論　文　内　容　要　旨

Metastatic Tumor Cells Detection and Anti-Metastatic Potential with Vesicular Stomatitis Virus in Immunocompetent Murine Model of Osteosarcoma

（水疱性口内炎ウイルスを用いた免疫応答性骨肉腫マウスモデル中の転移性腫瘍細胞の検出と抗転移効果の可能性）

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Sarcomas are aggressive cancers arising from mesenchymal tissues that are particularly lethal. American Cancer Society estimates that approximately 3,450 new cases of bone and joint cancers will be diagnosed and 1,550 Americans are expected to die of these cancers in the United States for 2018. Despite multimodality therapy with surgery, chemotherapy and radiation therapy, the overall survival of the bone cancers did not improve significantly over the last decade. Oncolytic recombinant Vesicular Stomatitis Virus is a promising new class of therapeutic agents that inherent tumor specificity to eliminate various malignancies by direct infection and lysis of cancer cells. This study aimed to evaluate the potential of systemic administration of recombinant Vesicular Stomatitis Virus (VSV) containing Katushka gene, a far-red fluorescent protein (RFP), as an effective agent for treating spontaneous lung metastasis in a syngeneic osteosarcoma mouse model. In addition, the present study evaluated the feasibility of this recombinant VSV-Katushka (rVSV-K) as a viral-based diagnostic platform for detection of circulating tumor cells (CTCs) from the blood of this osteosarcoma mouse model. Recombinant VSV-Katushka was generated and characterized *in vitro* on various human and murine osteosarcoma cells as well as normal human mesenchymal stromal cells (MSC). For virus infection assays, cells were examined microscopically for cytopathic effect and fluorescence expression. The Cytotoxicity assay was evaluated with microplate reader using a WST-8 method at indicated time points after infection. Viral RNA genome replication assay was determined by real-time reverse transcription polymerase chain reaction (RT-PCR). Spontaneous lung metastasis of osteosarcoma bearing mouse was established in immune-competent C3H male mice by subcutaneous implantation of syngeneic LM8 murine osteosarcoma cells into the back space of mice. The virus was injected into the tumor-bearing animals in either single injection or multiple injections, once a week for four consecutive weeks, via the jugular vein. To assess *in vivo* effectiveness, we measured the volume of primary tumors weekly and analyzed the histology of lung metastasis at five weeks after tumor implantation. A separate group of mice was followed for survival, and the results were analyzed by the *log-rank* test. To evaluate the feasibility of CTCs detection, blood samples from this osteosarcoma mouse model at which spontaneous lung metastasis had developed (5 weeks after LM8 tumor cells implantation) were transfected *ex vivo* with the virus. Following CTCs enrichment using RBC lysis buffer and immunofluorescence staining using anti-CD45 antibody and DAPI staining, the slides

with cell suspension were checked for CTCs under a fluorescence microscope. We found that rVSV-K induced cytopathic effects in all human and murine osteosarcoma cell lines at a 0.01 multiplicity of infection (MOI) whereas the morphology of normal mesenchymal cells was not changed even at a MOI as high as 1. Infected murine osteosarcoma cell line, LM8, uniformly showed red fluorescence expression at all MOIs. Infection with rVSV-K at 0.01 MOI also led to efficient cell killing in all osteosarcoma cell lines for up to 60 hours, whereas normal MSCs were refractory. Moreover, the results showed that rVSV-K is fully capable of replicating its RNA genome in all osteosarcoma cell lines within 48 hours of transfection, but its ability to promote so is attenuated in normal MSC. These data demonstrated that rVSV-K could preferentially infect and eradicate various osteosarcoma cell lines. When rVSV-K was administered systemically either single or repeated injections into mice bearing syngeneic LM8 tumors, the primary tumor volumes showed no significant decreased (*t-test*, $p>0.05$) compared to the corresponding control groups. Further quantification of the metastatic burden of the explanted lungs showed that, relative to the corresponding control groups, a single injection of the virus did not show significant inhibition of lung metastasis progression ($n=6$; $p=0.331$). Interestingly, however, repeated systemic injection demonstrated significant decreased of metastatic burden in the lungs ($p<0.01$) and was likely responsible for the observed increase in survival study (*log-rank* test, $p<0.01$). Furthermore, we demonstrated that *ex vivo* rVSV-K transfection into the blood samples of LM8 osteosarcoma bearing mouse model resulted in the visualization of red fluorescent signals from infected cells. All of the red fluorescent-positive cells contained nuclei ($DAPI^+$) and showed a negative signal by CD45 staining, indicating that the infected cells were not leucocytes. In conclusion, repeated systemic administration of rVSV-K is effective in the treatment of lung metastasis in immune-competent osteosarcoma bearing mouse model and successfully detects the circulating tumor cells in blood samples of this mouse model, thereby warranting further development for future viral-based theranostic strategy in patients with aggressive osteosarcoma.