

Oncolytic Efficacy of Thymidine Kinase-Deleted Vaccinia Virus Strain Tiantan (oncoVV-TT) in Glioma

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Abstract : Oncolytic viruses, which only replicate in tumor cells, are being extensively studied for their use in cancer therapy. A particular virus known as the vaccinia virus, a member of the poxvirus family, has demonstrated oncolytic abilities glioma. Treating Glioma with traditional methods such as chemotherapy and radiotherapy is quite challenging. Even though oncolytic viruses have shown immense potential in cancer treatment, their effectiveness in glioblastoma treatment is still low. Therefore, there is a need to improve and optimize immunotherapies for better results. In this study, we have designed oncoVV-TT, which can more effectively target tumor cells while minimizing replication in normal cells by replacing the thymidine kinase gene with a luc-p2a-GFP gene expression cassette. Human glioblastoma cell line U251 MG, rat glioblastoma cell line C6, and non-tumor cell line HFF were plated at 105 cells in a 12-well plates in 2 mL of DMEM-F2 medium with 10% FBS added to each well. Then incubated at 37°C. After 16 hours, the cells were treated with oncoVV-TT at an MOI of 0.01, 0.1 and left in the incubator for a further 24, 48, 72 and 96 hours. Viral replication assay, fluorescence imaging and viability tests, including trypan blue and crystal violet, were conducted to evaluate the cytotoxic effect of oncoVV-TT. The finding shows that oncoVV-TT had significantly higher cytotoxic activity and proliferation rates in tumor cells in a dose and time-dependent manner, with the strongest effect observed in U251 MG. To conclude, oncoVV-TT has the potential to be a promising oncolytic virus for cancer treatment, with a more cytotoxic effect in human glioblastoma cells versus rat glioma cells. To assess the effectiveness of vaccinia virus-mediated viral therapy, we have tested U251mg and C6 tumor cell lines taken from human and rat gliomas, respectively. The study evaluated oncoVV-TT's ability to replicate and lyse cells and analyzed the survival rates of the tested cell lines when treated with different doses of oncoVV-TT. Additionally, we compared the sensitivity of human and mouse glioma cell lines to the oncolytic vaccinia virus. All experiments regarding viruses were conducted under biosafety level 2. We engineered a Vaccinia-based oncolytic virus called oncoVV-TT to replicate specifically in tumor cells. To propagate the oncoVV-TT virus, HeLa cells (5 × 10⁴/well) were plated in 24-well plates and incubated overnight to attach to the bottom of the wells. Subsequently, 10 MOI virus was added. After 48 h, cells were harvested by scraping, and viruses were collected by 3 sequential freezing and thawing cycles followed by removal of cell debris by centrifugation (1500 rpm, 5 min). The supernatant was stored at -80 °C for the following experiments. To measure the replication of the virus in HeLa, cells (5 × 10⁴/well) were plated in 24-well plates and incubated overnight to attach to the bottom of the wells. Subsequently, 5 MOI virus or equal dilution of PBS was added. At the treatment time of 0 h, 24 h, 48 h, 72 h and 96 h, the viral titers were determined under the fluorescence microscope (BX-X700; Keyence, Osaka, Japan). Fluorescence intensity was quantified using the imagej software according to the manufacturer's protocol. For the isolation of single-virus clones, HeLa cells seeded in six-well plates (5×10⁵ cells/well). After 24 h (100% confluent), the cells were infected with a 10-fold dilution series of TianTan green fluorescent protein (GFP)virus and incubated for 4 h. To examine the cytotoxic effect of oncoVV-TT virus on U251mg and C6 cell, trypan blue and crystal violet assay was used.

Keywords : oncolytic virus, immune therapy, glioma, vaccinia virus

Conference Title : ICOVI 2023 : International Conference on Oncolytic Viruses and Immunity

Conference Location : Berlin, Germany

Conference Dates : July 17-18, 2023