides. By contrast, C-REV did not induce significant changes in PD-1 expression on $CD4^+$ T cells. In addition, no change was seen in other immune checkpoint receptors such TIGIT and TIM3 on $CD8^+$ TILs in the SCC-VII model. We also confirmed similar results in Pan02 after C-REV treatment by observing a significant decrease of PD-1 expression on $CD8^+$ TILs on the injected and contralateral sides.

The correlation between C-REV and PD-1 expression on $CD8^+$ T cells: No direct relation between PD-1 expression on $CD8^+$ T cells and viral infection through co-culturing SCC-VII infected with C-REV-GFP with $CD8^+$ T cells was observed. A replicate of C-REV in $CD8^+$ T cells or BMDCs was not found. Moreover, detectable changes in PD-1 expression on $CD8^+$ $PD\text{-}1^+$ T cells was not observed, even after co-culture with virus-infected tumor cells or triple co-culture with virus-infected tumor cells, BMDCs, and $CD8^+$ T cells. On contract, IFNγ expression was increased after the blockage of the PD-1 / PD-L1 axis. To trace the PD-1 expression on $CD8^+$ T cells, we confirmed that most $CD8^+$ T cells were PD-1 negative in the spleen, blood, and tumor-draining lymph nodes (TDLNs) in both control and C-REV treated mice. The total $CD8^+$ T cells in the blood and total DCs in TDLNs were increased.

【Discussion】

We demonstrated that C-REV treatment induced infiltration of $CD8^+PD\text{-}1^-$ into tumors. Furthermore, we confirmed high levels of infiltrating macrophages and DCs expressing PD-L1. Infiltration by myeloid immune cells, including DCs and macrophages, was significantly elevated in C-REV–treated tumors, but returned to baseline at later times after treatment. On the other hand, $CD8^+$ TILs were significantly more abundant in tumors after C-REV treatment, even at later time points. Surprisingly, most infiltrated $CD8^+$ T cells had low PD-1 expression after C-REV treatment relative to the non-treated group. $CD8^+PD\text{-}1^-$ TIL infiltration was accompanied by high levels of IFNγ, indicating an activated state.

$CD8^+$ T cell activity plays an essential role in the antitumor efficacy of C-REV. Importantly, infiltration of $CD8^+PD\text{-}1^-$ T cells was significantly elevated on the contralateral side, revealing the tumor specificity of $CD8^+PD\text{-}1^-$ T cells. C-REV treatment increases cytotoxic activity of lymphocytes with high expression of IFNγ that recognize tumor-specific antigens. In this study, we confirmed that infected tumor cells co-cultured with BMDCs induced proliferation of $CD8^+$ T cells with high levels of IFNγ.

OVs cannot replicate in normal cells such as immune cells, and we confirmed that C-REV could not replicate in either $CD8^+$ T cells or BMDCs. Moreover, C-REV did not directly change PD-1 expression levels in $CD8^+$ T cells. Furthermore, tracing of PD-1 expression on $CD8^+$ T cells in the blood, spleen, and lymph nodes revealed that most $CD8^+$ T cells were PD-1–negative, whereas most $CD8^+$ TILs were PD-1–positive. Moreover, the number of circulating $CD8^+$ T cells in peripheral blood increased after C-REV treatment, accompanied by an increase in the DC population in TDLNs. Together, these results suggest that C-REV remodels the tumor microenvironment, concomitant with an increase in the activated DC population in lymph nodes, which may lead to selective activation of $CD8^+PD\text{-}1^-$ T cells in LNs followed by migration and accumulation in the tumor microenvironment. Consequently, the presence of $CD8^+PD\text{-}1^-$ TILs after C-REV treatment may compensate for high expression of PD-L1 on the tumor or APCs, and may contribute to C-REV antitumor activity even PD-L1 expression persists.

【Conclusion】

We observed persistence of $CD8^+PD\text{-}1^-$ TILs after treatment with C-REV. Several OVs, including C-REV, are currently undergoing clinical trials. Our findings may provide insight into the roles of $CD8^+PD\text{-}1^-$ TILs in patients treated with oncolytic viruses.