COMPARISON OF THE ONCOLYTIC ACTIVITY OF RECOMBINANT VACCINIA VIRUS STRAINS LIVP-RFP AND MVA-RFP AGAINST SOLID TUMORS

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Among oncolytic viruses, modified vaccinia virus Ankara (MVA), a highly attenuated vaccinia virus (VV) is a well-studied variant with promising results in preclinical and clinical trials. The Lister VV strain from the Moscow Institute of Viral Preparations (LIVP) has been studied to a lesser extent than MVA and has a different oncolytic property from MVA. The aim of this work was to compare the oncolytic efficacy of LIVP and MVA strains against solid tumors. We developed recombinant variants LIVP-RFP and MVA-RFP; to enhance onco-selectivity thymidine kinase (TK) gene was inactivated by insertion of red fluorescent protein (RFP) gene to the TK locus. The replication kinetics and oncolytic activity of the obtained recombinant strains were evaluated *in vitro* and in vivo on tumor cell lines and mouse syngeneic tumor models of metastatic mouse 4T1 mammary adenocarcinoma, CT26 colon adenocarcinoma, and B16 melanoma. Both MVA-RFP and LIVP-RFP showed high replication efficiency in tumor cells and pronounced oncolytic activity against B16 melanoma and 4T1 breast adenocarcinoma allografts. In relation to 4T1, which is a model of triple negative human breast cancer, LIVP-RFP showed more than 50% increased cytotoxicity in *in vitro* tests compared to MVA-RFP, as well as a significant slowdown in the progression of 4T1 allografts and an increase in animal survival in experiments *in vivo*. Thus, the LIVP strain may be more promising than MVA as a platform for the development of recombinant oncolytic viruses for the breast cancer treatment.

Keywords: vaccinia virus, LIVP, MVA, viral oncolytic therapy, breast cancer, colon carcinoma, melanoma

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СРАВНЕНИЕ ОНКОЛИТИЧЕСКОЙ АКТИВНОСТИ РЕКОМБИНАНТНЫХ ШТАММОВ ВИРУСА ОСПОВАКЦИНЫ LIVP-RFP И MVA-RFP В ОТНОШЕНИИ СОЛИДНЫХ ОПУХОЛЕЙ

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Среди онколитических вирусов одним из наиболее изученных является вирус осповакцины (VV), штамма модифицированного высокоаттенуированного вируса Анкара (MVA), показавшего многообещающие результаты в доклинических и клинических испытаниях. Штамм Lister VV из Московского Института вирусных препаратов (LIVP) исследован в меньшей степени, чем MVA и имеет отличный от MVA тропизм. Целью работы было сравнить онколитическую эффективность штаммов LIVP и MVA в отношении солидных опухолей. Для повышения селективности LIVP и MVA к опухолевым клеткам нами были получены рекомбинантные варианты с инактивацией гена тимидинкиназы (TK), MVA-RFP и LIVP-RFP, экспрессирующие красный флуоресцентный белок. Кинетику репликации и онколитическую активность полученных рекомбинантных штаммов оценивали *in vitro* и *in vivo* на линиях опухолевых клеток и аллотрансплантатах мышиных сингенных моделей метастатической аденокарциномы молочной железы мыши 4T1, аденокарциномы толстой кишки CT26 и меланомы B16. Как MVA-RFP, так и LIVP-RFP показали высокую эффективность репликации в опухолевых клетках и выраженную онколитическую активность в отношении аллотрансплантатов меланомы B16 и аденокарциномы молочной железы 4T1. В отношении 4T1, являющейся моделью тройного негативного рака молочной железы человека, LIVP-RFP по сравнению с MVA-RFP показал более чем на 50% повышенную цитотоксичность в тестах *in vitro*, а также достоверное замедление прогрессирования аллотрансплантатов 4T1 и повышение выживаемости животных в экспериментах *in vivo*. Применение штамма LIVP в качестве платформы при разработке рекомбинантных онколитических вирусов для терапии рака молочной железы может быть более перспективным, чем применение штамма MVA.

Ключевые слова: вирус осповакцины, LIVP, MVA, вирусная онколитическая терапия, рак молочной железы, карцинома толстой кишки, меланома

Финансирование: разработка онколитических вирусов и эксперименты *in vitro* были выполнены при поддержке Российского научного фонда (грант РНФ № 20-75-10157); эксперименты *in vivo* также выполнены при поддержке Российского научного фонда (грант РНФ № 22-64-00057).

Вклад авторов: Я. Шакиба — анализ литературы, выполнение преаналитического этапа работы, проведение экспериментов *in vitro* и *in vivo*, анализ и интерпретация данных, подготовка рисунков и графиков; Е. Р. Набережная — проведение экспериментов *in vitro*, анализ и интерпретация данных; Д. В. Кочетков — уход за животными, интерпретация данных, Г. М. Юсубалева — визуализация данных, редактирование рукописи; П. О. Воробьев — наработка препаративных количеств вируса для *in vivo* исследований; В. П. Баклаушев — планирование исследования, выполнение преаналитического этапа работы, анализ данных; А. В. Липатова, П. М. Чумаков — руководство исследованием, разработка дизайна, создание рекомбинантных штаммов, интерпретация данных, редактирование рукописи.

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Oncolytic viruses represent a new class of drugs for the treatment of malignant neoplasms that are resistant to classical approaches of anticancer therapy. Oncolytic viruses selectively infect tumor cells, causing a direct cytopathic effect and indirect activation of cytotoxic cells, which ultimately leads to tumor regression [1]. The vaccinia virus (VV) is an oncolytic vector with excellent characteristics, including high tropism and cytolytic activity against tumor cells, rapid replication without integration into the host cell genome, resistance to the hypoxic tumor microenvironment, and a well-characterized safety profile [2, 3].

The LIVP strain demonstrated significant cytotoxic activity against tumors of various histological affiliations (colorectal cancer, gastric cancer, malignant mesothelioma, lung cancer, thyroid and breast cancer) [4, 5]. The biodistribution of the LIVP strain was also studied - the virus selectively infects tumor cells without being detected in the ovaries, spleen, or brain tissues after intravenous injection [6, 7]. The vaccinia virus expresses several immunomodulatory proteins to evade the body's immune response, such as interferon decoy receptors or inhibitors of innate immune regulatory pathways such as toll-like receptors or NF-κB signaling [8]. The Lister strain has been reported to encode more genes involved in immune evasion, such as A53R, the soluble tumor necrosis factor receptor, or T1/35kDa, an inhibitor of CC chemokines, which are absent in other strains such as MVA or WR (Western Reserve), resulting in less adverse inflammatory side effects after introduction to the host's body [9, 10]. LIVP is an attenuated sub variant of the English Lister strain obtained by adaptation to calf skin [11]. This strain was partly used in the smallpox eradication program after 1971 and is reported to have oncolytic properties and significantly less virulence compared to other Lister strain sub variants [12, 13]. This strain has not been studied in a number of preclinical or clinical trials [14–19].

Modified vaccinia virus Ankara (MVA) is one of the most widely studied VV strains with a promising potential in oncolytic viral therapy. MVA is a highly attenuated strain that does not replicate well in human cells, and its ability to reproduce is mainly limited to avian embryonic cells, making it quite safe [20]. In addition, MVA is a potent type I interferon inducer and elicits a strong humoral and cellular immune response. These properties of MVA make it an important candidate for the development of antitumor therapy [20]. MVA has been approved by the US Food and Drug Administration (FDA) as a safe smallpox vaccine [21]. In addition, the recombinant version MVA-BN vaccine vector has been approved by the European Medicines Agency (EMA) as part of the Ebola vaccine and is actively used in clinical trials of infectious diseases and tumor immunotherapy [22].

In this study, we obtained recombinant strains MVA-RFP and LIVP-RFP with inactivation of the viral thymidine kinase (TK) gene to increase specificity for tumor cells [23] by inserting the reporter gene tagRFP (red fluorescent protein) into the TK gene locus. Inactivation of the TK gene makes virus replication dependent on cellular TK, which is expressed only during the S-phase of the cell cycle, while transformed cells constantly express it. For example, recombinant viruses with a defective TK gene selectively replicate in rapidly dividing tumor cells that constantly express cellular thymidine kinase [24].

The aim of this work was to compare the oncolytic efficacy of MVA-RFP and LIVP-RFP in solid tumors of mouse syngeneic models of 4T1 mammary adenocarcinoma, B16 melanoma, and CT26 colon carcinoma.

METHODS

Cell cultures

Hamster kidney BHK-21 (ATCC # CCL-10), CT26 colon carcinoma (ATCC # CRL-2639), 4T1 mammary adenocarcinoma (ATCC # CRL-3406), B16 melanoma (ATCC # CRL-6475) and HEK293T (ATCC # CRL-3216) cell lines were purchased from the American Culture Collection (ATCC; USA). Rat fibroblasts deficient in TK (Rat2 TK-/-) were taken from the collection of the Cell Proliferation Laboratory of the IMB RAS (Moscow, Russia). All cells were cultured in DMEM supplemented with glutamine (Gibco; USA) and 10% fetal bovine serum (FBS) (Gibco; USA) and incubated at 37 °C under 5% CO_2.

Viruses

The vaccinia virus strain LIVP was obtained from the collection of the Cell Proliferation Laboratory of the IMB RAS (Moscow, Russia). Modified vaccinia virus Ankara (MVA) (ATCC № VR-1508) was purchased from ATCC.

A shuttle plasmid carrying the tagRFP gene was cloned to construct the MVA-RFP and LIVP-RFP strains. The tagRFP gene sequence was amplified by PCR from the pTagRFP-C plasmid construct (Evrogen; Russia) using primers 5-AGA GAGCCTGGATGGTGTCTAAGGGCGAAGAG and 5-AGAG AGGGATCCTTAATTAAGTTTGTGCCCCAGTTTG (Evrogen; Russia). tagRFP was expressed under the control of the 7.5k promoter. The frame was flanked by the TK gene region; the initial plasmid construct for recombination was created at the Cell Proliferation Laboratory of the IMB RAS (Moscow, Russia) [6]. Recombinant strains were obtained by lipofection of HEK293T cells with Lipofectamine 3000 (Thermo Fischer; USA) and subsequent infection with a wild-type vaccinia strain. After 48 h, a cryolysate of infected cells was prepared and viral particles were selected on Rat-2 TK-/- cells treated with bromodeoxyuridine at a concentration of 25 μg/mL [24]. After several rounds of selection, the virus was cloned by the plaque method to dissociate the wild strain. The resulting recombinant strains were grown in BHK-21 cells and purified by centrifugation in a sucrose density gradient [25]. The correctness of the inserts in the recombinant variants was confirmed by Sanger sequencing of the corresponding genome region. DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 (Thermo Fischer; USA) followed by analysis of the reaction products on an Applied Biosystems 3730 DNA Analyzer automatic sequencer (Thermo Fischer; USA) at the Genome Shared Use Center of the IMB RAS.

Titration of the virus

BHK-21 cells were seeded at 10,000 cells per well in a 96-well plate, the next day the medium was removed and the cells were infected with 10-fold dilutions of the viruses and incubated in DMED medium supplemented with 2% FBS. After 48 hours, when the cytopathic effect developed, the 50% infectious dose of tissue culture (TCID50) was evaluated according to the Reed and Muench method [26].

Assessment of cytotoxic activity of viruses

4T1, B16, CT26, and BHK-21 cells were seeded at 10,000 cells/well in 96-well plates, then infected at 1 and 10 MOI (multiplicity of infection) of the MVA-RFP or LIVP-RFP strains. Cytotoxic activity was assessed using the MTT test 24, 48,

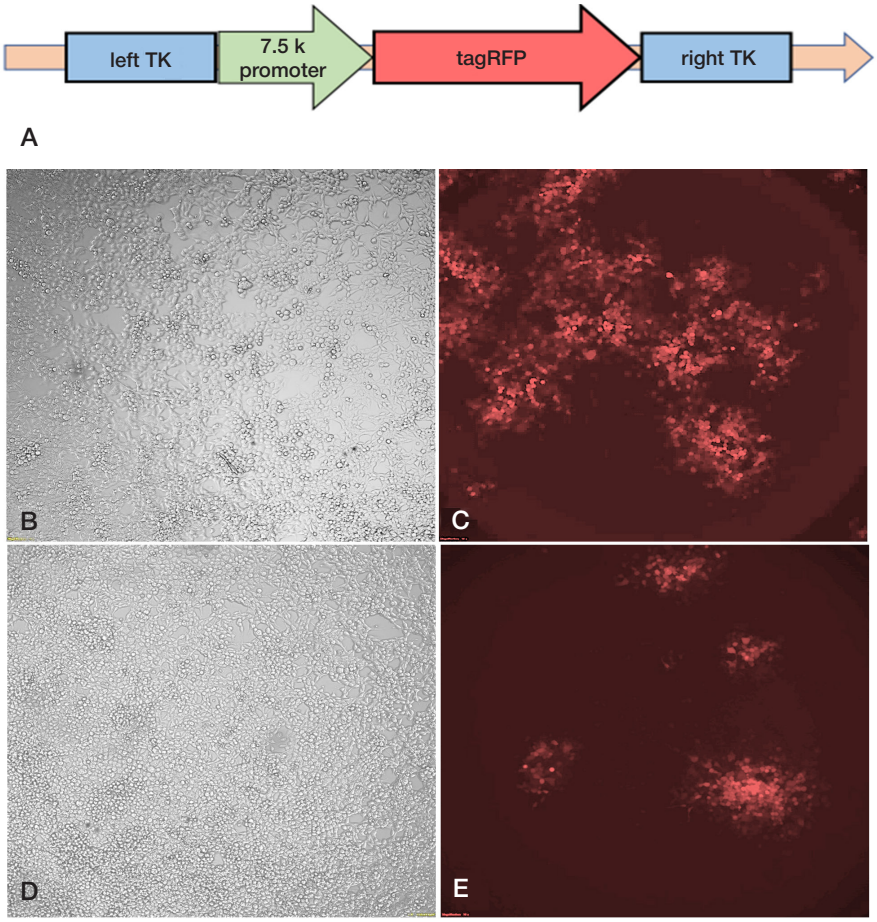

Fig. 1. Characterization of recombinant LIVP-RFP and MVA-RFP strains *in vitro*. A. Schematic of the plasmid vector used in the development of the LIVP-RFP and MVA-RFP strains. **B**, **C**. Fluorescence microscopy of HEK293T cells infected with the recombinant LIVP-RFP strain. **D**, **E**. Fluorescence microscopy of HEK293T infected with MVA-RFP (×100 magnification)

and 72 h after infection. The percentage of viable cells was calculated as the ratio of cell viability in infected wells to cell viability in uninfected control wells multiplied by 100 [27].

Estimation of virus replication rate by flow cytometry

The level of RFP expression in infected cells correlates with the level of viral replication. 4T1, B16, CT26, and BHK-21 cells were seeded at 100,000 cells per well in 24-well plates, infected with MVA-RFP or LIVP-RFP strains with MOIs of 1 and 10. 24 and 48 h after infection, cells removed from the surface with trypsin and resuspended in phosphate-buffered saline (PBS) (PanEco, Russia) with the addition of 2% FBS. The number of the brightly fluorescent cells in the RFP range was measured using a BD LSRFortessa Cell Analyzer (Beckman Dickinson; USA). Analysis was performed using Flowing Software 2.0 (Perttu Terho; Finland). The results are based on three independent experiments with three repetitions, and at least 10,000 events per sample.

Assessment of oncolytic activity of viruses *in vivo*

Six-week-old female BALB/c and C57BL/6 mice were used in the experiments. Mice had free access to food and water and were kept in standard conditions with controlled temperature (21–23 °C) and air ventilation, as well as a 12/12 light regimen. For tumor formation, 10^6 CT26 colon carcinoma or 4T1 breast cancer cells were implanted subcutaneously in the right flank of BALB/c mice, and 10^6 B16 melanoma cells were implanted in the right flank of C57BL/6 mice. Prior to virotherapy, mice with verified tumor allografts of CT26 (n = 15), 4T1 (n = 15), and B16 (n = 15) mice were divided into three subgroups (n = 5 each). 5 × 10^7 PFU of the viruses in 50 µl of PBS were injected intratumorally on the 7th and 9th days after tumor implantation. Control groups received intratumoral injections of PBS. Tumor volume was measured using a modified ellipsoidal formula: V = ½ (length × width^2) [28] every two days until the tumor volume reached 2000 mm^3. After reaching the maximum allowed volume, mice were euthanized and based on these data, survival curves were built.

Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using unpaired t-tests and two-way analysis of variance, differences were considered significant at $p < 0.05$. GraphPad Prism 8.0.2 (GraphPad Software, Inc.; USA) was used to prepare all graphs and perform statistical analysis.

RESULTS

Construction of recombinant viruses

TK inactivated LIVP-RFP and MVA-RFP strains containing an insertion of red fluorescent protein (tagRFP) were generated by recombination of the viral genome with a plasmid construct. Fluorescence microscopy of HEK293T cells infected with recombinant strains of LIVP-RFP and MVA-RFP showed that the viruses replicate and produce functionally active RFP (Fig. 1).

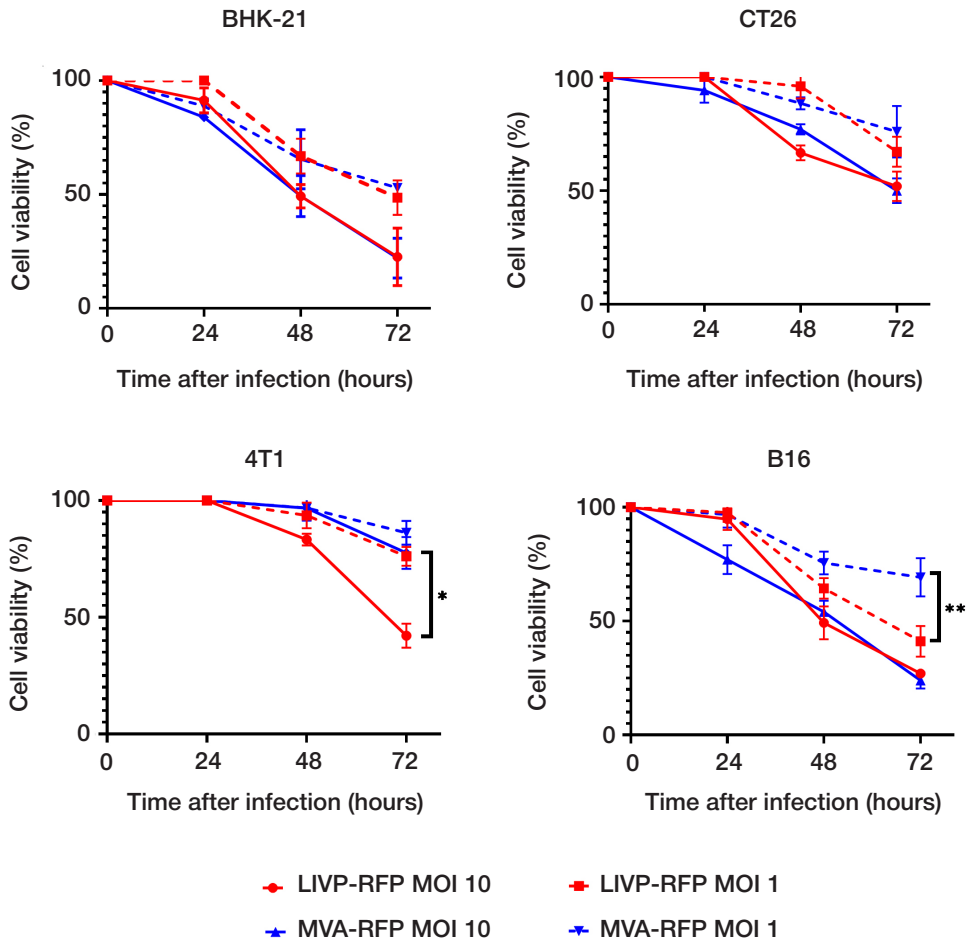

Fig. 2. Cytotoxicity of recombinant LIVP-RFP and MVA-RFP strains in various tumor cell cultures. BHK-21, B16, CT26 and 4T1 cells were infected with MOI 1 and 10 of LIVP-RFP and MVA-RFP viruses and cell viability was assessed using the MTT assay at 24, 48 and 72 hours post-infection. Statistical analysis was performed using a *t*-test; * — $p < 0.05$ and ** — $p < 0.01$ indicate significance.

Cytotoxic activity of LIVP-RFP and MVA-RFP strains against mouse tumor cells

The cytotoxic activity of recombinant vaccinia virus strains LIVP-RFP and MVA-RFP was assessed for 72 h using the MTT assay in mouse B16 melanoma, CT26 colon carcinoma, and 4T1 mammary adenocarcinoma cell cultures, as well as in the VV-sensitive BHK-21 cell line, which we used as a positive control. In BHK-21 culture, LIVP-RFP and MVA-RFP strains caused more than 75% cell death at MOI 10 and more than 50% death at MOI 1 (MOI 1) after 72 h (Fig. 2). B16 melanoma was the most sensitive of the studied metastatic tumor lines, in culture of which 50% cell death was observed 72 h after infection with MOI 10 LIVP-RFP or MVA-RFP (Fig. 2B; solid lines). Upon infection with B16 MOI 1, the recombinant LIVP-RFP strain showed significantly higher cytotoxicity after 72 h compared to MVA-RFP (Fig. 2; dotted lines). The most resistant to oncolytic virotherapy was CT26 colorectal carcinoma line, in culture of which less than 50% cell death was observed at a multiplicity of infection of 10 LIVP-RFP or MVA-RFP (Fig. 2). In 4T1 mammary adenocarcinoma, a cytopathic effect was detected only in infection with a multiplicity of 10. At the same time, a significantly higher cytotoxicity (> 50%) was noted for the LIVP-RFP strain compared to MVA-RFP (Fig. 2).

Assessment of viral replication by flow cytometry

The replication efficiency of viral strains in the studied cell lines was assessed by the number of fluorescent RFP-positive cells, which was determined using flow cytometry. It was found that the level of infection of the control line BHK-21 approaches 100% already after 24 h and does not change significantly after 48 h (Fig. 3). In the B16 melanoma cell line, an increase in the number of RFP-positive cells was observed, and the MVA-RFP strain, which infected more than 60% of the cells within 48 hours, showed a significantly higher replication efficiency. The 4T1 breast adenocarcinoma line, on the contrary, was characterized by the lowest replication efficiency of vaccinia virus, with the highest level of infection was observed when infected with the LIVP-RFP strain and it reached almost 30% after 48 hours. The efficiency of the viral replication in CT26 cell culture (about 40% for MOI 10) did not differ between LIVP-RFP and MVA -RFP.

Evaluation of the antitumor activity of LIVP-RFP and MVA-RFP strains in experiments *in vivo*

The oncolytic activity of the recombinant LIVP-RFP and MVA-RFP strains was studied in BALB/c mice with allografts of 4T1 breast or CT26 colon carcinomas, as well as in C57BL/6 mice with allografts of B16 melanoma. Double intratumoral injection of oncolytic viruses on days 7 and 9 after tumor inoculation resulted in a slowdown in tumor growth (Fig. 4) and an increase in animal survival (Fig. 5) in all groups treated with both LIVP-RFP and MVA-RFP compared to control groups that were injected with PBS. The most noticeable slowdown in tumor growth was found in the treatment of B16 melanoma allografts with the intratumoral injection of MVA-RFP, as well

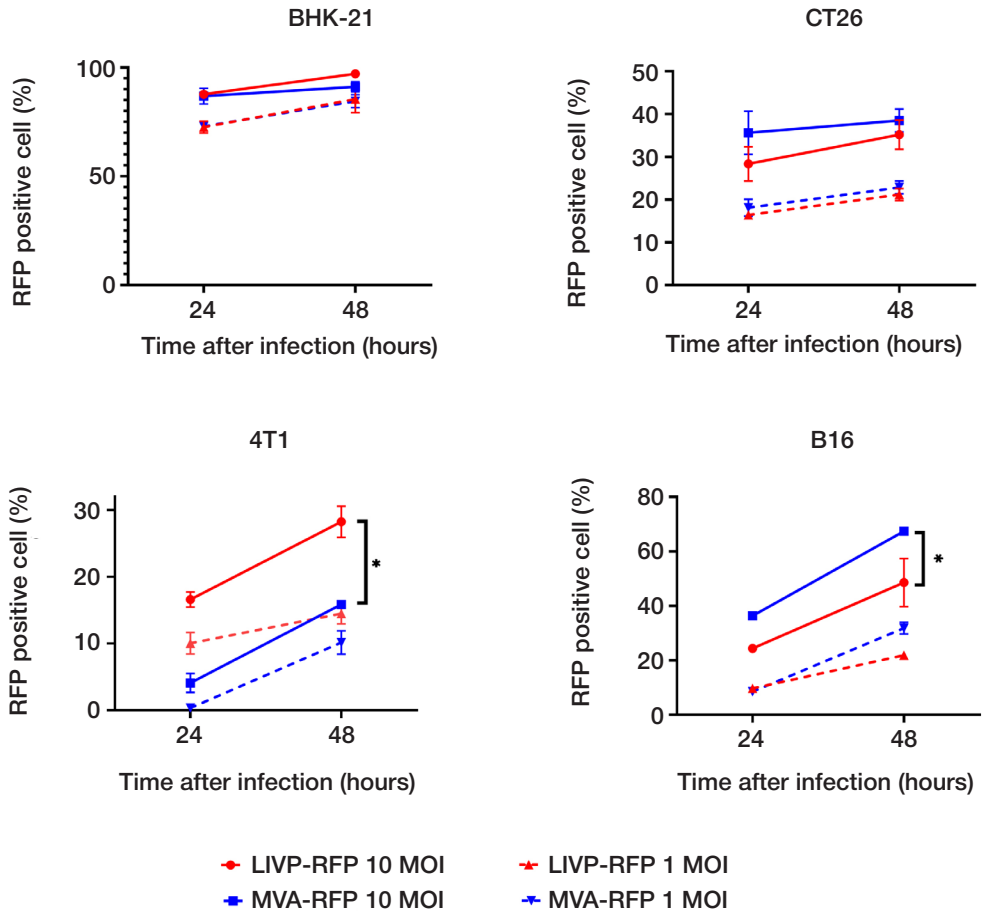

Fig. 3. Replication efficiency of viral strains in cell lines BHK-21, B16, CT26, 4T1 infected with MOI 1 and 10 LIVP-RFP and MVA-RFP based on the results of flow cytofluorometry after 24 and 48 h. The y-axis shows the number of cells in percent expressing RFP. Statistical analysis was performed using a *t*-test; * — $p < 0.05$ indicate significant

as 4T1 carcinoma allografts with the introduction of LIVP-RFP, which fully corresponded to the results obtained *in vitro*. Survival in the 4T1 and B16 subgroups (after virotherapy) was significantly higher compared to the control, while the animals treated with LIVP-RFP had a longer life expectancy than in the MVA-RFP subgroups (Fig. 5). Progression of CT26 carcinoma was not altered in any way by both LIVP-RFP and MVA-RFP therapy (Fig. 4), although both experimental subgroups showed an increase in survival of animals injected with recombinant viruses (Fig. 5).

Thus, data obtained from both *in vitro* and *in vivo* experiments confirm the superior oncolytic activity of the recombinant LIVP-RFP strain against the 4T1 breast adenocarcinoma model.

DISCUSSION

In this comparative study, we evaluated the cytotoxicity and replication capacity *in vitro*, and *in vivo* therapeutic potential against solid mouse tumors of recombinant LIVP-RFP and MVA-RFP strains derived from vaccinia virus strains LIVP and MVA, respectively, containing an insert of red fluorescent protein gene in the structural part of the viral thymidine kinase gene.

The effectiveness of the therapy with oncolytic viruses consists of two main components: the activation of the immune system in response to the introduction of viruses and the direct cytotoxic effect of viruses on tumor cells [29]. Activation of immunocompetent cytotoxic $CD8^+$ lymphocytes, $CD56^+$ NK cells, and tissue macrophages is of critical importance due to the fact that the most resistant and malignant tumors are characterized by the most pronounced immunosuppressive effect on the tumor microenvironment [30]. Therefore, systemic or intratumoral administration of viral particles that infect tumor cells and activate antigen-presenting cells is accompanied by increased production of inflammatory cytokines and recruitment of cytotoxic immune cells, which ultimately can slow down tumor progression. Antitumor immune responses are supplemented by a direct cytopathic effect of oncolytic viruses on tumor cells due to increased proliferation rate, inhibition of apoptosis, and other oncogenic mechanisms [30].

One of the key difficulties in the use of oncolytic viruses for therapy is a pronounced host immune response to the viral infection, which causes adverse side effects and reduces the effectiveness of the virotherapy. Poxviruses are unique in their ability to evade the host's immune response, making them generally safe for use in therapy, in particular, the Lister strain has proven to be highly safe in humans as it has been used during the worldwide smallpox eradication program [7, 31]. This strain has been shown to induce less pro-inflammatory cytokines such as IL8, IL6 and IFNγ in the host and induce higher levels of anti-inflammatory cytokines such as IL10 compared to other strains such as WR [5, 32].

Increasing the onco-selectivity of the virus limits viral infection at the site of the tumor and prevents infection of other organs, resulting in fewer inflammatory side effects. One of the strategies for increasing tumor selectivity and reducing the vaccinia virus virulence is deletion of the viral thymidine kinase gene [33].

In our study, we have shown that LIVP-RFP replicates and lyses 4T1 cells more efficiently than the MVA-RFP strain. In subsequent in vivo experiments, we were able to demonstrate the relationship between the ability of the virus to replicate in

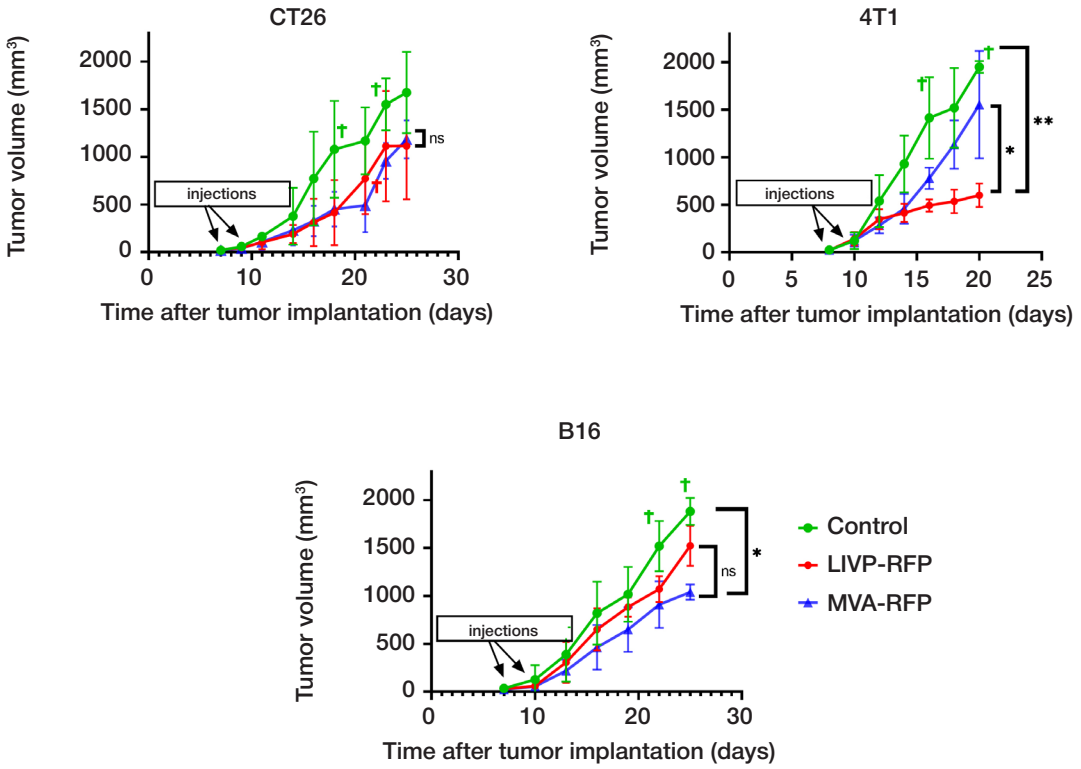

Fig. 4. Dynamics of changes in tumor volume in mice with allografts of colon carcinoma CT26, breast carcinoma 4T1, and melanoma B16 after treatment with recombinant strains of LIVP-RFP or MVA-RFP. Tumor measurements were taken every two days after treatment. The symbol † indicates the euthanasia of the animal. Statistical analysis was performed using a t-test; * — $p < 0.05$; ns — no statistically significant differences

tumor cells in vitro and its ability to slow tumor progression *in vivo*. A significantly smaller volume of tumor allografts of 4T1 adenocarcinoma and an increase in the survival of animals after LIVP-RFP therapy compared to MVA-RFP indicate a more pronounced oncolytic activity of LIVP-RFP in relation to 4T1 adenocarcinoma.

The 4T1 breast cancer cell line is a highly invasive and metastatic cell model of triple negative breast cancer (TNBC) [34]. TNBC is considered the most aggressive form of breast cancer with the worst prognosis and the absence of targeted treatment options [35]. Our results indicate that the LIVP strain has greater potential for the treatment of TNBC compared to MVA.

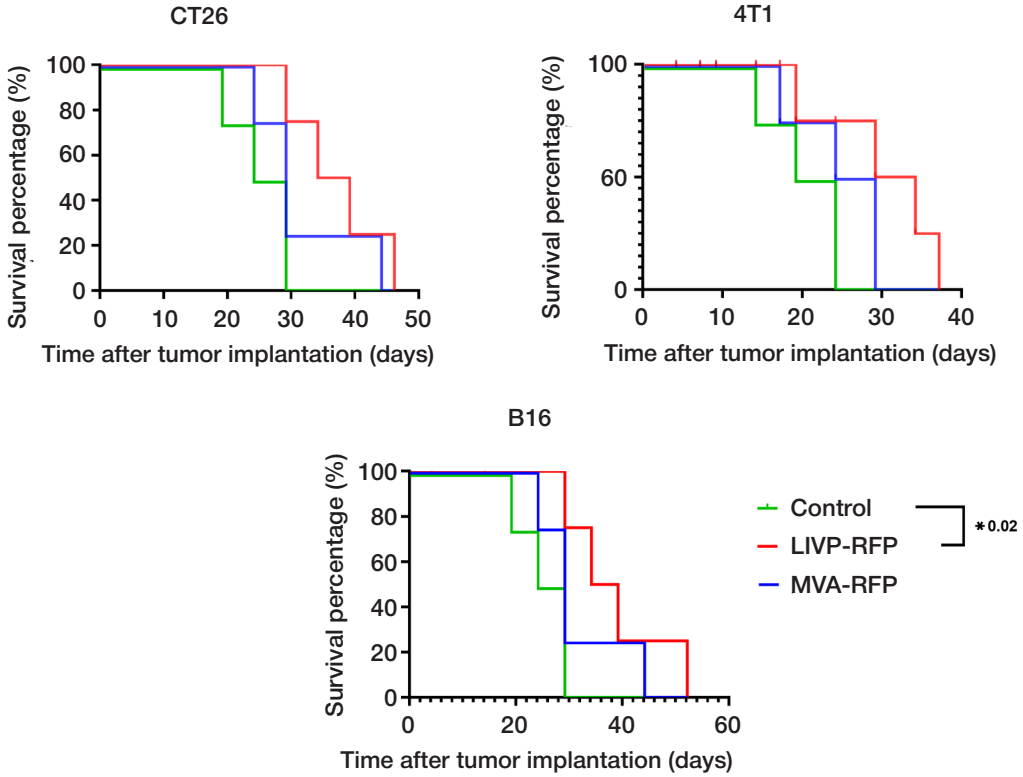

Fig. 5. Kaplan-Meier survival curves in experimental subgroups of mice with allografts of adenocarcinoma CT26, 4T1, and melanoma B16 after two intratumoral injections of recombinant LIVP-RFP or MVA-RFP viruses

CONCLUSIONS

A comparative study of the oncolytic properties of LIVP-RFP and MVA-RFP strains with an inactivated thymidine kinase gene showed that the LIVP-RFP strain is more effective for oncolytic virotherapy of 4T1 breast cancer. The use of the LIVP strain as a platform for the development of recombinant oncolytic viruses for the treatment of triple-negative breast cancer may be more promising than the use of the MVA strain.

References

1. Thorne SH, Hwang TH, Kirn DH. Vaccinia virus and oncolytic virotherapy of cancer. Curr Opin Mol Ther. 2005; 7 (4): 359–65.
2. Ho TY, et al. Deletion of immunomodulatory genes as a novel approach to oncolytic vaccinia virus development. Mol Ther Oncolytics. 2021; 22: 85–97.
3. Kirn DH, et al. Enhancing poxvirus oncolytic effects through increased spread and immune evasion. Cancer Res. 2008; 68 (7): 2071–5.
4. Haddad D, et al. A novel genetically modified oncolytic vaccinia virus in experimental models is effective against a wide range of human cancers. Ann Surg Oncol. 2012; 19: 665–74.
5. Hughes J, et al. Lister strain vaccinia virus with thymidine kinase gene deletion is a tractable platform for development of a new generation of oncolytic virus. Gene Ther. 2015; 22 (6): 476–84.
6. Shakiba Y, et al. Oncolytic efficacy of a recombinant vaccinia virus strain expressing bacterial flagellin in solid tumor models. Viruses. 2023; 15 (4): 828. DOI: 10.3390/v15040828.
7. Tysome JR, et al. Lister vaccine strain of vaccinia virus armed with the endostatin–angiostatin fusion gene: an oncolytic virus superior to dl 1520 (ONYX-015) for human head and neck cancer. Hum Gene Ther. 2011; 22 (9): 1101–8.
8. Smith GL. Vaccinia virus immune evasion. Immunol Lett. 1999; 65 (1–2): 55–62.
9. Bahar MW, et al. How vaccinia virus has evolved to subvert the host immune response. J Struct Biol. 2011; 175 (2): 127–34.
10. Smith GL, et al. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J Gen Virol. 2013; 94 (11): 2367–92.
11. Shvalov AN, et al. Complete genome sequence of vaccinia virus strain L-IVP. Genome Announc. 2016; 4 (3): e00372–16.
12. Gentschev I, et al. Preclinical evaluation of oncolytic vaccinia virus for therapy of canine soft tissue sarcoma. 2012; 7 (5): e37239.
13. Shchelkunov SN, et al. Enhancing the protective immune response to administration of a LIVP-GFP live attenuated vaccinia virus to mice. PLoS One. 2021; 10 (3): 377.
14. Kochneva G, et al. Engineering of double recombinant vaccinia virus with enhanced oncolytic potential for solid tumor virotherapy. Oncotarget. 2016; 7 (45): 74171.
15. Zonov E, et al. Features of the antitumor effect of vaccinia virus lister strain. Viruses. 2016; 8 (1): 20.
16. Koval O, et al. Recombinant vaccinia viruses coding transgenes of apoptosis-inducing proteins enhance apoptosis but not immunogenicity of infected tumor cells. Biomed Res Int. 2017; 2017: 3620510. DOI: 10.1155/2017/3620510.
17. Tkacheva A, et al. Targeted therapy of human glioblastoma combining the oncolytic properties of parvovirus H-1 and attenuated strains of the vaccinia virus. Molecular Genetics, Microbiology and Virology. 2019; 37 (2): 83–91.
18. Gholami S, et al. Vaccinia virus GLV-1h153 is a novel agent for detection and effective local control of positive surgical margins for breast cancer. Breast Cancer Res. 2013; 15 (2): R26.
19. Holloway R, et al. 837P Phase II trial of oncolytic vaccinia virus primed immunochemotherapy in platinum-resistant/refractory ovarian cancer (PRROC)(NCT02759588). Annals of Oncology. 2020; 31: 628.
20. Suter M, et al. Modified vaccinia Ankara strains with identical coding sequences actually represent complex mixtures of viruses that determine the biological properties of each strain. Vaccine. 2009; 27 (52): 7442–50.
21. Pittman PR, et al. Phase 3 efficacy trial of modified vaccinia Ankara as a vaccine against smallpox. N Engl J Med. 2019; 381 (20): 1897–908.
22. Gatti-Mays ME, et al. A phase I dose-escalation trial of BN-CV301, a recombinant poxviral vaccine targeting MUC1 and CEA with costimulatory molecules. Clin Cancer Res. 2019; 25 (16): 4933–44.
23. Parato KA, et al. The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. Mol Ther. 2012; 20 (4): 749–58.
24. Byrd CM, et al. Construction of recombinant vaccinia virus: cloning into the thymidine kinase locus. Methods Mol Biol. 2004: 31–40.
25. Cotter CA, et al. Preparation of cell cultures and vaccinia virus stocks. Curr Protoc Mol Biol. 2017; 117 (1): 16.16.1–16.16.18.
26. Ramakrishnan MAJ. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016; 5 (2): 85.
27. Morgan DM. Tetrazolium (MTT) assay for cellular viability and activity. Methods Mol Biol.1998: 179–84.
28. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol. 1989; 24: 148–54.
29. Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine. 2013; 31 (39): 4247–51.
30. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol. 2006; 6 (10): 715–27.
31. Thorne SH. Immunotherapeutic potential of oncolytic vaccinia virus. Immunol Res. 2011; 50: 286–93.
32. Matsuda T, et al. A comparative safety profile assessment of oncolytic virus therapy based on clinical trials. Ther Innov Regul Sci. 2018; 52 (4): 430–7.
33. Buller RML, et al. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. Nature. 1985; 317 (6040): 813–5.
34. Schrörs B, et al. Multi-omics characterization of the 4T1 murine mammary gland tumor model. Front Oncol. 2020; 10: 1195.
35. Li Z, et al. Immunotherapeutic interventions of triple negative breast cancer. J Transl Med. 2018; 16 (1): 147.

Литература

1. Thorne SH, Hwang TH, Kirn DH. Vaccinia virus and oncolytic virotherapy of cancer. Curr Opin Mol Ther. 2005; 7 (4): 359–65.
2. Ho TY, et al. Deletion of immunomodulatory genes as a novel approach to oncolytic vaccinia virus development. Mol Ther Oncolytics. 2021; 22: 85–97.
3. Kirn DH, et al. Enhancing poxvirus oncolytic effects through increased spread and immune evasion. Cancer Res. 2008; 68 (7): 2071–5.
4. Haddad D, et al. A novel genetically modified oncolytic vaccinia virus in experimental models is effective against a wide range of human cancers. Ann Surg Oncol. 2012; 19: 665–74.
5. Hughes J, et al. Lister strain vaccinia virus with thymidine kinase gene deletion is a tractable platform for development of a new generation of oncolytic virus. Gene Ther. 2015; 22 (6): 476–84.
6. Shakiba Y, et al. Oncolytic efficacy of a recombinant vaccinia virus strain expressing bacterial flagellin in solid tumor models. Viruses. 2023; 15 (4): 828. DOI: 10.3390/v15040828.
7. Tysome JR, et al. Lister vaccine strain of vaccinia virus armed

with the endostatin–angiostatin fusion gene: an oncolytic virus superior to dl 1520 (ONYX-015) for human head and neck cancer. Hum Gene Ther. 2011; 22 (9): 1101–8.
8. Smith GL. Vaccinia virus immune evasion. Immunol Lett. 1999; 65 (1–2): 55–62.
9. Bahar MW, et al. How vaccinia virus has evolved to subvert the host immune response. J Struct Biol. 2011; 175 (2): 127–34.
10. Smith GL, et al. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J Gen Virol. 2013; 94 (11): 2367–92.
11. Shvalov AN, et al. Complete genome sequence of vaccinia virus strain L-IVP. Genome Announc. 2016; 4 (3): e00372–16.
12. Gentschev I, et al. Preclinical evaluation of oncolytic vaccinia virus for therapy of canine soft tissue sarcoma. 2012; 7 (5): e37239.
13. Shchelkunov SN, et al. Enhancing the protective immune response to administration of a LIVP-GFP live attenuated vaccinia virus to mice. PLoS One. 2021; 10 (3): 377.
14. Kochneva G, et al. Engineering of double recombinant vaccinia virus with enhanced oncolytic potential for solid tumor virotherapy. Oncotarget. 2016; 7 (45): 74171.
15. Zonov E, et al. Features of the antitumor effect of vaccinia virus lister strain. Viruses. 2016; 8 (1): 20.
16. Koval O, et al. Recombinant vaccinia viruses coding transgenes of apoptosis-inducing proteins enhance apoptosis but not immunogenicity of infected tumor cells. Biomed Res Int. 2017; 2017: 3620510. DOI: 10.1155/2017/3620510.
17. Tkacheva A, et al. Targeted therapy of human glioblastoma combining the oncolytic properties of parvovirus H-1 and attenuated strains of the vaccinia virus. Molecular Genetics, Microbiology and Virology. 2019; 37 (2): 83–91.
18. Gholami S, et al. Vaccinia virus GLV-1h153 is a novel agent for detection and effective local control of positive surgical margins for breast cancer. Breast Cancer Res. 2013; 15 (2): R26.
19. Holloway R, et al. 837P Phase II trial of oncolytic vaccinia virus primed immunochemotherapy in platinum-resistant/refractory ovarian cancer (PRROC)(NCT02759588). Annals of Oncology. 2020; 31: 628.
20. Suter M, et al. Modified vaccinia Ankara strains with identical coding sequences actually represent complex mixtures of viruses that determine the biological properties of each strain. Vaccine. 2009; 27 (52): 7442–50.
21. Pittman PR, et al. Phase 3 efficacy trial of modified vaccinia Ankara as a vaccine against smallpox. N Engl J Med. 2019; 381 (20): 1897–908.
22. Gatti-Mays ME, et al. A phase I dose-escalation trial of BN-CV301, a recombinant poxviral vaccine targeting MUC1 and CEA with costimulatory molecules. Clin Cancer Res. 2019; 25 (16): 4933–44.
23. Parato KA, et al. The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. Mol Ther. 2012; 20 (4): 749–58.
24. Byrd CM, et al. Construction of recombinant vaccinia virus: cloning into the thymidine kinase locus. Methods Mol Biol. 2004: 31–40.
25. Cotter CA, et al. Preparation of cell cultures and vaccinia virus stocks. Curr Protoc Mol Biol. 2017; 117 (1): 16.16.1–16.16.18.
26. Ramakrishnan MAJ. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016; 5 (2): 85.
27. Morgan DM. Tetrazolium (MTT) assay for cellular viability and activity. Methods Mol Biol.1998: 179–84.
28. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol. 1989; 24: 148–54.
29. Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine. 2013; 31 (39): 4247–51.
30. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol. 2006; 6 (10): 715–27.
31. Thorne SH. Immunotherapeutic potential of oncolytic vaccinia virus. Immunol Res. 2011; 50: 286–93.
32. Matsuda T, et al. A comparative safety profile assessment of oncolytic virus therapy based on clinical trials. Ther Innov Regul Sci. 2018; 52 (4): 430–7.
33. Buller RML, et al. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. Nature. 1985; 317 (6040): 813–5.
34. Schrörs B, et al. Multi-omics characterization of the 4T1 murine mammary gland tumor model. Front Oncol. 2020; 10: 1195.
35. Li Z, et al. Immunotherapeutic interventions of triple negative breast cancer. J Transl Med. 2018; 16 (1): 147.