主論文の要旨

Metformin enhances the antitumor activity of oncolytic herpes simplex virus HF10 (canerpaturev) in a pancreatic cell cancer subcutaneous model

（メトホルミンは、膵臓細胞癌皮下モデルにおける腫瘍溶解性単純ヘルペスウイルス HF10 (canerpaturev) の抗腫瘍活性を増強します）

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【Introduction】

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and it represents the fourth leading cause of cancer deaths in Japan, with forecasts indicating a further escalation of mortality rates in the coming decades. Now, the current standard of care for patients with PDAC focuses on chemotherapeutic regimens and pancreatic cancer surgery. However, limited treatment options, advanced tumor stages due to late diagnosis, and the aggressive behavior of PDAC contribute to the high mortality of the disease. Consequently, there is an urgent need for an alternative approach to pancreatic cancer treatment. Recently, immunotherapy has been considered a promising approach to cancer treatment. Even though advancements in cancer immunotherapy, it showed limited preclinical and clinical response against pancreatic cancer. In addition to this, PDAC patients showed no response to a single treatment by immune checkpoint inhibitors (ICIs). Additionally, combination ICIs with chemotherapy have not either induced marked effects in patients. The limitations of treatments are attributed to its cold tumor microenvironment (TME) with low MHC-I expression. These are accompanied by high infiltration of suppressive immune cells, as well as the physical limitations like the presence of fibrotic tissue and stellate cells. These factors impair cytotoxic $CD8^+$ T cells infiltration and lead to T cell exhaustion. Therefore, to improve the outcomes, other approaches are critically needed to beat PDAC resistance.

One of the immune therapeutic targeted agents that has the potential against cold tumors is Oncolytic viruses (OVs). OV therapy is a promising alternative for patients who does not respond to ICIs due to their dual benefit in one therapy. OVs are attenuated viruses that cause anticancer effects by directly killing cancer cells (oncolysis) because they preferentially replicate in the tumor cells and can be engineered to express transgenes that enhance their cytotoxic effect. Moreover, OVs can modulate the TME and promote antitumor immunity. Canerpaturev (C-REV) is a promising OV, which was originally isolated from herpes simplex virus-1 (HSV-1) strain HF as clone 10 (Previously known as HF10). C-REV showed potent antitumor effects against various preclinical models, including pancreatic cancer. C-REV combined with anti-PD-L1 showed a greater antitumor effect with high infiltration of $CD8^+$ $PD\text{-}1^-$ tumor-infiltrating lymphocyte cells (TILs) in SCC-VII model. C-REV combined with chemotherapeutic S-1 also enhanced antitumor efficacy in a murine triple-negative breast cancer model. It also demonstrated its safety and efficacy in phases I, and II clinical trials targeting melanoma, pancreatic, breast, head, and neck cancer. C-REV combined with gemcitabine and nab-paclitaxel in phase I clinical trial against unresectable stage III or IV pancreatic cancer, and showed a favorable benefit/risk profile with antitumor activity. Furthermore, C-REV combined with cetuximab and bevacizumab synergistically inhibited the growth of human colorectal cancer as well as human breast carcinoma xenograft, respectively. Concluding with those results that the combination approach is promising and highlighting the need of new combination therapy to overcome cold tumors.

Metformin is an FDA-approved, commonly prescribed systemic antidiabetic medication for type II diabetes patients. Recently, metformin demonstrated its cancer antitumor effectiveness. Several epidemiological studies showed that metformin reduces cancer incidence in type II diabetes patients and improves their prognosis. Metformin improved the survival of diabetic patients with pancreatic cancer. Metformin affects the growth of cancer cells, and it showed a potent immune modulator effect. Metformin mainly modulates immune suppressive cells, which could be ideal for combining with OVs against cold tumors such as pancreatic cancer.

【Hypothesis】

we hypothesized that the combination of C-REV as an oncolytic virus plus metformin might be a good option for pancreatic cancer treatment.

【Material and Methods】

We used a low immunogenic Pan02 murine PDAC model due to its high morbidity and mortality. A bilateral tumor model of Pan02 was used to evaluate antitumor effects. Tumors were cut into cubes (2 mm^3). Pan02 tumors were inoculated into mice; one tumor cube was inoculated into each flank (right and left). When the average tumor size reached 100 mm^3, treatments were then started on day 0. Mice were randomly divided into four groups (n= 4 mice/group) with an equal average tumor volume among the groups. C-REV was injected three times ($1X10^6$ PFU/100 ul PBS) with three days intervals (D0, D3, and D6) on only one side (injected side). Metformin was continuously supplied in the drinking water (5 mg/ml) when C-REV treatment started.

【Results】

In vitro, metformin does not enhance the C-REV cell cytotoxic effect and it had no effect on C-REV replication in Pan02 cell line. However, in *in vivo* model, intratumoral administration of C-REV with the systemic administration of metformin led to synergistic antitumor effect on both sides of tumor and prolonged survival. Interestingly, combination therapy showed synergism from the calculation of the synergistic effect on both the injected and contralateral sides. Combination therapy enhanced the infiltration and IFN-γ production from $CD8^+$ $CD3^+$ TILs. Moreover, the combination therapy increased the effector $CD44^+$ $CD8^+$ $PD1^-$ and $CD69^+$ $CD8^+$ $PD1^-$ subsets as well as decreased the proportion of terminally-differentiated $CD103^+$ $KLRG\text{-}1^+$ T-regulatory cells on both sides of tumor. In addition, Combination treatment significantly increased tumor-infiltrating DCs ($CD11c^+$ $MHC\text{-}II^+$) on both the injected and contralateral sides. Interestingly, combination therapy efficiently modulates conventional dendritic cells type-1 (cDC1) on tumors, and tumor-drained lymph nodes. Combination therapy significantly enhanced XCR-1 expression on cDC1 on both the injected and contralateral sides. Moreover, combination treatment significantly increased the

cDC1 population in the TDLNs, which is essential for $CD8^+$ T cell activation that infiltrated into the tumors after activation.

【Conclusion】

In summary, we observed that the combination of C-REV with metformin may be a novel therapeutic combination. C-REV mainly enhances $CD8^+$ TILs infiltration and function, which could be upregulated by metformin. Metformin also increases cDC1 infiltration as well as modulates suppressor terminally-differentiated tumor-infiltrating T regulatory cells. Thus, this combination can enhance the antitumor effect. Currently, many OVs are undergoing clinical trials including C-REV in addition metformin is a highly prescribed medicine and millions of diabetic patients use this drug on a daily base. Our findings may provide new insights into the role of combination treatment in the modulation of immune-suppressive tumors.