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**RESEARCH ARTICLE**

# Knockdown of *FLT4*, *Nup98*, and *Nup205* cellular genes as a suppressor for the viral activity of Influenza A/WSN/33 (H1N1) in A549 cell culture

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***Abstract***

***Objectives.*** *To evaluate the effect of cellular genes FLT4, Nup98, and Nup205 on the reproduction of the influenza A virus in A549 human lung cancer cell line.*

***Methods.*** *The work was carried out using the equipment of the center for collective use of the I.I. Mechnikov Research Institute of Vaccines and Sera (Russia). The virus-containing fluid was collected within three days from the moment of transfection and infection and the intensity of viral reproduction was assessed by viral titration and hemagglutination reaction. The viral RNA concentration was determined by real-time reverse-transcription polymerase chain reaction (RT-PCR). To calculate statistically significant differences between groups, the nonparametric Mann–Whitney test was used.*

***Results.*** *In cells treated with small interfering RNAs (siRNAs) targeted at FLT4, Nup98, and Nup205 genes, a significant decrease in their expression and indicators of viral reproduction (virus titer, hemagglutinating activity, viral RNA concentration) was observed at a multiplicity of infection (MOI) = 0.1. Additionally, it was found that a decrease in the expression of target genes using siRNA does not lead to a significant decrease in cell survival. The viral titer in cells treated with siRNA FLT4.2, Nup98.1, and Nup205 on the first day was lower by an average of 1.0 lg, and on the second and third days, by 2.2–2.3 lg, compared to cells treated with nonspecific siRNA. During real-time RT-PCR, a significant decrease in the concentration of viral RNA was observed*

*with siRNA Nup98.1 (up to 190 times) and Nup205 (up to 30 times) on the first day, 26 and 29 times on the second day, and 6 and 30 times on the third day, respectively. For FLT4.2 siRNA, the number of viral RNA copies decreased by 23, 18, and 16 times on the first, second, and third days. Similar results were obtained when determining the hemagglutinating activity of the virus. The hemagglutinating activity on the third day most strongly decreased in cells treated with siRNA Nup205 and FLT4.2 (16 times). In cells treated with siRNA FLT4.1, Nup98.1, and Nup98.2, hemagglutinating activity decreased by 8 times.*

***Conclusions.*** *In the present study, three cellular genes (FLT4, Nup98, and Nup205) were identified—the decrease in the expression of which effectively suppresses viral reproduction—and the original siRNA sequences were obtained. The results obtained are important for creating therapeutic and prophylactic medication, whose action is based on the RNA interference mechanism.*

***Keywords:*** *influenza A virus, RNA interference, gene, messenger RNA, small interfering RNAs*

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**НАУЧНАЯ СТАТЬЯ**

# Нокдаун клеточных генов *FLT4*, *Nup98* и *Nup205* как супрессор вирусной активности гриппа A/WSN/33 (H1N1) в культуре клеток A549

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***Аннотация***

***Цели.*** *Оценка влияния подавления экспрессии клеточных генов FLT4, Nup98 и Nup205 на динамику репродукции вируса гриппа А в культуре легочных клеток человека А549.*
***Методы.*** *Работа выполнена с использованием оборудования центра коллективного пользования Научно-исследовательского института вакцин и сывороток им И.И. Мечникова (Россия). Вируссодержащую жидкость отбирали в течение трех дней с момента трансфекции и заражения и оценивали интенсивность вирусной репродукции методами титрования по цитопатическому действию и в реакции гемагглютинации. Концентрацию вирусной РНК определяли методом полимеразной цепной реакции (ПЦР) в реальном времени с обратной транскрипцией (ОТ-ПЦР-РВ). Для вычисления статистически значимых различий между группами использовали непараметрический критерий Манна–Уитни.*

***Результаты.*** *В клетках, обработанных малыми интерферирующими РНК (миРНК) к генам FLT4, Nup98 и Nup205, отмечалось достоверное подавление экспрессии целевых генов и показателей вирусной репродукции (титр вируса, гемагглютинирующая активность, концентрация вирусной РНК) при коэффициенте множественности заражения, равном 0.1. Дополнительно было установлено, что подавление экспрессии целевых генов с помощью миРНК не приводит к значительному снижению выживаемости клеток. Вирусный титр в клетках, обработанных миРНК FLT4.2, Nup98.1 и Nup205, на первые сутки был меньше в среднем на 1.0 lg, а на вторые и третьи – на 2.2–2.3 lg, по сравнению с клетками, обработанными неспецифической миРНК. При проведении ОТ-ПЦР-РВ отмечено достоверное уменьшение концентрации вирусной РНК с миРНК Nup98.1 (до 190 раз) и Nup205 (до 30 раз) на первые сутки, в 26 и в 29 раз на вторые и в 6 и 30 раз на третьи сутки, соответственно. Для миРНК FLT4.2 количество копий вирусной РНК уменьшилось в 23, 18 и 16 раз на первые, вторые и третьи сутки. Схожие результаты были получены при определении гемагглютинирующей активности вируса. Наиболее сильно, в 16 раз, гемагглютинирующая активность на третьи сутки снизилась в клетках, обработанных миРНК Nup205 и FLT4.2. В клетках, обработанных миРНК FLT4.1, Nup98.1 и Nup98.2, гемагглютинирующая активность уменьшилась в 8 раз.*

***Выводы.*** *В ходе исследования были выявлены три клеточных гена (FLT4, Nup98 и Nup205), подавление экспрессии которых позволяет эффективно уменьшить вирусную репродукцию, а также получены оригинальные последовательности миРНК. Полученные результаты имеют важное значение для создания терапевтических и профилактических препаратов, чье действие основано на механизме РНК-интерференции.*

***Ключевые слова:*** *вирус гриппа А, РНК-интерференция, ген, матричная РНК, малые интерферирующие РНК*

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## INTRODUCTION

Influenza infection is one of the most significant problems in global health today. According to the World Health Organization, up to 1 billion new cases of influenza are reported worldwide each year, with 3–5 million cases of severe illness and 0.5 million deaths [1]. Influenza viruses of the genus *Alphainfluenzavirus* (*Influenza A virus*, (HAV))—with high epidemiological significance and capable of causing pandemics—are of particular clinical significance [2]. In addition to causing respiratory system failure, influenza can cause complications in the cardiovascular system, central nervous system, and urinary system [3–7]. The risk of developing bacterial and fungal complications post influenza is no exception [8–10].

Despite the currently available specific anti-influenza drugs, their use is often unjustified since new viral strains resistant to these drugs are detected every year [11, 12]. The problem with the use of influenza vaccines is acute, as it is necessary to create vaccines adapted to new strains of the influenza virus every year, and the development of a universal vaccine is far from complete [13–15]. In addition, influenza vaccination as prophylaxis is challenging for people who are allergic to chicken eggs [16].

To date, several etiotropic, symptomatic, and specific drugs are used to treat influenza. Currently, many influenza virus strains have 95% resistance to derivatives of the adamantane series [17]. Certain circulating strains are also known to be resistant to fusion inhibitors (*umifenovir*) [18]. In different epidemic seasons, the sensitivity of influenza A and B virus strains varied dramatically in relation to neuraminidase inhibitors. In 2008–2009, all circulating influenza A (H1N1) viruses were resistant to oseltamivir, but in 2018 they were fully susceptible to oseltamivir, peramivir, and zanamivir [19–21]. Thus, despite the widespread knowledge of biological function, structural organization, and pathogenesis of the influenza virus, no effective means of therapy and prevention exist as of yet [11, 12].

RNA interference (RNAi) is a sequential regulatory reaction in eukaryotic cells caused by an exogenous double-stranded RNA molecule [22]. A. Fire and C. Mell discovered RNAi in 1998 in the nematode *Caenorhabditis elegans*. They put forward several provisions on the properties of RNAi: mRNA degrades with it; the efficiency of double-stranded RNA (dsRNA) fragment, which determines the recognition of the complementary region of the target messenger RNA (mRNA), is higher than that of single-stranded RNA (ssRNA); a short dsRNA fragment is required to suppress gene expression [23].

The mechanism of RNAi is to cleave exogenous double-stranded RNA into small sequences ranging in size from 21 to 25 base pairs; small interfering RNAs (siRNAs). The size of the resulting siRNAs is small based on the fact that larger siRNAs increase the chance of interferon production. After the formation of siRNA, it binds to the RISC (RNA-induced silencing complex) complex, which consists of three proteins: *AGO2*, *PACT*, and *TRBP*. The resulting complex recognizes and cleaves the target mRNA [24–26].

Several antiviral drugs based on the RNAi mechanism are currently known and are at different stages of clinical trials, namely: *Miravirsen*, hepatitis C (*Santaris Pharma*); *ALN-RSV01*, respiratory syncytial viral infection (*Alnylam Pharmaceuticals*); and *pHIV7-shITAR-CCR5RZ*, HIV infection (*City of Hope Medical Center*) [27, 28]. Patisiran and Givosiran (*Alnylam Pharmaceuticals*) were also approved for clinical use to treat amyloid polyneuropathy and acute hepatic porphyria, respectively[1] [29].

A critical factor that compromises the efficiency of RNA interference might be the development of resistance to the siRNAs directed against viral genes [30]. To overcome the drug resistance ability of the influenza virus, the search for novel antiviral siRNAs with antiviral activity and targeted to host cell components is required for the replication of the virus.

This study has shown that using siRNAs directed to the cellular *FLT4*, *Nup98*, and *Nup205* genes can inhibit the reproduction of the influenza A virus in the A549 lung cancer cell line. Here the cellular gene *FLT4* plays a vital role in the process of endocytosis in the virus. While *Nup98* and *Nup205* encode proteins of the nuclear pore complex that are involved in the import and export of viral RNA segments into the nuclear cavity.

[1] Multi-Discipline Review. Center for Drug Evaluation and Research. Appl. No. 212194Orig1s000. 167 p. URL: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf (Accessed August 24, 2021).

## MATERIALS AND METHODS

### Selecting target genes for suppressing viral reproduction

In this study, the criteria used to select genes that encode the expression of cellular factors necessary for viral reproduction are as follows: (i) genes reported as a potentially successful target for siRNA in publications related to siRNA screening; (ii) genes reported as effective in early independent studies; and (iii) genes showing low cytopathic effect from the temporary suppression their expression [26–28].

### siRNA

The siRNA was selected from the web-based software, siDirect 2.0[2]. All oligonucleotides were synthesized by *Syntol* (Russia). Oligoribonucleotides were diluted with water to a concentration of 100 pmol/μL. Then, complementary oligonucleotides were mixed, incubated in a thermostat at 60°C for 1 min, and then cooled to room temperature. Prepared RNA duplexes were stored at –80°C. All work with finished duplexes was carried out using a cold tripod. The sequences of the siRNAs are presented in Table 1. As a nonspecific control, we used siRNA *L2*, specific to the firefly luciferase gene and not affecting the life cycle of A549 cells.

### Evaluation of the suppression of cellular gene expression

The expression level of the target genes was determined after siRNA transfection. The cells were treated with a lysis solution, and then cellular RNA was isolated using the MagnoSorb kit (*Interlabservice*, Russia), 24 h post-transfection. OT-1 reagent kit (*Syntol*, Russia) was used to set up the reverse-transcription reaction. Changes in gene expression dynamics were monitored using quantitative real-time PCR with a set of primers for the *FLT4*, *Nup98*, *Nup205*, and *GAPDH* genes [32]. To assess the effect of siRNAs on target genes, the relative expression level of the *FLT4*, *Nup98*, and *Nup205* genes was calculated according to the standard $2^{-\Delta\Delta CT}$ method.[3] For each siRNA, primers were synthesized according to the gene regions affected by siRNA. Primers were selected using Integrated DNA Technologies website[4] and synthesized by *Syntol* (Table 2).

### Virus

The influenza virus used in this study is A/WSN/33 (**H1N1**) (St. Jude Children's Research Hospital, USA). Cultivation and determination of the virus titer were carried out on the culture of canine kidney cells (Madin-Darby Canine Kidney (MDCK)).

[2] http://sidirect2.rnai.jp/ (Accessed February 02, 2021).

[3] Bradburn S. How to Perform the Delta-Delta Ct Method. URL: https://toptipbio.com/delta-delta-ct-pcr/ (Accessed August 27, 2021).

[4] https://eu.idtdna.com/ (Accessed March 02, 2021).

**Table 1.** siRNA sequences

| siRNA | Sequence |
|---|---|
| *FLT4.1* | AAUGACAUCUGAAUCUCAG**dGdG**<br>CUGAGAUUCAGAUGUCAUU**dTdA** |
| *FLT4.2* | UGAAGUUCUGUUGAAAAAG**dAdC**<br>CUUUUUCAACAGAACUUCA**dCdA** |
| *Nup98.1* | AGUCUUUGUUUCAGAAAGC**dGdC**<br>GCUUUCUGAAACAAAGACU**dCdA** |
| *Nup98.2* | UCCAAAUGUUGAAGUUGUG**dCdC**<br>CACAACUUCAACAUUUGGA**dCdA** |
| *Nup205* | UCAAAAUCUUAUCAAGAAG**dGdT**<br>CUUCUUGAUAAGAUUUUGA**dAdG** |
| *L2* (nonspecific siRNA) | UUUCCGUCAUCGUCUUUCC**dTdT**<br>GGAAAGACGAUGACGGAAA**dTdT** |

### Cell culture

MDCK cells (Institut Pasteur, France) and A549 human lung adenocarcinoma cells were used (ATCC® CCL-185, USA) in this study. MDCK cells were grown in minimum essential medium (MEM) (*PanEco*, Russia), supplemented with 5% fetal bovine serum (*Gibco*) (*ThermoFisher Scientific*, USA), 40 μg/mL gentamicin (*PanEco*), and 300 μg/mL L-glutamine (*PanEco*) at 37°C in a $CO_2$-incubator. A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (*PanEco*), supplemented with 5% fetal bovine serum (*Gibco*), 40 μg/mL gentamicin (*PanEco*), and 300 μg/mL L-glutamine (*PanEco*) at 37°C in a $CO_2$-incubator.

### MTT assay

The cell viability of A549 cells treated with siRNA was assessed with the MTT (methylthiazolyltetrazolium bromide) assay. On days 1, 2, 3 after transfection, 20 μL of MTT solution, 5 mg/mL (*PanEco*), was added to the wells containing cells in a 96-well plate and incubated at 37°C in a 5% $CO_2$ incubator for 2 h. Further, the supernatant and 100 μL of dimethyl sulfoxide (*Sigma-Aldrich*, cat. # D4540-1L) was added to each well. The optical density values were determined at 530 nm using a microplate reader Varioscan (*Thermo Fisher Scientific*, USA), with the background values set at 620 nm.

### Transfection of siRNA cells with subsequent infection

For siRNA transfection, A549 cells were plated on 12-well plates at a density of $1 \times 10^5$ cells/mL. After achieving 80% confluence, the cells were washed with phosphate-buffered saline and serum-free Opti-MEM medium (*Thermo Fisher Scientific*, USA). Then a mixture of 24 μL Lipofectamine 2000 (*Thermo Fisher Scientific*) and 600 μL Opti-MEM (*Thermo Fisher Scientific*) was added to a solution of siRNA in Opti-MEM medium and incubated at room temperature for 20 min. The siRNA concentration required for gene knockdown was 40 pmol/μL per well. After incubation, the complexes were added to the wells. siRNA *L2* was used as a nonspecific control. The cells were then incubated at 37°C in a $CO_2$ incubator. Four hours later, the culture medium was removed from all wells, except for the negative control, and 1 mL of viral liquid consisting of DMEM, 0.001% chymotrypsin inhibitor (Tosyl phenylalanyl chloromethyl ketone (TPCK)) (*Sigma-Aldrich*, Germany), and 40 μg/mL gentamicin was added with a MOI of 0.1. After that, the cells were again placed in the $CO_2$ incubator. Over the next three days, supernatant samples were taken for the subsequent staging of the hemagglutination, titration, and Reverse Transcription Polymerase Chain Reaction (RT-PCR).

### Detection of viral RNA

Viral RNA (vRNA) was isolated from the selected supernatants using the High Pure RNA Isolation Kit (*Roche*, Germany). OT-1 reagent kit (*Syntol*, Russia) was used to set up the reverse-transcription reaction. The concentration of viral RNA in the culture was measured using quantitative real-time RT-PCR with a set of primers and probes for the hemagglutination assay (HA) M-gene [33]. For real-time PCR, a reagent kit containing EVA Green dye, a reference ROX dye (*Syntol*), and a 2.5-fold reaction mixture (*Syntol*) were used. The working concentration of primers and probes was 10 and 5 pmol/μL, respectively. The real-time PCR reaction was carried out in a DT-96 thermal

cycler (*DNA-Technology*, Russia). The temperature-time regime for real-time PCR was as follows: 95°C–5 min (1 cycle); 62°C–40 s; 95°C–15 s (40 cycles). Table 2 shows primers and probes synthesized by *Syntol*.

**Determination of HAV hemagglutinating titer**

Saline (50 μL) was added to each well of a 96-well round-bottom plate. Then, 50 μL of samples were added to the wells, and subsequent 2-fold dilutions were made. After that, 50 μL of 0.5% erythrocyte mixture was added to the wells and left at room temperature for 40 min. The viral titer was expressed in agglutinating units.[5]

**Virus titration at the endpoint of the cytopathic effect**

The viral titer was determined using the endpoint assay to assess the cytopathic effect in the MDCK cells. MDCK cells were seeded in 96-well plates at a density of ($1 \times 10^4$ cells/mL). After 2 days, the nutrient medium was removed from the wells; 10-fold serial dilutions of viral samples were added in a support medium without trypsin and incubated for 4 days in a $CO_2$ incubator at 37°C. On the fourth day, the titration results were visually recorded under a microscope for the presence of a specific cytopathic effect for the influenza virus (change, deformation, detachment of dead cells from the bottom of the well). The viral titer was calculated according to the method described in [34] and was expressed as the logarithm of tissue cytotoxic doses—$TCD_{50/mL}$.

**Statistical data processing**

The statistical significance of the results obtained was assessed using the Mann-Whitney test. The difference was considered significant if $0.01 \leq p \leq 0.05$. Reliability indicators were calculated using Psychol-ok[6].

**Table 2.** Primers for real-time RT-PCR for the IAV M-gene

| Primer | Sequence |
|---|---|
| *FLT4.1F* | AAUGACAUCUGAAUCUCAGdGdG |
| *FLT4.1R* | CUGAGAUUCAGAUGUCAUUdTdA |
| *FLT4.2F* | UGAAGUUCUGUUGAAAAAGdAdC |
| *FLT4.2R* | CUUUUUCAACAGAACUUCAdCdA |
| *Nup98.1F* | UGAGUAUGUUAGACUAUUGdAdT |
| *Nup98.1R* | CAAUAGUCUAACAUACUCAdCdC |
| *Nup98.2F* | AUUAAGGUUCUUCAAAACCdAdA |
| *Nup98.2R* | GGUUUUGAAGAACCUUAAUdAdA |
| *Nup205F* | UUAUUCACAUCAAUCUGUGdAdC |
| *Nup205R* | CACAGAUUGAUGUGAAUAAdTdG |
| *IAV M F*: | GGAATGGCTAAAGACAAGACCAAT |
| *IAV M R*: | GGGCATTTTGGACAAAGCGTCTAC |
| *IAV M Pr*: *FAM* | AGTCCTCGCTCACTGGGCACGGTG-BHQ1 |
| *GAPDH F* | AGCCACATCGCTCAGACAC |
| *GAPDH R* | GCCCAATACG ACCAAATCC |

[5] MU 3.3.2.1758–03 Methods for determining the quality indicators of immunobiological drugs for the prevention and diagnosis of influenza. https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=4727 (Accessed August 27,2021).
[6] https://www.psychol-ok.ru/statistics/mann-whitney/ (Accessed August 05, 2021).

## RESULTS

### Justification for the choice of siRNA targets

Three target genes were selected for siRNA experiments. All three genes actively interact with the influenza virus at several stages of its reproduction. The *FLT4* gene encodes the Epidermal Growth Factor (*EGF*) receptor protein of the tyrosine kinase receptor. According to Eierhoff, the *EGF* protein is actively involved in the process of viral endocytosis [35]. Proteins *Nup98* and *Nup205* (encoded by genes of the same name) import and export viral mRNA from nucleoplasm [36, 37].

We tested the ability of the synthesized siRNAs to suppress the expression of their target genes. Compared to cells treated with nonspecific siRNA *L2*, gene expression decreased by more than 80% on the first day for each of the five siRNAs. Figure 1 shows the efficiency of mRNA knockdown in A549 cells. Evaluation of the suppression of gene expression was carried out using the $2^{-\Delta\Delta CT}$ method.[7]

### Influence of siRNA on the survival of transfected cells

The survival rate of A549 cells transfected with siRNA was measured within three days (Table 3). The survival threshold was set at 70%, according to a similar study [26]. After 24 h, the viability of cells treated with siRNA remained practically unchanged. On the second day, the survival rate of cells treated with all siRNAs, except for *Nup205* and siRNA *L2*, decreased by 14–21%. The survival rate of untransfected cells was taken as 100%. All values were normalized to the mean optical density of untransfected cells at each time interval following transfection. Treatment of cells with siRNA did not decrease cell survival compared with the negative control.

### Influence of siRNA on hemagglutinating activity

Table 4 shows the changes in the hemagglutinating titer of the influenza virus on day 3 in the hemagglutination assay (HA). The hemagglutinating activity in cells treated with siRNAs *Nup205* and *FLT4.2* decreased 16 times compared to 8 times in cells treated with siRNAs *FLT4.1*, *Nup98.1*, and *Nup98.2*.

### Influence of siRNA on the titer of the virus

The next step was to determine whether the change in the infectious titer of the virus was due to the siRNA's effect on the target genes. Within three days after transfection, the supernatant was removed and then titrated on a monolayer of MDCK cells in 96-well plates. It was found that the use of all siRNAs at MOI = 0.1 led to a significant decrease in viral reproduction compared to siRNA *L2*. As seen in Fig. 1, virus titers increased

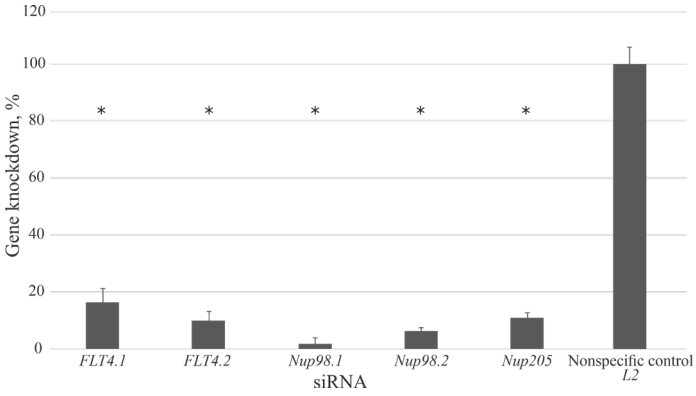


**Fig. 1.** Influence of siRNA on the expression of genes *FLT4*, *Nup98*, and *Nup205*.

with time in nontransfected cell culture, reaching peak values at 48 and 72 hours. The same was observed in cells transfected with nonspecific siRNA *L2*. When the *FLT4* gene expression was suppressed using *FLT4.1* siRNA, the viral titer decreased by about 0.9–1.0 lg $TCD_{50/mL}$ compared to the control on the first, second, and third day. With siRNA *FLT4.2*, there was a decrease by 1.0 lg $TCD_{50/mL}$ on the first day; however, there was a decrease by 2.2 lg $TCD_{50/mL}$ on the second and third day, compared to the control groups. Upon transfection of siRNA to the *Nup205* gene, the viral titer decreased by 1.0 lg $TCD_{50/mL}$ on the 1st day and by 2.3 lg $TCD_{50/mL}$ on the next day relative to the control and nonspecific control. In cells treated with *Nup98.1* siRNA, a significant decrease in the virus titer was observed (2.3 lg $TCD_{50/mL}$) on the 2nd day compared to the controls, while for *Nup98.2*, it was 2.2 lg $TCD_{50/mL}$ on the 3rd day, compared to the controls. The dynamics of changes in the viral titer are shown in Fig. 2.

### Influence of siRNA on the concentration of viral RNA

The effect of siRNA on the concentration of viral RNA is shown in Fig. 3. On the first day, a decrease in the concentration of viral RNA was observed with *Nup98.1* siRNA (up to 190 times) and *Nup205* siRNA (up to 30 times) during real-time RT-PCR. A 29-fold decrease in the vRNA concentration for *Nup205* and 26-fold for *Nup98.1* was noted on the second day. While on the third day, the vRNA concentration decreased by 6 and 30 times for *Nup98.1* siRNA and *Nup205* siRNA, respectively. For *FLT4.2* siRNA, the viral RNA concentration decreased by 23, 18, and 16 times on the first, second, and third day, respectively. In contrast, there was no significant decrease in the concentration of viral RNA using *FLT4.1* siRNA on the first, second, and third days.

[7] Bradburn S. How to Perform the Delta-Delta Ct Method. URL: https://toptipbio.com/delta-delta-ct-pcr/ (Accessed August 27, 2021).

**Table 3.** Cell survival after siRNA transfection in percentage, %

| siRNA | 1st day | 2nd day | 3rd day |
|---|---|---|---|
| *FLT4.1* | 96 | 81 | 74 |
| *FLT4.2* | 94 | 80 | 81 |
| *Nup98.1* | 100 | 79 | 79 |
| *Nup98.2* | 97 | 86 | 87 |
| *Nup205* | 94 | 95 | 94 |
| *L2* | 94 | 99 | 99 |
| *K*-(untranslated) | 100 | 100 | 100 |

**Table 4.** Viral reproduction on the 3rd day according to HA data

| siRNA | Viral reproduction to HA ($log_2$) |
|---|---|
| | A/WSN/33 (MOI = 0.1) |
| *FLT4.1* | 1:8 |
| *FLT4.2* | 1:4 |
| *Nup98.1* | 1:8 |
| *Nup98.2* | 1:8 |
| *Nup205* | 1:4 |
| *K*-(*L2*) | 1:64 |
| *K-IAV* | 1:64 |

## DISCUSSION

Influenza is an acute infectious respiratory disease caused by viruses of the *Orthomyxoviridae* family. Diseases caused by the influenza virus are one of the most pressing global public health problems today. The search for new anti-influenza medication is relevant because the influenza virus rapidly develops resistance to known specific anti-influenza drugs [38].

In this work, we performed a series of cell culture experiments to assess the anti-influenza activity of small interfering RNAs directed at *FLT4*, *Nup98*, and *Nup205* genes. A pronounced antiviral activity of siRNAs directed to the mRNA of these genes was observed, and consistent data were obtained on the correlation between the expression of cellular genes and viral reproduction, assessed by different methods (virus titration by cytopathic effect, real-time RT-PCR, HA).

An important factor for the successful use of siRNA is that the knockdown of the target gene should not affect the vital activity of cells. siRNAs targeting the genes *FLT4*, *Nup98*, and *Nup205* did not decrease cell viability below the threshold level of 70%, by analogy with the paper [26].

The use of siRNAs to suppress the expression of cellular genes to reduce viral reproduction has an advantage over siRNAs directed to the whole viral genome. This is due to the fact that influenza viruses have a higher tendency for mutational variability that often leads to substitutions of nucleotide sequences in their genome [39]. This can cause siRNA to be ineffective

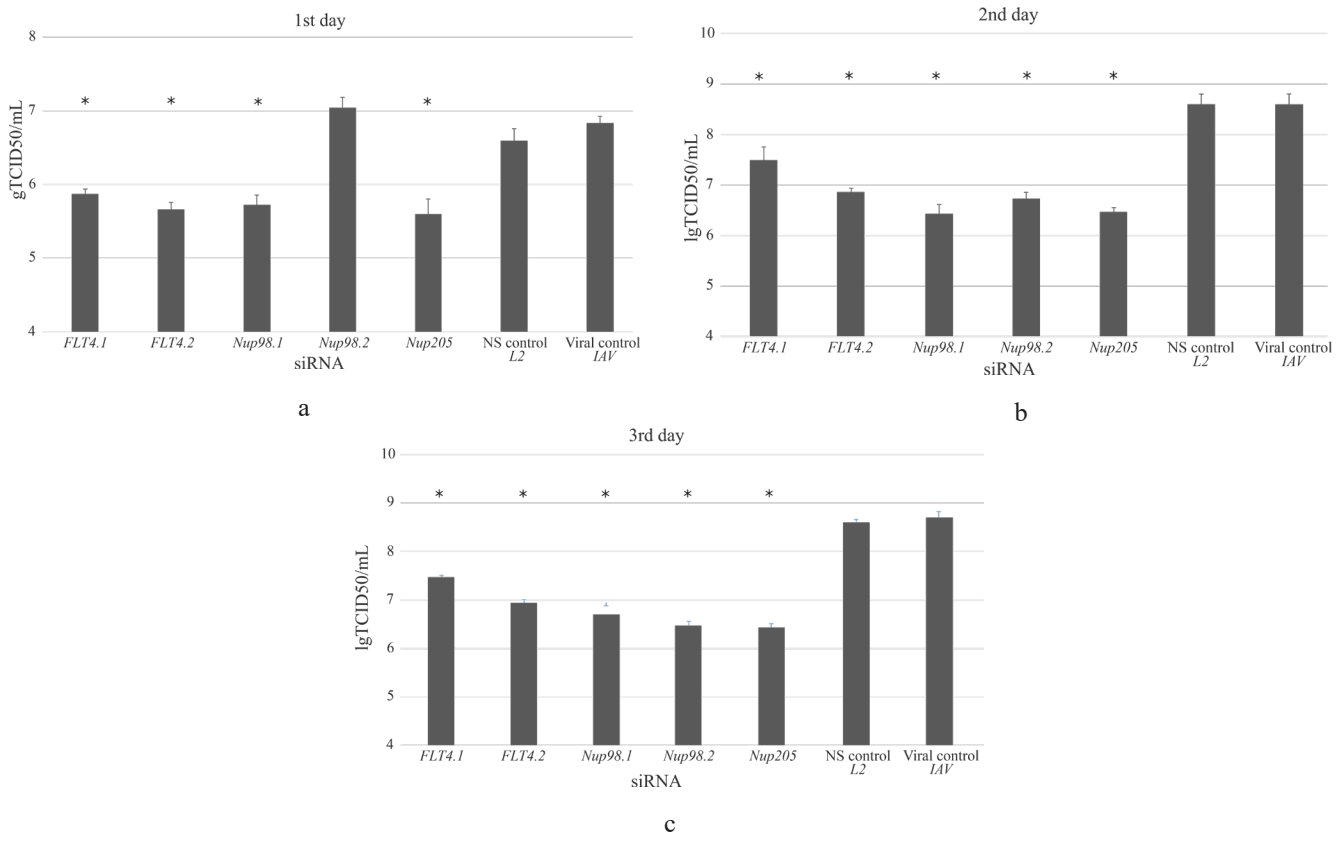


**Fig. 2.** Effect of siRNAs directed to the *FLT4*, *Nup98*, and *Nup205* genes on the reproduction of the influenza virus (MOI = 0.1). (a) 1st day post infection (p.i.), (b) 2nd day p.i., and (c) 3rd day p.i.

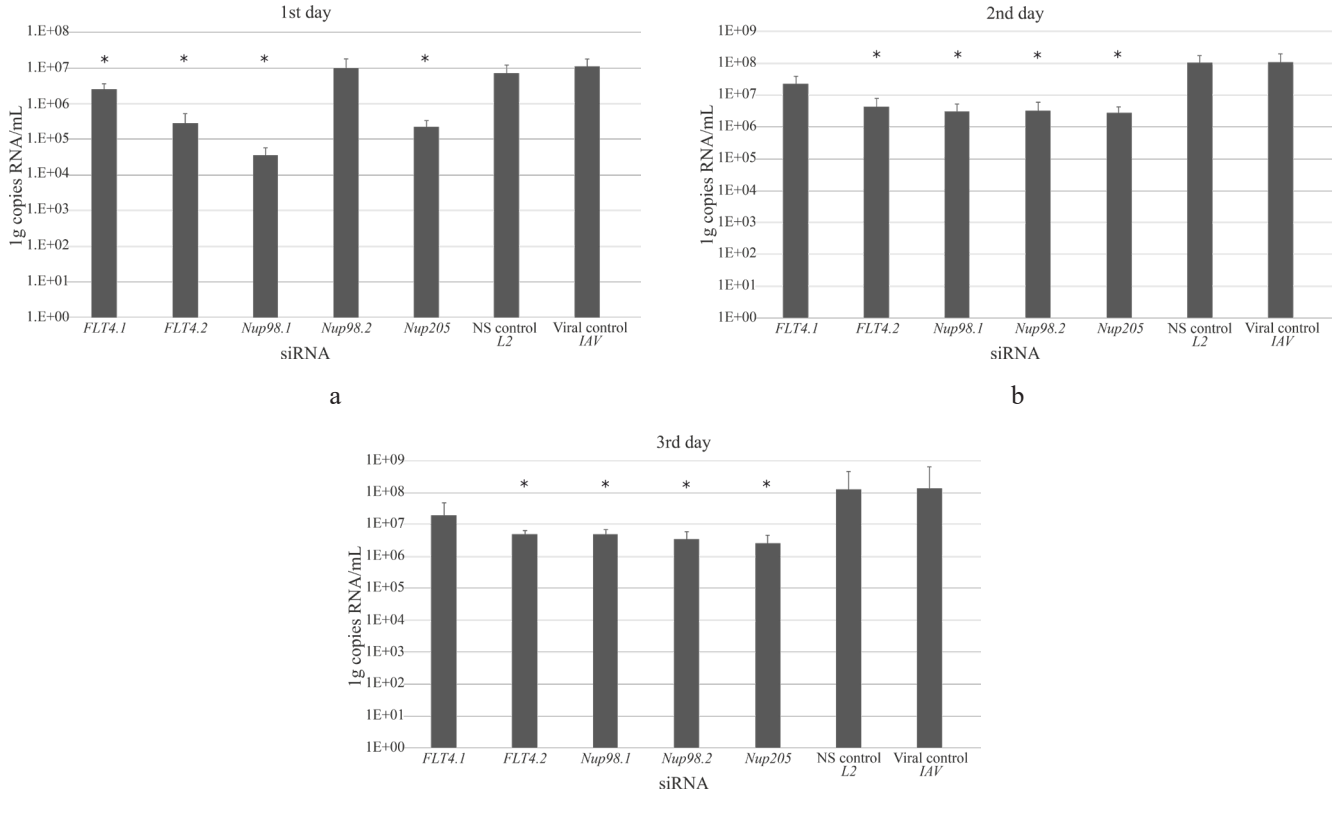


**Fig. 3.** The effect of siRNA on the concentration of viral RNA. (a) 1st day post infection (p.i.), (b) 2nd day p.i., and (c) 3rd day p.i.

toward the virus since even a single substitution in the target RNA sequence can completely neutralize the action of siRNA [43]. For example, the ability to elude the action of siRNA was experimentally demonstrated in a model of HIV-1 infection, where nucleotide substitutions occurred in the *tat*, *nef*, *int*, and *att* genes [41]. In view of this, A. Karlas and M. Lesch suggest that using siRNAs directed at the cellular genome is more justified since the possibility of an alternate viral reproduction pathway is very low [30]. The promise of this approach—based on the suppression of the activity of cellular genes necessary for the reproduction of the influenza virus—has been shown in several independent studies [26, 30, 42, 43].

## CONCLUSIONS

Currently, there remains a need to create highly effective drugs to treat influenza and its complications. The present study shows that siRNAs directed to cellular genes that play essential roles in viral endocytosis and nuclear import and export of vRNA significantly reduce the reproduction of the influenza virus *in vitro*. These data confirm that the investigated human genes *FLT4*, *Nup98*, and *Nup205* are promising targets for developing anti-influenza drugs. The findings of this study give hope that siRNA medication will be implemented in the future.

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### *Authors' contributions*

***E.A. Pashkov, E.R. Korchevaya, A.A. Rtishchev, and B.S. Cherepovich*** – conducting the experiments;

***E.A. Pashkov, E.P. Bystritskaya, and Yu.E. Dronina*** – writing the text of the article and the analysis of the obtained results;

***E.B. Fayzuloev, A.V. Poddubikov, and A.V. Sidorov*** – scientific editing;

***A.S. Bykov, O.A. Svitich, and V.V. Zverev*** – idea of the study and general management.

*The authors declare no obvious and potential conflicts of interest related to the publication of this article.*

## REFERENCES

1. Hussain M., Galvin H.D., Haw T.Y., Nutsford A.N., Husain M. Drug resistance in influenza A virus: the epidemiology and management. *Infect. Drug Resist.* 2017 Apr 20;10:121–134. https://doi.org/10.2147/IDR.S105473

2. Peasah S.K., Azziz-Baumgartner E., Breese J, Meltzer M.I., Widdowson MA. Influenza cost and cost-effectiveness studies globally – a review. *Vaccine*. 2013;31(46):5339–5348. https://doi.org/10.1016/j.vaccine.2013.09.013

3. Rezkalla S.H., Kloner R.A. Influenza-related viral myocarditis. *WMJ*. 2010;109(4):209–213. PMID: 20945722. URL: https://wmjonline.org/wp-content/uploads/2010/109/4/209.pdf

4. Nguyen J.L., Yang W., Ito K., Matte T.D., Shaman J., Kinney P.L. Seasonal Influenza Infections and Cardiovascular Disease Mortality. *JAMA Cardiol*. 2016;1(3):274–281. PMID: 27438105; PMCID: PMC5158013 https://doi.org/10.1001/jamacardio.2016.0433

5. Ekstrand J.J. Neurologic complications of influenza. *Semin. Pediatr. Neurol*. 2012;19(3):96–100. https://doi.org/10.1016/j.spen.2012.02.004

6. Edet A., Ku K., Guzman I., Dargham H.A. Acute Influenza Encephalitis/Encephalopathy Associated with Influenza A in an Incompetent Adult. *Case Rep. Crit. Care*. 2020;2020:6616805. https://doi.org/10.1155/2020/6616805

7. Err H., Wiwanitkit V. Emerging H6N1 influenza infection: renal problem to be studied. *Ren. Fail*. 2014;36(4):662. https://doi.org/10.3109/0886022X.2014.883934

8. Leneva I.A., Egorov A.Yu., Falynskova I.N., Makhmudova N.R., Kartashova N.P., Glubokova E.A., Vartanova N.O., Poddubikov A.V. Induction of secondary bacterial pneumonia in mice infected with pandemic and laboratory strains of the H1N1 influenza virus. *Zhurnal mikrobiologii, epidemiologii i immunobiologii = Journal of Microbiology, Epidemiology and Immunobiology*. 2019;(1):68–74 (in Russ.). https://doi.org/10.36233/0372-9311-2019-1-68-74

9. Metersky M.L., Masterton R.G., Lode H., File T.M. Jr., Babinchak T. Epidemiology, microbiology, and treatment considerations for bacterial pneumonia complicating influenza. *Int. J. Infect. Dis*. 2012;16(5):e321–31. https://doi.org/10.1016/j.ijid.2012.01.003

10. Vanderbeke L., Spriet I., Breynaert C., Rijnders B.J.A., Verweij P.E., Wauters J. Invasive pulmonary aspergillosis complicating severe influenza: epidemiology, diagnosis and treatment. *Curr. Opin. Infect. Dis*. 2018;31(6):471–480. https://doi.org/10.1097/QCO.0000000000000504

11. Van der Vries E., Schutten M., Fraaij P., Boucher C., Osterhaus A. Influenza virus resistance to antiviral therapy. Adv. Pharmacol. 2013;67:217–246. https://doi.org/10.1016/B978-0-12-405880-4.00006-8

12. Han J., Perez J., Schafer A., Cheng H., Peet N., Rong L., Manicassamy B. Influenza Virus: Small Molecule Therapeutics and Mechanisms of Antiviral Resistance. *Curr. Med. Chem*. 2018;25(38):5115–5127. https://doi.org/10.2174/0929867324666170920165926

13. Looi Q.H., Foo J.B., Lim M.T., Le C.F., Show P.L. How far have we reached in development of effective influenza vaccine? *Int. Rev. Immunol*. 2018;37(5):266–276. https://doi.org/10.1080/08830185.2018.1500570

14. Pleguezuelos O., James E., Fernandez A., Lopes V., Rosas L.A., Cervantes-Medina A., Cleath J., Edwards K., Neitzey D., Gu W., Hunsberger S., Taubenberger J.K., Stoloff G., Memoli M.J. Efficacy of FLU-v, a broad-spectrum influenza vaccine, in a randomized phase IIb human influenza challenge study. *NPJ Vaccines*. 2020;5(1):22. https://doi.org/10.1038/s41541-020-0174-9

15. Wang F., Chen G., Zhao Y. Biomimetic nanoparticles as universal influenza vaccine. *Smart Mater. Med.* 2020;1:21–23. https://doi.org/10.1016/j.smaim.2020.03.001

16. Smith M. Vaccine safety: medical contraindications, myths, and risk communication. *Pediatr. Rev*. 2015;36(6):227–238.

17. Wang J., Wu Y., Ma C., Fiorin G., Wang J., Pinto L.H., *et al*. Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *Proc. Natl. Acad. Sci. USA*. 2013;110(4):1315–1320. https://doi.org/10.1073/pnas.1216526110

18. Leneva I.A., Russell R.J., Boriskin Y.S., Hay A.J. Characteristics of arbidol-resistant mutants of influenza virus: Implications for the mechanism of anti-influenza action of arbidol. *Antiviral Res.* 2009;81(2):132–140. https://doi.org/10.1016/j.antiviral.2008.10.009

19. Hurt A.C., Ernest J., Deng Y.M., Iannello P., Besselaar T.G., Birch C., *et al*. Emergence and spread of oseltamivir-resistant influenza A (H1N1) viruses in Oceania, Southeast Asia and South Asia. *Antiviral Res*. 2009;83(1):90–93. https://doi.org/10.1016/j.antiviral.2009.03.003

20. Hurt A.C. The epidemiology and spread of drug resistant human influenza viruses. *Curr. Opin. Virol*. 2014;8:22–29. https://doi.org/10.1016/j.coviro.2014.04.009

21. Lampejo T. Influenza and antiviral resistance: an overview. *Eur. J. Clin. Microbiol. Infect. Dis.* 2020;39(7):1201–1208. https://doi.org/10.1007/s10096-020-03840-9

22. Fire A.Z. Gene silencing by double-stranded RNA. *Cell Death Differ*. 2007;14(12):1998–2012. https://doi.org/10.1038/sj.cdd.4402253

23. Fire A., Xu S.Q., Montgomery M.K., Kostas S.A., Driver S.E., Mell C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998;391(6669): 806–811. https://doi.org/10.1038/35888

24. Faizuloev E.B., Nikonova A.A., Zverev V.V. Prospects for the development of antiviral drugs based on small interfering RNAs. *Voprosy virusologii = Problems of Virology*. 2013;(S1):155–169 (in Russ.). URL: https://cyberleninka.ru/article/n/perspektivy-sozdaniya-protivovirusnyh-preparatov-na-osnove-malyh-interferiruyuschih-rnk/viewer

25. McManus M.T., Sharp P.A. Gene silencing in mammals by small interfering RNAs. *Nat. Rev. Genet*. 2002;3(10):737–747. https://doi.org/10.1038/nrg908

26. Estrin M.A., Hussein I.T.M., Puryear W.B., Kuan A.C., Artim S.C., Runstadler J.A. Host-directed combinatorial RNAi improves inhibition of diverse strains of influenza A virus in human respiratory epithelial cells. *PLoS One*. 2018;13(5):e0197246. https://doi.org/10.1371/journal.pone.0197246

27. Janssen H.L., Reesink H.W., Lawitz E.J., Zeuzem S., Rodriguez-Torres M., Patel K., van der Meer A.J., Patick A.K., Chen A., Zhou Y., Persson R., King B.D., Kauppinen S., Levin A.A., Hodges M.R. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med*. 2013;368(18):1685–1394. https://doi.org/10.1056/nejmoa1209026

28. Qureshi A., Tantray V.G., Kirmani A.R., Ahangar A.G. A review on current status of antiviral siRNA. *Rev. Med. Virol*. 2018;28(4):e1976. https://doi.org/10.1002/rmv.1976

29. Hoy S.M. Patisiran: First Global Approval. *Drugs*. 2018;78(15):1625–1631. https://doi.org/10.1007/s40265-018-0983-6

30. Lesch M., Luckner M., Meyer M., Weege F., Gravenstein I., Raftery M., Sieben C., Martin-Sancho L., Imai-Matsushima A., Welke R.W., Frise R., Barclay W., Schönrich G., Herrmann A., Meyer T.F, Karlas A. RNAi-based small molecule repositioning reveals clinically approved urea-based kinase inhibitors as broadly active antivirals. *PLoS Pathog*. 2019;15(3):e1007601. https://doi.org/10.1371/journal.ppat.1007601

31. Karlas A., Machuy N., Shin Y., Pleissner K.P., Artarini A., Heuer D., *et al*. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature*. 2010;463(7282):818–822. https://doi.org/10.1038/nature08760

32. Arenas-Hernandez M., Vega-Sanchez R. Housekeeping gene expression stability in reproductive tissues after mitogen stimulation. *BMC Res. Notes*. 2013;6:285. https://doi.org/10.1186/1756-0500-6-285

33. Lee H.K., Loh T.P., Lee C.K., Tang J.W., Chiu L., Koay E.S. A universal influenza A and B duplex real-time RT-PCR assay. *J. Med. Virol*. 2012;84(10):1646–1651. https://doi.org/10.1002/jmv.23375

34. Ramakrishnan M.A. Determination of 50% endpoint titer using a simple formula. *World J. Virol*. 2016;5(2):85–86. https://doi.org/10.5501/wjv.v5.i2.85

35. Eierhoff T., Hrincius E.R., Rescher U., Ludwig S., Ehrhardt C. The Epidermal Growth Factor Receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. *PLoS Pathog*. 2010;6(9):e1001099. https://doi.org/10.1371/journal.ppat.1001099

36. Shaw M.L., Stertz S. Role of Host Genes in Influenza Virus Replication. In: Tripp R., Tompkins S. (Eds.). Roles of Host Gene and Non-coding RNA Expression in Virus Infection. *Current Topics in Microbiology and Immunology.* 2017;419:151–189. https://doi.org/10.1007/82_2017_30

37. Watanabe T., Watanabe S., Kawaoka Y. Cellular networks involved in the influenza virus life cycle. *Cell Host & Microbe*. 2010;7(6):427–439. https://doi.org/10.1016/j.chom.2010.05.008

38. Hussain M., Galvin H.D., Haw T.Y., Nutsford A.N., Husain M. Drug resistance in influenza A virus: the epidemiology and management. *Infect. Drug Resist.* 2017;10:121–134. https://doi.org/10.2147/IDR.S105473

39. Sanjuán R., Domingo-Calap P. Mechanisms of viral mutation. *Cell. Mol. Life Sci*. 2016;73(23):4433–4448. https://doi.org/10.1007/s00018-016-2299-6

40. Presloid J.B., Novella I.S. RNA viruses and RNAi: quasispecies implications for viral escape. *Viruses*. 2015;7(6):3226–3240. https://doi.org/10.3390/v7062768

41. Das A.T., Brummelkamp T.R., Westerhout E.M., Vink M., Madiredjo M., Bernards R., *et al*. Human immunodeficiency virus type 1 escapes from RNA interference-mediated inhibition. *J. Virol.* 2004;78(5):2601–5. https://doi.org/10.1128/JVI.78.5.2601-2605.2004

42. Rupp J.C., Locatelli M., Grieser A., Ramos A., Campbell P.J., Yi H., *et al*. Host cell copper transporters CTR1 and ATP7A are important for Influenza A virus replication. *Virol . J*. 2017;14(1):11. https://doi.org/10.1186/s12985-016-0671-7

43. Wang R., Zhu Y., Zhao J., Ren C., Li P., Chen H., *et al*. Autophagy Promotes Replication of Influenza A Virus *In Vitro*. *J. Virol*. 2019;93(4):e01984–18. https://doi.org/10.1128/JVI.01984-18

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