論文の内容の要旨

論文題目

In Vivo Molecular Imaging of Oncolytic Virus Therapy Using
Luciferase-expressing Recombinant Herpes Simplex Viruses

和訳

ルシフェラーゼ発現型遺伝子組換え単純ヘルペスウイルスを用いた
ウイルス療法の生体分子イメージング

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Oncolytic virus therapy is an emerging treatment strategy for cancer. The third generation oncolytic Herpes Simplex Virus type 1 (HSV-1) named G47? was designed to selectively replicate in tumor cells by introducing three genomic alterations: deletions in both copies of the ?34.5 gene, inactivating lacZ insertion in the infected-cell protein 6 gene, and a deletion in the a47 gene along with the overlapping US11 promoter region. Previous studies have demonstrated that G47? exhibits an efficient oncolytic activity in various tumor cells, yet minimal toxicity in normal tissues.

In the course of drug development, it is necessary to study the kinetics such as absorption, distribution, and excretion of the therapeutics from a biological system. Conventionally, virus biodistribution studies were performed by obtaining tissue biopsies at certain time points from virus treated animals. However, with this method, one animal provides information for one time point, therefore only limited information can be obtained due to the unavailability of longitudinal studies, and the possibility of missing unpredicted replication sites or time points. To overcome these limitations, molecular imaging technologies have been developed to visualize viral infection and replication in living animals. In this study, we aim to elucidate the biodistribution and kinetics of G47? through *in vivo* imaging of a newly developed luciferase-expressing recombinant HSV-1. We constructed two new viruses, T-luc^{CMV} and T-luc^{US11}, by

inserting the CMV-promoter-driven luciferase gene and the Us11-promoter-driven luciferase gene, respectively, into the backbone of G47? . Theoretically, cytomegalovirus (CMV) immediate-early promoter allows a short expression of the luciferase gene immediately upon infection of the virus regardless of its replication capability, whereas the Us11 promoter, a strict late promoter, drives the luciferase gene only when the virus is replicating: The luciferase expression by T-luc^{CMV} should indicate fresh virus infection, and that by T-luc^{US11} should indicate ongoing virus replication.

After confirming the genome structures of the T-luc^{CMV} and T-luc^{US11} by Southern blot analyses, we studied the basic characters of T-luc^{CMV} and T-luc^{US11} in comparison to a control virus T-01. *In vitro* viral replication capabilities and cytopathic efficacies of T-luc^{CMV} and T-luc^{US11} showed comparable results compared to those of T-01. The *in vivo* antitumor efficacies of T-luc^{CMV} and T-luc^{US11} were also similar to that of T-01 when tested in A/J mice bearing subcutaneous Neuro2a tumors. Therefore, we concluded that the expression of the luciferase gene does not interfere with viral infection, replication and its cytopathic efficacy.

The *in vitro* luciferase assay showed that, while T-luc^{CMV} expressed luciferase immediately after infection as predicted, the luciferase expression pattern of T-luc^{US11} did not seem to reflect the course of virus replication precisely. However, since luciferase expression under the CMV promoter rapidly declines after viral DNA synthesis takes place, a persistent luminescence reflects continuously occurring fresh infections by progeny viruses resulting from virus replication. Therefore, with T-luc^{CMV}, we were able to monitor both viral infection and replication. When T-luc^{CMV} was injected intratumorally into subcutaneous tumors, the local luciferase expression was detected up to day 7 in Neuro2a tumors in A/J mice, and for more than 14 days in U87MG tumors in athymic mice. The T-luc^{CMV} infection was not detected from any other organs at any time point after an intratumoral administration. An intravenous administration of T-luc^{CMV} in both A/J and athymic mice resulted in a high expression of luciferase in the liver, but rapidly vanished within 48 hours. Contrastingly, when athymic mice bearing subcutaneous U87MG tumors were injected intravenously with T-luc^{CMV}, the luciferase expression in tumors became detectable after one day, and grew stronger and persisted for more than two weeks. When T-luc^{CMV} was injected into intracerebral tumors, the luciferase expression was detectable up to day 6 for Neuro2a tumors, and for more than two weeks for U87MG tumors.

We conclude that *in vivo* imaging of the luciferase-expressing recombinant HSV-1 is a reliable and efficient method to investigate viral biodistribution and kinetics in living animals, and has vast applications in future studies of oncolytic virus therapy.