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Comparative analysis of oncolytic potential of vesicular stomatitis virus serotypes Indiana and New Jersey in cancer cell lines

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Abstract

Aim. Compare the lytic efficiency and the kinetics of accumulation of vesicular stomatitis virus serotypes Indiana (VSV-IND) and New Jersey (VSV-NJ) on cell lines of mouse melanoma B16F10, human hepatocellular carcinoma HepG2 and human mammary adenocarcinoma MCF7.

Materials and methods. The viability of mouse melanoma B16F10, human hepatocellular carcinoma HepG2 and human mammary adenocarcinoma MCF7 cell lines infected with VSV-IND and VSV-NJ viruses at different multiplicity of infection (10 MOI; 1 MOI; 0.1 MOI) was assessed after 24, 48 and 72 hours, and the half maximal inhibitory concentration (IC50) values were measured using the methyl tetrazolium test. The relationship with virus accumulation in cell culture was determined using reverse transcription – quantitative polymerase chain reaction; 50% tissue culture infectious dose (TCID50) of VSV-IND and VSV-NJ for B16F10, HepG2, MCF7 were calculated using the Reed-Muench method.

Results. The most susceptible cell line for both viruses was B16F10: cell viability 72 hours after infection at 10 MOI was only 10.4% and 5.7% for VSV-IND and VSV-NJ, respectively. HepG2 cell viability at 72 hours post-infection at 10 MOI was 10.8% and 9.8% for VSV-IND and VSV-NJ, and for MCF7 adenocarcinoma it was 46.6% and 36.2%, respectively. Moreover, only in the B16F10 culture was a positive statistically significant correlation of medium strength established between the inhibition of cell viability and the accumulation of viral RNA: for VSV-IND $r = 0.601$ ($p < 0.05$); for VSV-NJ $r = 0.668$ ($p < 0.05$). HepG2 and MCF7 showed no significant correlation.

Conclusion. The research results indicate the potential of using oncolytic viruses of the VSV-IND and VSV-NJ as a platform for the development of new recombinant viruses for virotherapy of solid tumors in combination with other types of treatment.

Keywords: immunotherapy; viral vectors; oncolytics; animal viruses; cytopathic effect; polymerase chain reaction
MeSH terms:
CELL LINE, TUMOR – VIROLOGY
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ONCOLYTIC VIROTHERAPY – METHODS
VESICULAR STOMATITIS INDIANA VIRUS – IMMUNOLOGY
VESICULAR STOMATITIS INDIANA VIRUS – PATHOGENICITY
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VESICULAR STOMATITIS NEW JERSEY VIRUS – PATHOGENICITY
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Data availability. The data that support the findings of this study are available from the corresponding authors on reasonable request. Data and statistical methods used in the article were examined by a professional biostatistician on the Sechenov Medical Journal editorial staff.
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Сравнительный анализ цитолитического потенциала вируса везикулярного стоматита серотипов Indiana и New Jersey в отношении опухолевых клеточных линий

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Аннотация

Цель. Провести сравнительный анализ эффективности лизиса раковых клеток и кинетику накопления двумя серотипами вируса везикулярного стоматита Indiana (VSV-IND) и New Jersey (VSV-NJ) на клеточных линиях меланомы мыши B16F10, гепатоцеллюлярной карциномы человека HepG2 и аденокарциномы молочной железы человека MCF7.

Материалы и методы. Была оценена жизнеспособность клеточных линий меланомы мыши B16F10, гепатоцеллюлярной карциномы человека HepG2 и аденокарциномы молочной железы человека MCF7, инфицированных вирусами VSV-IND и VSV-NJ при разной множественности заражения (Multiplicity of infection, MOI) (10 MOI; 1 MOI; 0,1 MOI), через 24, 48 и 72 часа, а также вычислены значения половины максимальной ингибирующей концентрации (IC50) с помощью метил-тетразолиевого теста. Взаимосвязь с накоплением вируса в клеточной культуре определяли с помощью полимеразной цепной реакции с обратной транскрипцией в режиме реального времени, 50%-ные инфицирующие дозы (TCID50) VSV-IND и VSV-NJ для B16F10, HepG2, MCF7 рассчитывали с помощью метода Рида – Менча.

Результаты. Наиболее восприимчивой клеточной линией для обоих вирусов оказалась B16F10: жизнеспособность клеток через 72 часа после заражения 10 MOI составила лишь 10,4 и 5,7% для VSV-IND и VSV-NJ соответственно. Жизнеспособность клеток HepG2 составила 10,8 и 9,8% для VSV-IND и VSV-NJ, а для аденокарциномы MCF7 – 46,6 и 36,2% соответственно через 72 часа после заражения 10 MOI. При этом лишь в культуре B16F10 установлена положительная статистически значимая корреляция средней силы между ингибированием жизнеспособности клеток и накоплением вирусной РНК: для VSV-IND r = 0,601 (p < 0,05); для VSV-NJ r = 0,668 (p < 0,05). HepG2 и MCF7 не продемонстрировали значимой корреляции.

Заключение. Результаты исследований свидетельствуют о возможности использования серотипов VSV-IND и VSV-NJ в качестве платформы для разработки новых рекомбинантных вирусов для виротерапии солидных опухолей в комбинации с другими видами лечения.

Ключевые слова: иммунотерапия; вирусные векторы; онколитики; вирусы животных; цитопатическое действие; полимеразная цепная реакция

Рубрики MeSH:
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КЛЕТОЧНЫЕ ЛИНИИ НОВООБРАЗОВАНИЙ – ДЕЙСТВИЕ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ
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ВЕЗИКУЛЯРНОГО СТОМАТИТА НЬЮ-ДЖЕРСИ ВИРУС – ПАТОГЕННОСТЬ
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Соответствие принципам этики. Данное исследование с использованием биологического материала проводилось исключительно в соответствии с Хельсинкской декларацией Всемирной медицинской ассоциации об этических принципах проведения биомедицинских исследований.
Доступ к данным исследования. Данные, подтверждающие выводы этого исследования, можно получить у авторов по обоснованному запросу.
Данные и статистические методы, представленные в статье, прошли статистическое рецензирование редактором журнала – сертифицированным специалистом по биостатистике.
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Abbreviations

ATCC – American Type Culture Collection
IC50 – Half maximal inhibitory concentration
RT-qPCR – real-time reverse transcription – quantitative polymerase chain reaction
MOI – Multiplicity of infection
TCID50 – 50% Tissue Culture Infectious Dose
VSV – Vesicular stomatitis virus
VSV-IND – Vesicular stomatitis virus Indiana
VSV-NJ – Vesicular stomatitis virus New Jersey
HCC – hepatocellular carcinoma
MTT – methyl tetrazolium assay
BC – breast cancer

Despite progress in the study of the molecular processes of oncogenesis, cancer mortality worldwide remains significant, with approximately 9.3 million cases reported annually. This places cancer as the second leading cause of mortality from non-communicable diseases, following cardiovascular diseases, which account for 17.2 million cases per year[1]. According to global statistics for 2020, the three most common and aggressive types of cancer are: breast cancer (BC), lung cancer, and colon/rectal cancer. The number of new cases per year is 2.26, 2.21, and 1.93 million, respectively[2].

In the fight against cancer, there remain a number of unsolved problems related to the effectiveness and safety of treatment – for example, chemotherapy and radiation therapy, despite their positive effects on cancer cells, cause clinically significant side effects that worsen the general condition of patients. The search for drugs with high efficiency that cause selective death of tumor cells but have minimal side effects on healthy cells and tissues is an urgent one [1–4].

Oncolytic viruses have long been considered as possible antitumor agents [5]. Among them, much attention is paid to viruses of the family *Rhabdoviridae*, which includes the vesicular stomatitis virus (VSV) [6]. VSV is an RNA-containing virus that can effectively penetrate different types of animal cells while being non-pathogenic for humans [7, 8]. VSV replication is enhanced in tumor cells with a defective interferon signaling pathway, in which the innate immune response does not work [9]. In addition, VSV has a rapid life cycle, does not integrate into the host genome [8], and is a potent inducer of apoptosis in infected cancer cells [10, 11]. VSV has demonstrated antitumor activity in a wide range of cancer cells, including osteosarcoma [12], cervical cancer [13], breast cancer [14], melanoma [15], hepatocellular carcinoma (HCC) [16], pancreatic cancer [17] and glioblastoma [4, 18].

There are two main VSV serotypes: New Jersey (VSV-NJ) and Indiana (VSV-IND), which differ in terms of their genetic and antigenic properties. The homology of the major capsid glycoprotein G of viruses of both serotypes is less than 50% [19]. Viruses of the VSV-NJ and VSV-IND serotypes also have different pathogenicity for farm animals [20, 21]. Additionally, phosphoprotein P which is an important component of the RNA-dependent RNA polymerase exhibits the highest variable among both viruses, with a nucleotide sequence homology of 30% [22]. Having a common origin and different antigenic properties, VSV-NJ and VSV-IND may differ in terms of their effectiveness in killing tumor cells.

The potential of virotherapy is attracting interest as it allows viruses to target a wide range of malignant cells. In this work, we compared the oncolytic potential of VSV-NJ and VSV-IND using the most widespread cell lines: mouse melanoma – B16F10, human HCC – HepG2, human breast cancer – MCF7. In addition to differences in the origin, structure, and nature of metastasis of melanoma, HCC, and breast cancer, these cell lines show different permissiveness towards oncolytic viruses. For example, B16F10 is not permissive to human adenovirus type 5 [23, 24], but is permissive to myxoma virus [25]. This difference is due to the absence of the CAR receptor (coxsackievirus and adenovirus receptor) on the cell surface which is necessary for adenovirus penetration, while the infectious cycle of the myxoma virus does not depend on membrane receptors [26].

Most modern studies are devoted to recombinant strains based on VSV-IND, but none of them has been approved in the clinic to date. To assess the prospects for using other VSV serotypes in tumor virotherapy, their comparative analysis is necessary, as is further study of various genetically different variants of natural VSV serotypes to select the most promising platform for the development of new armored oncolytic viruses.

Aim of the study: To study the cytolytic effect and kinetics of accumulation of VSV-IND and VSV-NJ on cell lines of mouse melanoma, HCC and human BC.

MATERIALS AND METHODS

Viruses

This study used vesicular stomatitis viruses VSV (serotype New Jersey (VSV-NJ) and Indiana (VSV-IND) from the working collection of the Institute of Medical Parasitology, Tropical and Vector-Borne Infections named after E.I. Martsinovsky, Federal State Autonomous Educational Institution of Higher Education "First Moscow State Medical University named after I.M. Sechenov, Ministry of Health of Russia (Sechenov University).

VSV viruses were obtained in hamster fibroblast cell culture BHK-21 (American Type Culture Collection (ATCC), CCL-10)[3].

[1] World Health Organization: Newsroom/Fact sheets/Detail/Noncommunicable diseases. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed: 22.09.2023).

[2] World Health Organization: Newsroom/Fact sheets/Detail/Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer (accessed: 22.09.2023).

[3] American Type Culture Collection (ATCC): Cell Products/Animal Cells/CCL-10. https://www.atcc.org/products/ccl-10 (accessed: 29.09.2023).

Cell lines

The cell lines chosen for the work were: mouse melanoma – B16F10 (ATCC CRL-6475)[4], human HCC – HepG2 (ATCC HB-8065)[5], Human breast cancer – MCF7 (ATCC HTB-22)[6]. Cancer cell lines were provided by the Institute of Gene Biology, Russian Academy of Sciences (Moscow, Russia) (Fig. 1).

Cell Culture and Viability Analysis

The experimental part of this work was carried out from August 2022 to February 2023. Cells were cultured under standard conditions (5% CO_2, 37 °C), according to ATCC protocols for each cell line, until 70–80% confluence was achieved in T25 and T75 flasks (Wuxi NEST Biotechnology Co., Ltd., China), in Dulbecco's Modified Eagle Medium, DMEM (PanEco LLC, Russia) with a high glucose content, with L-glutamine (PanEco LLC, Russia), by adding 10% fetal calf serum (Diaem LLC, Russia) and penicillin-streptomycin, 100x lyophile. (PanEco LLC, Russia).

Cell viability was determined on a C-100 cell counter (RWD Life Science, China) using trypan blue (PanEco LLC, Russia). The cytotoxicity of oncolytic virus serotypes was assessed by the decrease in the viability of cells of the studied lines in the methyl tetrazolium test (MTT)[7]. For each experiment, cells were seeded into three 96-well culture plates (SARSTEDT AG & Co., Germany) at a concentration of 10,000 cells per well, cultured for 24 hours, after which cells were removed from one well and the number of living cells were counted to calculate the Multiplicity of infection (MOI).

Cells in plate wells were infected with VSV-IND and VSV-NJ serotypes at doses of 10 MOI, 1 MOI and 0.1 MOI. As an uninfected control, wells with cells without added virus were left. For each virus dose (MOI), 4 to 8 wells were used. All work was carried out under sterile conditions.

The results of the experiment were considered after 24, 48 and 72 hours using the MTT assay (the final concentration of the MTT reagent in the well was 0.5 mg/ml). The optical density of the color reaction was measured at wavelengths of 550 and 620 nm on a Varioskan LUX plate spectrophotometer (ThermoFisher Scientific TM., USA). Cell viability in wells with uninfected cells was taken as 100% (control value). The viability of infected cells was calculated as a percentage of the optical density values of uninfected cells. Based on the MTT assay data, the half maximal inhibitory concentration (IC50) values were also calculated for each cell line at different MOI (10; 1; 0.1) and incubation durations (24, 48 and 72 hours).

Virus RNA Isolation

To conduct a polymerase chain reaction with reverse transcription in real time (real-time reverse transcription – quantitative polymerase chain reaction, RT-qPCR), 100 µl of the suspension was taken from the wells of the plates 24, 48, and 72 hours after infection of the cell cultures and isolated viral RNA using a set of reagents for isolating RNA/DNA from clinical material "RIBO-prep" (FBUN Central Research Institute of Epidemiology of Rospotrebnadzor, Russia) according to the manufacturer's protocol.

RT-qPCR. For RNA detection (VSV), a primer system was developed for the complementary sequence of the gene region VSV-IND and VSV-NJ. Forward (5'-GCTGCAGTGGACATGTTCTTC-3') and reverse primers (5'-CATGTATGAATCSGCCTTRTCAATT TC-3') were used to amplify the RT-qPCR product of 240 bp. in the presence of the SybrGreen intercalating dye with a reverse transcription polymerase chain reaction kit in one tube (Alpha Enzyme LLC, Russia) and the addition of 3% dimethyl sulfoxide at the following reaction parameters: 50 °C – 10 min (1 cycle), 95 °C – 5 min (1 cycle), 95 °C – 15 sec., 55 °C – 30 sec., 72 °C – 30 sec. (40 cycles) on a QuantStudio 5 device (ThermoFischer Scientific, USA).

Determination of 50% Tissue Infectious Dose of Virus

Determination of 50% tissue infectious dose of the virus (Tissue Culture Infectious Dose, TCID50) at 1 ml of sample was carried out using the titration method in BHK-21 cell culture (ATCC CCL-10) in a 96-well plate format (Fig. 1). 10,000 cells were added to the plate wells in complete Dulbecco's modified Eagle growth medium, cultured for 24 hours in an atmosphere of 5% CO_2 at 37 °C, then infected with VS-IND and VSV-NJ at doses of 10 MOI, 1 MOI and 0.1 MOI (8 wells per dilution) in a volume of 50 µl per well and incubated for 60 minutes (5% CO_2, 37 °C) for virus sorption on cells. Next, 150 µl of complete growth medium was added and incubated

[4] American Type Culture Collection (ATCC): Cell Products/Animal Cells/CRL-6475. https://www.atcc.org/products/crl-6475 (date of access: 29.09.2023).

[5] American Type Culture Collection (ATCC): Cell Products/Human Cells/HB-8065. https://www.atcc.org/products/hb-8065 (accessed: 29.09.2023).

[6] American Type Culture Collection (ATCC): Cell Products/Human Cells/HTB-22. https://www.atcc.org/products/htb-22 (accessed: 29.09.2023).

[7] Methods for studying cytotoxicity in drug screening. Educational and methodological manual / A.G. Iksanova, O.V. Bondar, K.V. Balakin. Kazan: Kazan University, 2016. 40 p. https://kpfu.ru/staff_files/F1213114794/Metody_skrininga_LS.pdf (accessed: 29.09.2023).

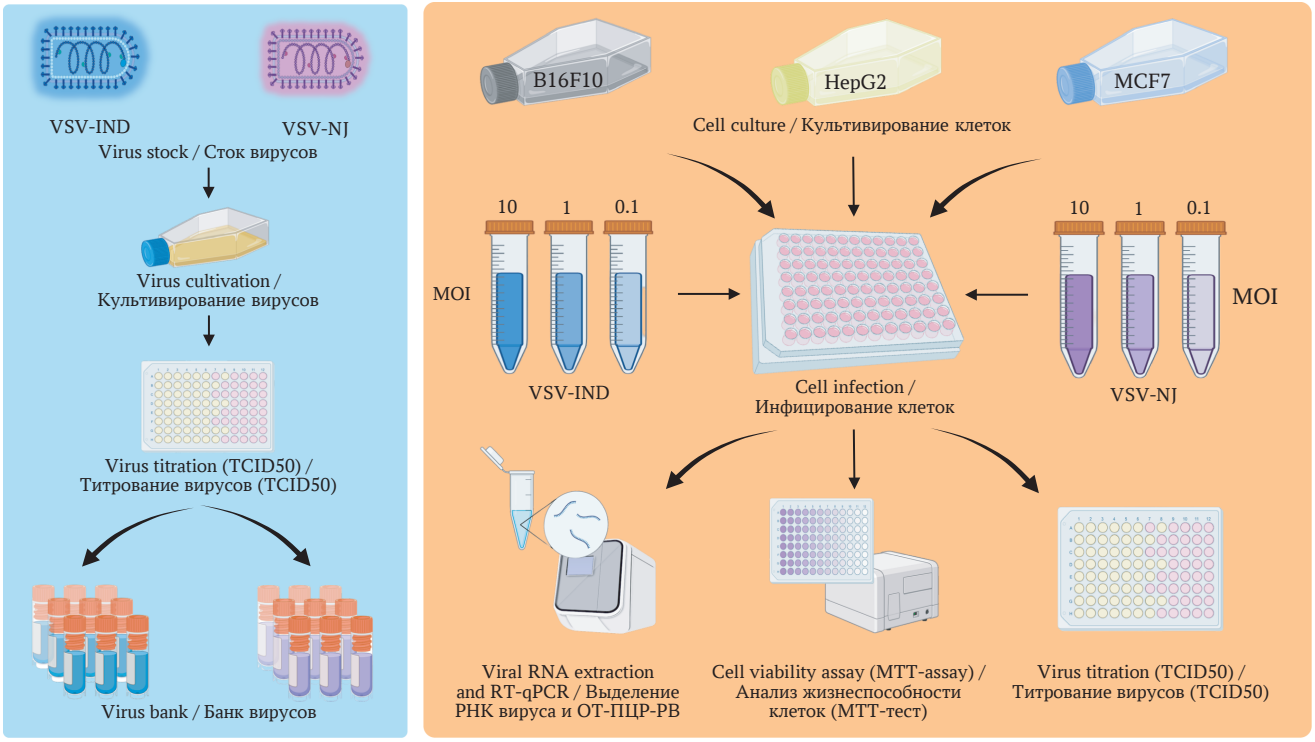

FIG. 1. Experimental design: study of oncolytic potential of vesicular stomatitis virus serotypes Indiana and New Jersey.
РИС. 1. Схема эксперимента: изучение онколитического потенциала вируса везикулярного стоматита серотипов Indiana и New Jersey.

Note: VSV-IND – Vesicular stomatitis virus Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey; TCID50 – 50% Tissue Culture Infectious Dose; B16F10 – Mouse melanoma cell line; HepG2 – Human hepatocellular carcinoma cell line; MCF7 – Human breast cancer cell line; MOI – Multiplicity of infection; RNA – Ribonucleic acid; RT-qPCR – real-time reverse transcription – quantitative polymerase chain reaction; MTT – methyl tetrazolium test.
Примечание: VSV-IND – Vesicular stomatitis virus Indiana, вирус везикулярного стоматита серотип Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey, вирус везикулярного стоматита серотип New Jersey; TCID50 – 50% Tissue Culture Infectious Dose, 50%-ная инфицирующая доза вируса; B16F10 – клеточная линия меланомы мыши; HepG2 – клеточная линия гепатоцеллюлярной карциномы человека; MCF7 – клеточная линия рака молочной железы человека; MOI – Multiplicity of infection, множественность заражения; РНК – рибонуклеиновая кислота; ОТ-ПЦР-РВ –полимеразная цепная реакция с обратной транскрипцией в режиме реального времени; МТТ – метил-тетразолиевый тест.

for 72 hours under the same conditions. The results were considered under an inverted microscope (Micromed I, Russia), noting wells with characteristic changes in cell morphology (rounded and compressed cells, partial cell detachment). Virus titer was calculated using the Reed–Muench method[8].

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. MTT assay and RT-qPCR data are presented as average values with sample standard deviation. The statistical significance of differences in cell viability was determined using Student's *t*-test with Bonferroni correction for multiple comparisons.

To identify the relationship between cell viability and virus accumulation in cell culture, correlation analysis was used to calculate the Pearson correlation coefficient (r). The interpretation of the correlation strength was carried out by assessing the range of values (r): from 0 to 0.299 – as a weak relationship; from 0.3 to 0.699 – as moderate; from 0.7 to 1.0 – as strong. To refute the null hypothesis, the level of statistical significance was adopted $p < 0.05$. Statistical data analysis and plotting were carried out using RStudio v.2023.03.1+446[9] using the rstatix and ggplot2 libraries.

RESULTS

Mouse melanoma cell line B16F10

Then transducing the B16F10 cell line in experiments with MTT, a significant decrease in cell viability was observed when infected with both serotypes of the virus – VSV-IND (Fig. 2A) and VSV-NJ (Fig. 2B).

[8] https://web.archive.org/web/20131119121647/http://aje.oxfordjournals.org/content/27/3/493.extract
[9] https://dailies.rstudio.com/rstudio/cherry-blossom/electron/windows-xcopy/2023-03-1-446/

When analyzing the relationship between cytotoxicity and the amount of viral RNA, a positive statistically significant correlation of medium strength was established for both serotypes: $r = 0.601$ for VSV-IND, $p < 0.05$ and $r = 0.668$ for VSV-NJ, $p < 0.05$.

TCID50 of viruses accumulated on this culture over 24 hours at an infection dose of 10 MOI was 1.0×10^4 and 1.47×10^5 TCID50 for VSV-IND and VSV-NJ, respectively (Fig. 2C, 2D). We also determined the IC50 dose (MOI) for viruses of both serotypes (Table).

In the B16F10 culture, after 24 hours of exposure to inhibit the viability of 50% of the cells, 5.31 MOI of VSV-IND and 1.08 MOI of VSV-NJ were determined, that is, the IC50 of VSV-IND was almost five times higher than the IC50 of VSV-NJ. On the contrary, when the exposure time was increased to 48 and 72 hours, it was no longer possible to determine the IC50 for VSV-IND due to the death of many cells (more than 80%) in the entire range of virus concentrations used.

At the same time, for VSV-NJ after 48 and 72 hours, the IC50 was determined within the range of 0.02–0.1 MOI (table), and the TCID50 indicator for VSV-IND turned out to be more than 3 times higher than for VSV-NJ (9.28×10^3 and 2.94×10^3 TCID50 for 10 MOI respectively).

Human hepatocellular carcinoma cell line HepG2

The viability of HepG2 cells after 24 hours of incubation with the virus of both serotypes is comparable to that of B16F10 (Fig. 3A, 3B). After 48 hours, over the entire range of virus concentrations, more viable HepG2 cells remain than in the B16F10 culture under the same conditions, except for VSV-NJ 0.1 MOI, for which the values for both cell lines are the same (Fig. 2B, 3B). The dynamics of the decrease in cell viability makes it possible to determine the IC50 after 48 hours of exposure to the virus of both serotypes (table). However, after 72 hours, the permissiveness of HepG2 to VSV-NJ exceeds that of the B16F10 line. Even at a minimal infectious dose of 0.1 MOI, more than 80% of cells are killed after 72 hours: IC50 is undetectable in this infectious dose range for both serotypes (Fig. 3B).

As a result of titration of VSV-NJ and VSV-IND using the TCID50 method, after infection of HepG2 cells, the values ranged from 2.15×10^4 up to 4.64×10^6 TCID50 for VSV-IND and from 1.0×10^6 to 3.16×10^9 TCID50 for VSV-NJ (Fig. 3C, 3D), which is several orders of magnitude higher than that for B16F10. No significant correlations were found between the decrease in viability and the change in the amount of viral RNA (RT-qPCR) for HepG2.

Human breast adenocarcinoma cell line – MCF7

In a series of our experiments, the MCF7 cell line was the least permissive to VSV (Fig. 4A, 4B), demonstrating resistance to VSV-NJ of more than 50% over the entire range of virus concentrations and incubation times, except for the maximum concentrations (10 MOI, 72

Table. **IC50 values for VSV-NJ and VSV-IND in cancer cell lines**

Таблица. **Определение IC50 для серотипов вируса VSV-NJ и VSV-IND на раковых клеточных культурах**

Cell line / Клеточная линия	Time, h / Время, ч	IC50	
		VSV-IND (MOI)	VSV-NJ (MOI)
B16F10	24	5.31	1.08
	48	n/a	0.02
	72	n/a	0.1
HepG2	24	12.07	4.80
	48	0.25	0.01
	72	n/a	n/a
MCF7	24	17.25	33.12
	48	n/a	0.01
	72	0.66	6.02

Note: IC50 – Half maximal inhibitory concentration; VSV-IND – Vesicular stomatitis virus Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey; MOI – Multiplicity of infection; n/a – not analyzed.

Примечание: IC50 – Half maximal inhibitory concentration, половина максимальной ингибирующей концентрации; VSV-IND – Vesicular stomatitis virus Indiana, вирус везикулярного стоматита серотип Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey, вирус везикулярного стоматита серотип New Jersey; MOI – Multiplicity of infection, множественность заражения; n/a – not analyzed, не определяется.

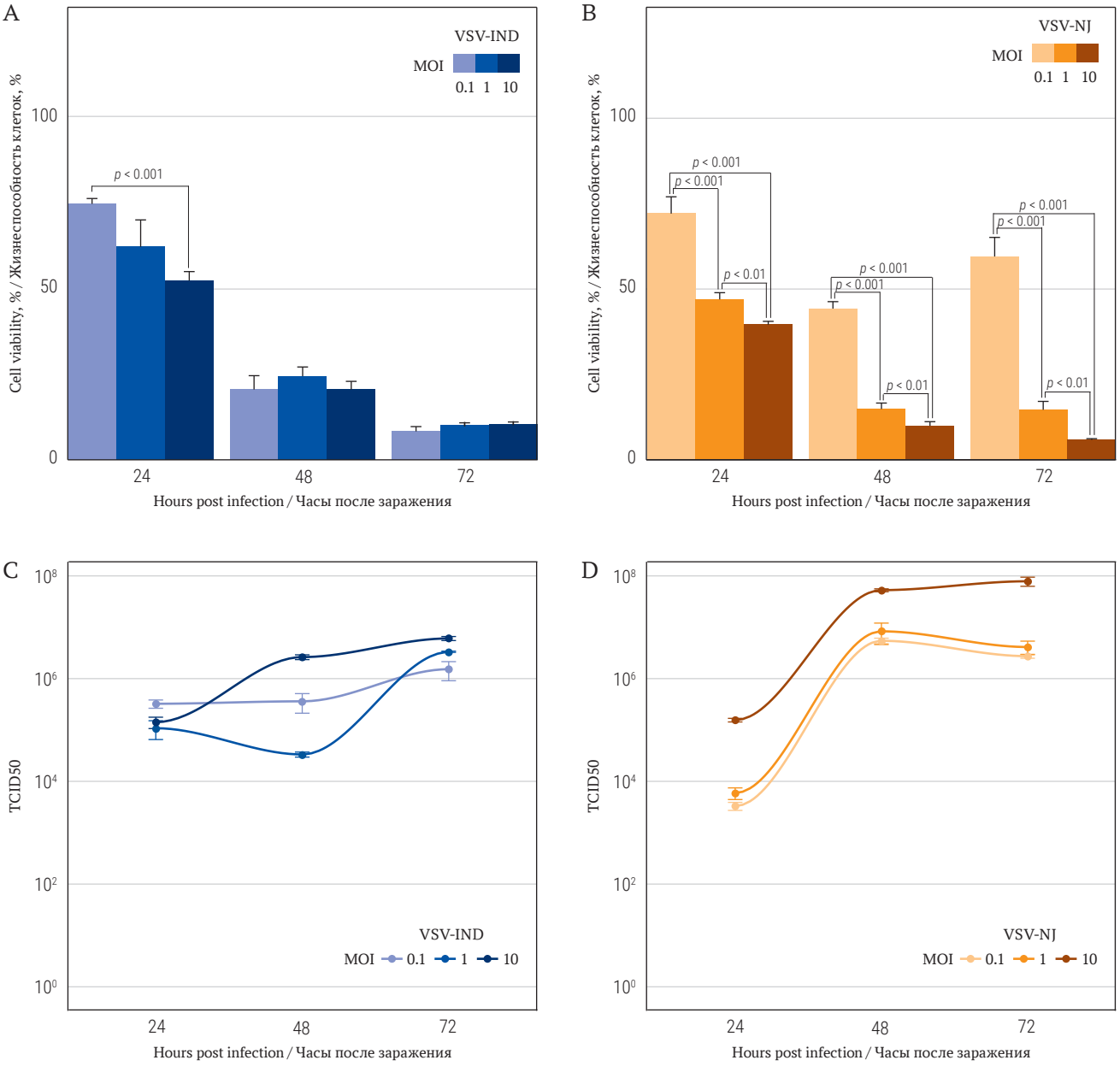

FIG. 2. B16F10 cells viability after VSV-IND and VSV-NJ infection.
РИС. 2. Оценка жизнеспособности клеток B16F10, инфицированных вирусами VSV-IND и VSV-NJ.
Note: the oncolytic effect is expressed as a proportion of living cells (%) after infection with VSV-IND (A) and VSV-NJ (B).
Quantitative analysis of RNA is expressed as titer (TCID50) VSV-IND (C) and VSV-NJ (D). As a reference standard for the quantitative determination of TCID50, VSV with a predetermined titer was used: 3.0×10^7 TCID50 and 6.23×10^6 TCID50 for VSV-IND and VSV-NJ, respectively.
VSV-IND – Vesicular stomatitis virus Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey; MOI – Multiplicity of infection; TCID50 – 50% Tissue Culture Infectious Dose.
Примечание: онколитический эффект представлен в % живых клеток после инфицирования VSV-IND (A) и VSV-NJ (B).
Количественный анализ РНК выражается в титре (TCID50) VSV-IND (C) и VSV-NJ (D). В качестве референс-стандарта для количественного определения TCID50 использовали VSV с известным титром: $3,0\times10^7$ TCID50 и $6,23\times10^6$ TCID50 для VSV-IND и VSV-NJ соответственно.
VSV-IND – Vesicular stomatitis virus Indiana, вирус везикулярного стоматита серотип Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey, вирус везикулярного стоматита серотип New Jersey; MOI – Multiplicity of infection, множественность заражения; TCID50 – 50% Tissue Culture Infectious Dose, 50%-ная инфицирующая доза вируса.

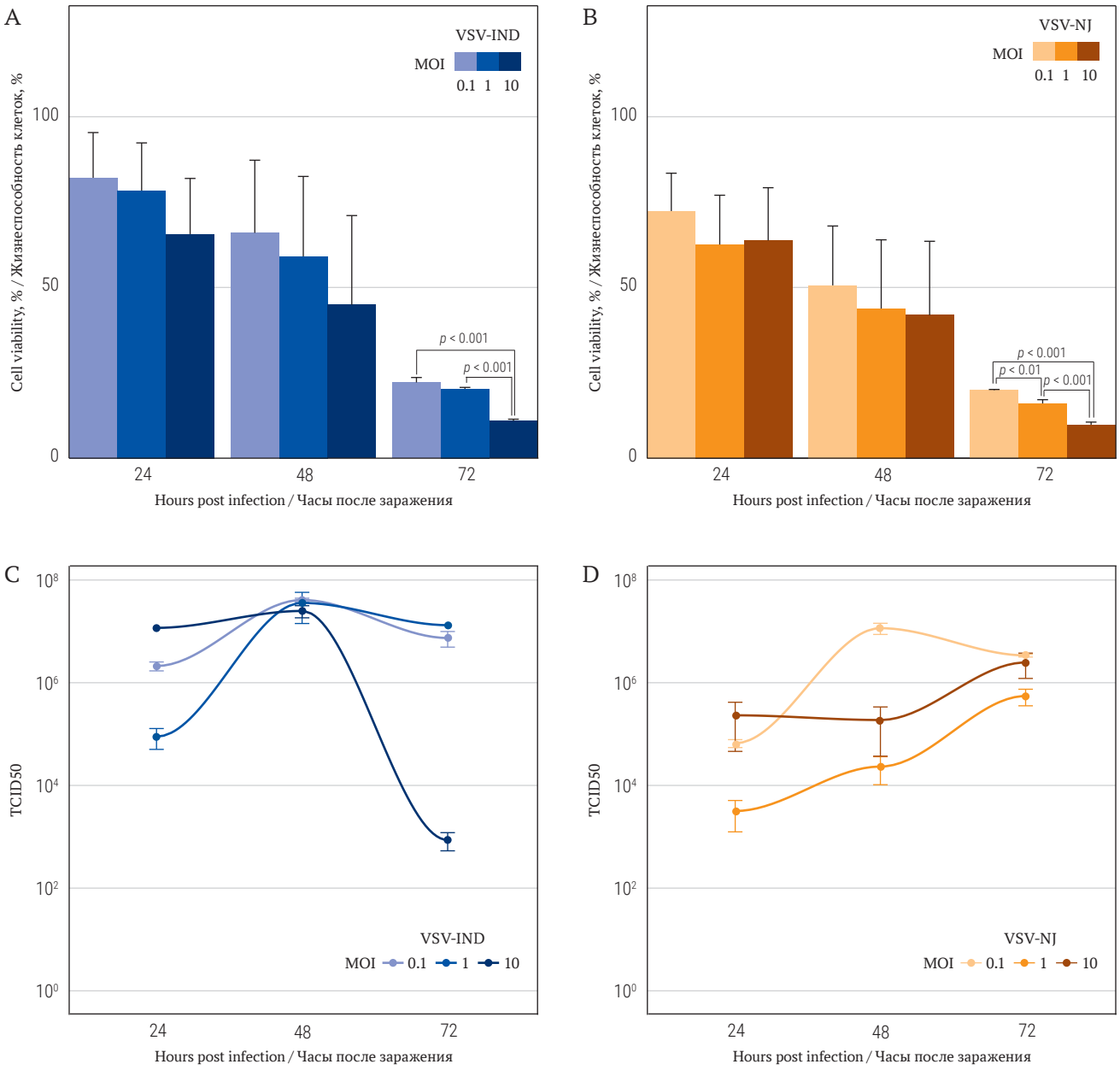

FIG. 3. HepG2 cells viability after VSV-IND and VSV-NJ infection.
РИС. 3. Оценка жизнеспособности клеток HepG2, инфицированных вирусами VSV-IND и VSV-NJ.

Note: the oncolytic effect is expressed as a proportion of living cells (%) after infection with VSV-IND (A) and VSV-NJ (B).
Quantitative analysis of RNA is expressed as titer (TCID50) VSV-IND (C) and VSV-NJ (D). As a reference standard for the quantitative determination of TCID50, VSV with a predetermined titer was used: 3.0×10^7 TCID50 and 6.23×10^6 TCID50 for VSV-IND and VSV-NJ, respectively.
VSV-IND – Vesicular stomatitis virus Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey; MOI – Multiplicity of infection; TCID50 – 50% Tissue Culture Infectious Dose.
Примечание: онколитический эффект представлен в % живых клеток после инфицирования VSV-IND (A) и VSV-NJ (B).
Количественный анализ РНК выражается в титре (TCID50) VSV-IND (C) и VSV-NJ (D). В качестве референс-стандарта для количественного определения TCID50 использовали VSV с известным титром: $3,0\times10^7$ TCID50 и $6,23\times10^6$ TCID50 для VSV-IND и VSV-NJ соответственно.
VSV-IND – Vesicular stomatitis virus Indiana, вирус везикулярного стоматита серотип Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey, вирус везикулярного стоматита серотип New Jersey; MOI – Multiplicity of infection, множественность заражения; TCID50 – 50% Tissue Culture Infectious Dose, 50%-ная инфицирующая доза вируса.

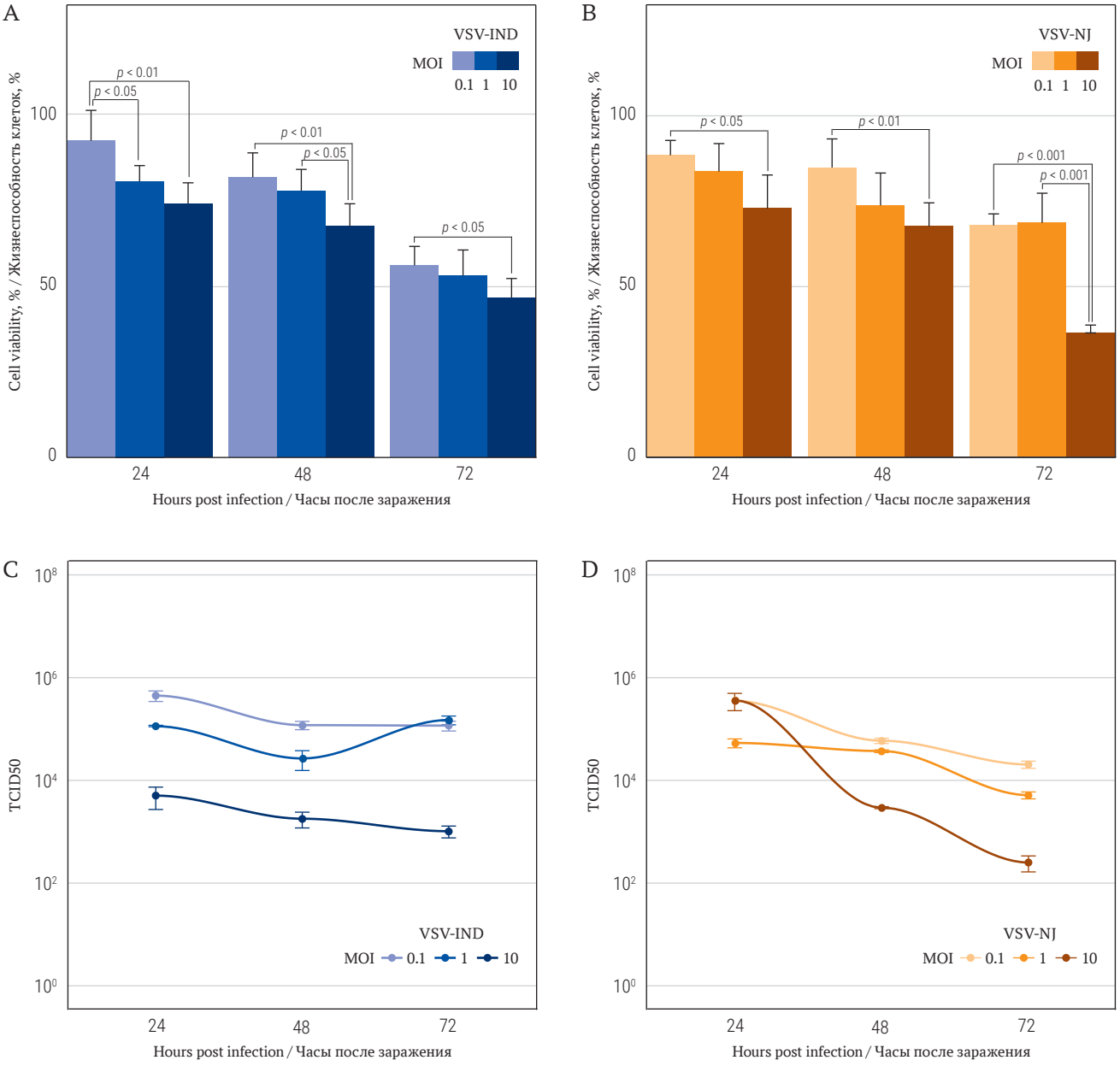

FIG. 4. MCF7 cells viability after VSV-IND and VSV-NJ infection.
РИС. 4. Оценка жизнеспособности клеток MCF7, инфицированных вирусами VSV-IND и VSV-NJ.
Note: the oncolytic effect is expressed as a proportion of living cells (%) after infection with VSV-IND (A) and VSV-NJ (B).
Quantitative analysis of RNA is expressed as titer (TCID50) VSV-IND (C) and VSV-NJ (D). As a reference standard for the quantitative determination of TCID50, VSV with a predetermined titer was used: 3.0×10^7 TCID50 and 6.23×10^6 TCID50 for VSV-IND and VSV-NJ, respectively.
VSV-IND – Vesicular stomatitis virus Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey; MOI – Multiplicity of infection; TCID50 – 50% Tissue Culture Infectious Dose.
Примечание: онколитический эффект выражается в % живых клеток после инфицирования VSV-IND (A) и VSV-NJ (B).
Количественный анализ РНК выражается в титре (TCID50) VSV-IND (C) и VSV-NJ (D). В качестве референс-стандарта для количественного определения TCID50 использовали VSV с известным титром: $3,0\times10^7$ TCID50 и $6,23\times10^6$ TCID50 для VSV-IND и VSV-NJ соответственно.
VSV-IND – Vesicular stomatitis virus Indiana, вирус везикулярного стоматита серотип Indiana; VSV-NJ – Vesicular stomatitis virus New Jersey, вирус везикулярного стоматита серотип New Jersey; MOI – Multiplicity of infection, множественность заражения; TCID50 – 50% Tissue Culture Infectious Dose, 50%-ная инфицирующая доза вируса.

hours). When exposed VSV-IND within 72 hours, the content of viable cells remained at least 40%. Accordingly, high IC50 values were observed (Table). The content of viral RNA in the MCF7 culture either remained virtually unchanged, as in VSV-IND (1 MOI), or decreased, and the decrease reached three orders of magnitude, as in VSV-NJ (10 MOI) (Figure 4C,D). There was no confirmed relationship between indicators of decreased cell viability and changes in the amount of viral RNA during infection with both serotypes.

DISCUSSION

VSV is an important subject of research that contributes to a comprehensive understanding of the interaction between viruses and cancer cells [10]. Due to the non-pathogenicity of VSV for humans and the ability to replicate in different cancer cells, the natural serotypes VSV-IND and VSV-NJ can serve as a source of new effective immunotherapy agents. VSV selectively induces cytolysis of numerous transformed human cell lines in vitro with all morphological characteristics of apoptotic cell death [27]. In addition, the VSV genome can accommodate the insertion of one or more functional genes [28–30] which means that the oncolytic potential can be significantly enhanced. In this work, we focused on comparing VSV-IND and VSV-NJ in terms of how efficient they were at destroying cells.

Based on the results of the MTT test, we assume that the mouse melanoma cell lines B16F10 and human HCC HepG2 are permissive to both serotypes of the VSV virus. Moreover, on the B16F10 cell line, the cytotoxicity of VSV-IND (72 hours after infection) reached more than 80%, regardless of the initial dose, and in the culture of HepG2 cells the same picture was observed for VSV-NJ. However, it was not possible to record 100% cell death in any cell line. According to the literature, the mouse melanoma cell line B16F10 may be partially resistant to VSV infection [15, 28]. The high cytotoxicity of VSV in HepG2 culture was reported in the work of BM Nagalo et al., 2020 [16], where the VSV glycoprotein G was modified with measles virus hemagglutinin.

In our hypothesis we assumed that: the presence of a direct statistically significant strong or moderate correlation between inhibition of cell viability (VSV cytotoxicity) and viral RNA accumulation may reflect death due to viral replication, while (2) high rates of cell death in the absence of RNA accumulation (and therefore virus replication) may indicate the cytotoxic effect of viral proteins. The first situation was observed in the B16F10 cell culture for both serotypes. On the contrary, in the HepG2 cell culture, no significant relationships were found between the decrease in cell viability and the accumulation of viral RNA. It can be assumed that the susceptibility of HepG2 cells to VSV-IND and VSV-NJ is largely due to the cytotoxic effect of their proteins rather than replication, which is consistent with the data from the literature [20].

Also, it should be noted that the human breast cancer line MCF7 demonstrated a survival rate of more than 50% in almost the entire range of VSV-IND and VSV-NJ concentrations and exposure times, except for the maximum ones (10 MOI and 72 hours). Previously, in the work of C. Rogers et al., 2017 [11], when studying the influence of matrix protein M of VSV to the highly oncogenic line MCF7, similar results were obtained. The authors were unable to achieve complete tumor regression *in vivo*, and they concluded that the cytolytic effect of VSV in breast cancer must be combined with more aggressive treatment [11]. In 2021, an attempt to use VSV in conjunction with immunotherapy with natural killer T-cells (NKT) turned out to be more successful [31]. At the same time, VSV monotherapy did not lead to complete regression of the tumor (as in our experiments), although it demonstrated the induction of cell death. The study was conducted on the 4T1 human adenocarcinoma line which does not allow us to compare the results with our experiment. However, it gives hope for success in the case of combination therapy for breast adenocarcinoma.

The content of viral RNA after 72 hours in the MCF7 cell culture in our study decreased for both VSV-IND and VSV-NJ. A statistically significant relationship between a decrease in cell viability and the accumulation of viral RNA was not detected during infection with both serotypes.

Limitations of the study

This study does not include genetically modified VSVs and is limited to naturally occurring (wild) virus serotypes. A greater variety of cancer cell lines can be used to test the oncolytic potential of viruses.

Directions for further research

Future studies may include assessing the effects of VSV-IND and VSV-NJ on a wider range of cancerous as well as non-cancerous cell lines, such as MCF10, fibroblasts, etc. It is also planned to determine the type of cell death resulting from exposure to VSV (necrosis, apoptosis, autophagy). Based on the results of a larger study, it is possible to determine the most universal and promising serotype as a platform for further genetic modifications of VSV in order to enhance the antitumor effect.

CONCLUSION

In our work, we showed that the susceptible cell line for the VSV-IND and VSV-NJ viruses turned out

to be B16F10 and HepG2, while the inhibition of the viability of MCF7 cells was only 63.8 and 53.4% for VSV-IND and VSV-NJ, respectively. The MCF7 line is moderately resistant to infection by VSV of both serotypes in terms of cell survival and viral RNA accumulation. The B16F10 and HepG2 lines turned out to be permissive when infected with VSV-IND and VSV-NJ at doses of 10, 1 and 0.1 MOI, despite the absence of total cell death. They reveal different dynamics of death and accumulation of viral RNA which can be used for the selection of agents for combination therapy of melanoma and liver cancer

AUTHOR CONTRIBUTIONS

Anastasia S. Isaeva and Natalya O. Porozova made equal significant contributions to research and data processing, statistical analysis, and preparation of the manuscript. Esther Idota: preparation and maintenance of cancer cell lines. Sofya I. Volodina: development of protocols for VSV serotypes titration and depositing viruses in the working collection. Alexander N. Lukashev study design, discussion and manuscript editing. Alexander S. Malogolovkin: study concept and methodology of the experiment, as well as writing the text of the article. All the authors approved the final version of the article.

ВКЛАД АВТОРОВ

А.С. Исаева и Н.О. Порозова внесли равный значительный вклад в проведение исследований и обработку данных, статистическую обработку результатов исследования и подготовку рукописи. Э. Идота внесла основной вклад в подготовку и поддержание раковых клеточных линий. С.И. Володина участвовала в разработке протоколов титрования серотипов VSV и депонирования вирусов в рабочую коллекцию. А.Н. Лукашев внес основной вклад в дизайн исследований, обсуждение и редактирование статьи. А.С. Малоголовкин внес основной вклад в разработку концепции идеи и методологии проведения эксперимента, а также написание текста статьи. Все авторы утвердили окончательную версию статьи.

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