Tumor Cell Death-Inducing Pretreatments Enhance the Distribution and Efficacy of Oncolytic Herpes Simplex Virus in Solid Tumors

Satoshi Nagano1,2, Jean Y. Perentes1, Setsuro Komiya2, Rakesh K. Jain1, Yves Boucher1
1Edwin L. Steele Laboratory, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA; 2Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan
sat4664@yahoo.co.jp

Introduction: Despite the recent progress of multimodal cancer treatment, there is still a need for the development of innovative and effective therapies especially for recurrent or metastatic diseases. Although the gene therapy with replication deficient virus could be effective for lung metastasis of a mouse tumor model, limited transduction efficiency of replication-deficient virus urged researchers to use replication-competent, oncolytic viruses. Oncolytic viruses, which selectively replicate in tumor cells, were expected to kill cells throughout the tumor mass. Unfortunately, most clinical trials showed limited response of tumors to oncolytic viral therapies. For successful eradication of the tumor by oncolytic gene therapy, initial widespread distribution of the virus within the tumor is crucial. However, viral distribution in the tumor is limited by the large size of viral vectors, which limits their penetration and distribution through the fine interstices of the interstitial matrix and the narrow spaces separating tumor cells. Our group recently showed that degradation of collagen matrix with bacterial collagenase improves viral distribution and therapeutic efficacy of oncolytic herpes-simplex virus (HSV) (2). In this study we tested the hypothesis that the void space resulting from tumor cell apoptosis would improve the distribution and efficacy of oncolytic HSV injected intratumorally.

Materials and Methods: In this study we used two different approaches to induce apoptosis, which are tet-regulated expression of apoptotic genes and cytotoxic agents. To establish tet-regulated apoptosis system, MDA-MB-435S cells were transfected with plasmids expressing tet-inducible CD8/Caspase-8, CD8/Empty (control gene for caspase-8) or TRAIL-R1. Tet-on expression was induced with addition of doxycycline (1 μg/ml) on culture media. For treatment with cytotoxic agents, paclitaxel (100 or 300 nM), TRAIL (100 nM) or sequential treatment of two agents were used to determine the cytotoxic effect on different cancer cells in vitro. Apoptosis was determined by hoechst33342 nuclear staining or DNA fragmentation ELISA. In vivo, collagen-poor MDA-MB-435S (wild type or tet-on transfectants) or collagen-rich MDA-MB-361HK cells were implanted into the mammary fat pad of SCID mice and tumor cell apoptosis was induced by doxycycline-regulated expression of CD8/Caspase-8 or cytotoxic agents. TUNEL and immunostaining for collagen I or hyaluronate were performed to assess apoptosis or change in extracellular matrix components. To study the effect of apoptosis-inducing pretreatments on the viral distribution, oncolytic HSV that expresses GFP was injected intratumorally following to different pretreatments. Viral distribution was evaluated by quantifying the GFP-expressing area on frozen tumor sections. Finally, to test if apoptosis induction before the HSV injection can enhance the effect of oncolytic viral therapy, tumor growth was assessed in MDA-MB-435S tumors.

Results: In vitro, both tet-regulated expression of apoptotic genes and cytotoxic treatments induced significant apoptosis on tumor cells. Paclitaxel (24h) followed by TRAIL (24h) induced significantly more apoptosis than single or other combinational treatment. In mice with MDA-MB-435S tumors, both the activation of caspase-8 and pretreatment with cytotoxic agents induced 9.0 % and 4.0 % apoptosis, respectively. In control tumors without any pretreatment, virus was distributed 13 % of the tumor section. Consistent with the increased apoptosis in pretreated tumors, viral distribution was significantly improved by both caspase-8 activation (42.4 %) and paclitaxel-TRAIL (30.3 %). Serial reconstruction of tumor sections revealed that apoptotic cells were heterogeneous distributed. In tumor areas with a high density of apoptotic cells, the apoptosis-induced cellular shrinkage produced interstitial void spaces and channels that facilitated HSV distribution. Similarly, in the necrotic areas of paclitaxel-pretreated MDA-MB-361HK tumors, channels lined by HSV particles were found to connect necrotic and virus infected areas. We also show that the intratumoral injection of oncolytic HSV after caspase-8 activation or the paclitaxel plus TRAIL pre-treatment produces a significantly longer tumor growth delay than the administration of HSV before the induction of cell death.

Discussion: It is envisioned that gene therapy approaches with viral vectors will be combined with radiation or chemotherapy. Previous studies showed that radiation or certain chemotherapy enhances viral replication of HSV. However, the effect of cell death before viral injection on distribution and efficacy of oncolytic viruses was not clear. We found that the paclitaxel-TRAIL treatment before HSV injection was significantly effective than the paclitaxel-TRAIL treatment after HSV injection, demonstrating the importance of sequence of the treatments. Our results also showed that both apoptosis and necrosis can improve the spread of HSV in tumors. In necrotic areas interstitial channels are lined by HSV particles, which facilitate the spread of oncolytic virus after their injection. Therefore, various chemotherapies that induce not only apoptosis but also necrosis may possibly be used as a pretreatment of oncolytic viral therapy.

In conclusion, cancer cell death improves the intratumoral spread and therapeutic efficacy of oncolytic HSV. Thus the administration of cytotoxic agents before the injection of virus could significantly enhance the efficacy of oncolytic viral therapy.


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