EXPRESSION OF MTCO1 IN CELL CULTURES FROM PATIENTS WITH LEIGH SYNDROME UNDER THE ACTION OF AAV9-SURF1

Adrianov MA[1,2] ✉, Gauthier MS[1], Degtyareva AV[1,3], Marey MV[1], Manukhova LA[1], Rastorguev SM[4], Simonov VS[4], Ushakova LV[1], Vysokikh MY[1,2]

[1] Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russia
[2] Belozersky Research Institute of Physico-Chemical Biology at Lomonosov Moscow State University, Moscow, Russia
[3] Filatov Clinical Institute of Child Health, Sechenov First Moscow State Medical University, Moscow, Russia
[4] Pirogov Russian National Research Medical University, Moscow, Russia

The most common biochemical defect in Leigh syndrome is aberrations in proteins involved in the assembly of the electron transport chain (ETC) complex IV subunits — cytochrome C oxidase (COX). Among these, mutations in the *SURF1* gene are the most common. The SURF1 protein is embedded in the inner mitochondrial membrane and plays a crucial role in the COX complex assembly. All mutations in the *SURF1* gene result in the truncated protein biosynthesis and damage to the COX complex. Adeno-associated viral vectors (AAV9), which carry the not mutated *SURF1* gene (AAV9-SURF1), are being investigated for the treatment of this disease. The aim of this study was to evaluate the expression levels of SURF1 and MTCO1 proteins in whole blood from patients with Leigh syndrome compared to reference values obtained for a pool of patients without mutations, as well as to evaluate the expression of the MTCO1 cytochrome c oxidase subunit in skin fibroblast cultures from patients with Leigh syndrome treated with AAV9. To investigate the gene therapy efficacy, AAV9-SURF1 was added to fibroblasts derived from the skin of a patient with a mutation in the Surf1 gene and to control skin fibroblasts at an optimal dose that did not impair cell viability in the MTT assay. We used Western blot analysis and quantitative PCR to evaluate changes in the relative amounts of the studied proteins after the addition of AAV9-SURF1 to control cells and cells obtained from the patient and identified significant compensatory changes in skin fibroblasts from a patient with a SURF1 mutation.

Keywords: Leigh syndrome, SURF1, adeno-associated viral vector, gene replacement therapy, cytochrome oxidase, COX, MTCO1

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✉ **Correspondence should be addressed:** Mikhail A. Adrianov
Academica Oparina, 4, Moscow, 117997, Russia, ya.litsch@gmail.com

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ЭКСПРЕССИЯ MTCO1 В КУЛЬТУРАХ КЛЕТОК ПАЦИЕНТОВ С СИНДРОМОМ ЛИ ПРИ ДЕЙСТВИИ AAV9-SURF1

М. А. Адрианов[1,2] ✉, М. С. Готье[1], А. В. Дегтярева[1,3], М. В. Марей[1], Л. А. Манухова[1], С. М. Расторгуев[4], В. С. Симонов[4], Л. В. Ушакова[1], М. Ю. Высоких[1,2]

[1] Национальный медицинский исследовательский центр акушерства, гинекологии и перинатологии имени В. И. Кулакова, Москва, Россия
[2] Научно-исследовательский институт физико-химической биологии имени А. Н. Белозерского, Московский государственный университет имени М. В. Ломоносова, Москва, Россия
[3] Клинический институт детского здоровья имени Н. Ф. Филатова, Первый московский государственный медицинский университет имени И. М. Сеченова, Москва, Россия
[4] Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

Самым частым биохимическим дефектом при синдроме Ли является нарушение белков, которые участвуют в сборке субъединиц IV комплекса электрон-транспортной цепи — цитохром-с-оксидазы (COX). Среди них наиболее часто встречаются мутации гена *SURF1*. Белок SURF1 встроен во внутреннюю мембрану митохондрии и играет решающую роль в сборке COX-комплекса. Все мутации в гене *SURF1* приводят к биосинтезу укороченного белка и повреждениям COX-комплекса. Для терапии данного заболевания исследуют возможность использования аденоассоциированных вирусных векторов (AAV9), в геном которых внесен немутированный *SURF1* (AAV9-SURF1). Целью исследования было дать оценку уровня экспрессии белков SURF1, MTCO1 в цельной крови пациентов с синдромом Ли на фоне полученных референсных значений для пула пациентов без мутаций, а также оценку экспрессии субъединицы цитохром-с-оксидазы MTCO1 в культурах фибробластов кожи пациентов с синдромом Ли при действии AAV9-SURF1. Для моделирования генотерапевтического воздействия к фибробластам, полученным из кожи пациента, и к контрольным фибробластам кожи был добавлен AAV9-SURF1 в подобранной оптимальной дозе, не приводящей к ухудшению жизнеспособности клеток в MTT-тесте. Методами вестерн-блота и количественной ПЦР мы оценили изменения в относительном количестве исследуемых белков после добавления AAV9-SURF1 на контрольные клетки и клетки, полученные от пациента. Нами были выявлены существенные компенсаторные изменения для фибробластов кожи пациента с мутацией SURF1.

Ключевые слова: синдром Ли, SURF1, аденоассоциированный вирусный вектор, генозаместительная терапия, цитохромоксидаза, COX, MTCO1

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✉ **Для корреспонденции:** Михаил Андреевич Адрианов
ул. Академика Опарина, д. 4, г. Москва, 117997, Россия; ya.litsch@gmail.com

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Mitochondrial encephalomyopathies that manifest in early infancy with severe, progressive neurological impairment include subacute necrotizing encephalomyelopathy, or Leigh syndrome, described by Professor Denis Archibald Leigh in 1951 following a postmortem examination of a 7-month-old boy with progressive neurological symptoms [1].

Leigh syndrome is a rare hereditary disorder, with an incidence of approximately 1 in 34,000-36,000 newborns, it affects both boys and girls equally. It is an extremely heterogeneous disease, caused by defects in multiple genes located on autosomes, the X chromosome, and mitochondrial DNA. It is known that the most common biochemical defect in this syndrome is a disruption of the amino acid sequence of proteins involved in the assembly of the subunits of complex IV of the electron transport chain (ETC) of the inner mitochondrial membrane — cytochrome c oxidase (COX), which catalyzes the transfer of electrons in the ETC from cytochrome c to molecular oxygen and formation of water, thus, plays a key role in mitochondrial oxidative phosphorylation.

The most common mutations are in the gene encoding the *SURF1* (Sea Urchin Retroposon Family 1) protein, which is embedded in the inner mitochondrial membrane and plays a crucial role in the assembly of the COX complex [2]. Normally, the 35 kDa precursor of SURF1 is imported into the mitochondria, where its mature form (30 kDa) is formed after processing. However virtually all mutations (more than 60) in the *SURF1* gene result in protein biosynthesis that is already shortened at the precursor stage and damage to the COX complex, destabilized by defective SURF1. About 50% of mutations in the *SURF1* gene are missense, nonsense, and small deletions, with the most common being a 10-nucleotide deletion with a 2-nucleotide insertion (311-321del10insAT) in exon 4 and a 2-nucleotide deletion (845delCT) in exon 9 of the gene [3, 4].

Although it has been established that SURF1-associated Leigh syndrome has a typical course, leading to early mortality before the age of 10 in most cases, approximately 10% of cases exhibit milder symptoms with a longer lifespan [5]. In all cases, the onset of the disease occurs in infancy and is characterized by impaired feeding associated with the onset of damage to the central nervous system, hypotonic and poor coordination of sucking and swallowing, followed by further regression of already established psychomotor development [6]. According to a number of researchers, gastroparesis and delayed gastric emptying, as well as gastroesophageal reflux, in case of Leigh syndrome caused by mutations in the *SURF1* gene and most likely due to neuromuscular and mitochondrial dysfunction caused by disturbances in the ETC with cytochrome oxidase deficiency [7]. Typical manifestations of the developing disease also include such disorders as neuropathy, ataxia, ophthalmoplegia and hypertrichosis [8]. To investigate the molecular mechanisms and potential therapies for Leigh syndrome, mouse models deficient in the *SURF1*(–/–) gene were created. These mice exhibit significant reduction in birth weight, which recovers by the age of one month, with manifestation of moderate motor developmental delay against the background of defective assembly and deficiency of a number of critical COX subunits, including mitochondria-encoded MT-CO1 [9]. Despite the described impairments, these mice have a lifespan similar to the wild type, and it has been suggested that the loss of a COX assembly factor such as Surf1 in mice leads to compensatory responses, including activation of mitochondrial biogenesis and the Nrf2-dependent signaling cascade, which contribute to the alleviation of the manifestations of the pathological phenotype [10]. Furthermore, in this model, AAV serotype 9 (AAV9) adenoviral vector therapy for SURF1-associated Leigh syndrome, in which mice were injected with a codon-optimized human *SURF1* gene (hSURF1opt) (AAV9/hSURF1v1), was shown to restore not only SURF1 function but also complex IV activity and correct exercise-induced lactic acidosis without any toxicity to wild-type mice [11, 12].

Here, we used a similar approach with aim to explore the potential of gene therapy for Leigh syndrome in a model of subcutaneous fibroblasts from patients with a *SURF1* gene mutation transfected with an adenoviral construct containing a codon-optimized human *SURF1* gene.

METHODS

The study included three patients with a genetically confirmed diagnosis of Leigh syndrome and typical clinical manifestations. The inclusion criteria for the study were: 1) a genetically confirmed diagnosis of Leigh syndrome with frequently occurring mutations in the *SURF1* gene (Table 1); 2) age of disease manifestation up to 3 years; 3) informed consent from the patient's legal representative; exclusion criteria were the presence of another genetically determined disease, including a syndromic form of the pathology. Two patients were born full-term, in satisfactory condition at birth (Apgar score 8/9 points), with normal weight and length for gestational age; one patient was born at 30 weeks. All patients exhibited psychomotor developmental delays from the first months of life. Over time, their condition progressively worsened, with loss of motor and speech skills. The first two patients experienced metabolic crises — acetonemic states characterized by vomiting, food refusal, weakness, and lethargy. Severe bulbar dysfunction necessitated gastrostomy placement in the first and second patients, a tracheostomy in the third patient, and the first patient required respiratory support (NIVL). All patients were diagnosed after 1.5 years of age. MRI data revealed changes in brain tissue characteristic of the disease: the first patient, aged 2 years, showed MRI signs of bilateral symmetrical structural changes in the substantia nigra, red nuclei, along the conduction pathways in the medulla oblongata and in the middle cerebellar peduncles. The second patient (aged 14 months) showed bilateral symmetrical increases in signal intensity in the basal ganglia, corticospinal tracts and brainstem structures. The third patient (aged 12 months) showed increased signal intensity in the lower parts of the brainstem (pons, medulla oblongata) without involvement of the basal ganglia. The diagnosis was confirmed by molecular genetic research methods (Table 1).

At the time of the study, all patients' neurological status included impaired eye coordination and fixation, rotatory nystagmus with a vertical component, floating eye movements, bulbar-pseudobulbar syndrome, diffuse generalized trunk and limb hypotonic/atonic associated with mixed tetraparesis, decreased tendon-periosteal reflexes, polyneuropathy syndrome with distal muscle wasting of the limbs, and myogenic contractures in large joints.

The control group consisted of healthy children (3–13 years old) with undetected mutations in the *SURF1* gene and no symptoms who presented to the children's outpatient department of the Kulakov National Research Center for Obstetrics, Gynecology and Perinatology as part of a routine pediatric examination.

Collection of blood samples

Whole blood obtained in vacuette with EDTA was treated with ammonium chloride and potassium lysing buffer (AbiLyse

Table 1. Clinical and anamnestic data of patients included in the study

Gender		Patient 1	Patient 2	Patient 3
		Female	Female	Male
Variant in gene *SURF1*		Deletions in the heterozygous state: c.845 84delCT; p.(Ser282Cysfs*9) c.901 902delTC; p.(Ter301Thrext27)	Variants in compound heterozygous state:c.845_846delCT c.752-2A>G	Variants in compound heterozygous state:c.240G>C(p.Q8OH) c.870dupT(p.K29I)
Age of onset of disease		1 month psychomotor developmental delay	1 year metabolic crisis	2 years old hand tremors, gait disturbance, motor clumsiness
Age at the time of disease diagnosis		2.5 years	1.5 years	2.5 years
Age at the time of study		6 years	5.5 years	12 years
Clinical manifestations at the time of the study	Psychomotor retardation	+	+	+
	Bulbar disorders	+ gastrostomy	+ gastrostomy	+ tracheostomy
	Polyneuropathic syndrome	+	+	+
	Metabolic crysis	+	+	+
	Respiratory therapy	NIVL 10 hours/day	–	–
	Palliative status	+	+	+

ACK Lysing Buffer, USA) for 15–60 minutes after intravenous collection from patients, following the manufacturer's protocol for subsequent sample collection for Western blot analysis.

Fibroblast Cultivation

Primary subcutaneous fibroblast cell cultures were kindly provided by the Pirogov Russian National Research Medical University. Starting from passage 3, the cells were cultured in a complete nutrient medium consisting of DMEM (PanEco, Russia): F12 (PanEco, Russia) at a 1 : 1 ratio, supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (PenStrep, GIBCO, USA), and 10% fetal bovine serum (FBS, GIBCO, USA). The culture medium was maintained at 37 °C in a 5% CO_2 atmosphere. Upon reaching 60% confluence, the cells were subcultured at a 1:3 ratio using 0.05% trypsin/EDTA (PanEco, Russia) in culture flasks. Trypsin activity was suppressed by adding an appropriate amount of fresh nutrient medium. Cell counts were determined at each passage using a Goryaev chamber (MINIMED, Russia). Cell morphology was assessed using a NIKON ECLIPSE TS100 inverted light microscope (JAPAN).

Construction of the AAV vector carrying the *hSURF1* gene

Total RNA was isolated using the commercial RNA-solo kit (Eurogen) according to the manufacturer's protocol. Tissue samples were homogenized and lysed with ExtractRNA reagent (Eurogen, Russia), followed by RNA extraction and purification. The concentration and purity of the obtained RNA were assessed spectrophotometrically at 260/280nm on “Nanodrop spectrophotometer” (USA). RNA integrity and the absence of degradation were verified by electrophoresis in a 2% agarose gel on horizontal system (Helicon, Russia) according standard protocol (http://molbiol.ru/protocol/07_01.html). The results were visualized using the GenoSens 2250 Touch gel documentation system (Clinx). First-strand cDNA synthesis was performed on the isolated RNA template using the Magnus reverse transcriptase reagent kit (Eurogen). To amplify the complete coding sequence of the *SURF1* gene, specific primers containing additional nucleotide sequences at the 5'-ends complementary to the terminal regions of the linearized pAAV-CAG plasmid were designed for subsequent assembly by the TLTC method. The primers had the following structure: forward primer SURF1-dir:5'-tgtccaggcggccgccATGGCGGCG GTGGCT-3' reverse primer SURF1-rev: 5'-aggcacagtcgagg cagatctTCACACACCAGGTGTCCCAC-3' (lowercase letters denote sequences homologous to the vector, and uppercase letters denote regions complementary to the beginning and end of the *SURF1* gene open reading frame). Primer annealing temperatures and specificities were calculated using Primer3. The *SURF1* gene was amplified using polymerase chain reaction (PCR). The amplification protocol included an initial denaturation at 95 °C for 5 minutes, 30 cycles (denaturation at 95 °C for 30 seconds, annealing at 62 °C for 30 seconds, elongation at 72 °C for 1 minute), and a final elongation at 72 °C for 5 minutes. PCR products were analyzed by electrophoresis in a 1% agarose gel.

The pAAV-CAG plasmid vector was prepared for cloning using PCR amplification with the Vec-dir (AGATCTGCCTCGACTGTGCCT) and Vec-rev (GGCGGCCGCCTGGACA) primers and Kapa polymerase (Rosche). This ensured the production of a linear molecule with terminal sequences homologous to the amplified *SURF1* gene. Recombinant plasmid assembly was performed using the T5 exonuclease-dependent assembly method. The purified *SURF1* gene PCR product was mixed with the linearized vector supplemented with T5 exonuclease and the mixture was incubated on ice for 5 minutes. Two microliters of the reaction mixture were used to transform chemically competent E. coli strain XL-Blue (Eurogen) cells according to the standard method [13].

Plasmid DNA was isolated from the cultures using the miniprep method and Plasmid Miniprep 2.0 kit (Eurogen). Primary screening of positive clones was performed using PCR with SURF1-specific primers. The accuracy of the resulting recombinant construct was further confirmed by restriction analysis with PstI endonuclease in buffer Orange (SibEnzyme), followed by restriction profile analysis by agarose gel electrophoresis.

Assessing the cytotoxic effect of the AAV vector carrying the *hSURF1* gene

The cytotoxic effect of the virus was assessed using the MTT assay on the viability of subcutaneous fibroblasts from patients

Table 2. Primer characteristics

Gene	Primer direction	Primer sequence 5'->3'	Primer length, n.p.	Product length, n.p	Annealing T, C
GAPDH	Direct	GGATTTGGTCGTATTGGG	18	205	55.5
	Reverse	GGAAGATGGTGATGGGATT	19		
SURF1	Direct	GCGGCCTCATCTCCT	15	281	58.6
	Reverse	CTCTGCGCCTGTGATTC	17		
MTCO1	Direct	CCTACTCCTGCTCGCATCTG	20	363	59.4
	Reverse	AGAATGGGGTCTCCTCCTCC	20		

without the Surf1 mutation. Cell cultures from passage 4 were seeded in 96-well transparent flat-bottomed plates (Greiner Bio-One, Austria) at a concentration of 1 × 10^5 cells/well. After 24 hours of cultivation at 37 °C and 5% CO_2, the cells were treated with various concentrations of the vector: 10^{13}, 10^{12}, 10^{10}, and 10^8 viral particles per ml of culture medium. Control wells were supplemented with culture medium in appropriate quantities instead of viral particles. After adding viral particles, the cells were incubated for 24 hours under the conditions described above. At the end of the incubation, 20 µl of a solution containing 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT, Santa Cruz) at a concentration of 5 mg/ml were added to each well of the microplate. After 4 hours, the medium with the MTT reagent was removed, and 100 µl of dimethyl sulfoxide (DMSO) were added to the wells to solubilize formazan crystals. Optical density was measured using an ELISA microplate reader at 570 nm. The assay was performed in triplicate to calculate the mean half-maximal inhibitory concentration (IC_{50}) of viral particles and standard deviation.

Electrophoresis and Western blot analysis of target proteins in whole blood and fibroblasts lysates

To analyze the relative content of the studied proteins in whole blood and subcutaneous fibroblasts lysates with and without viral infection, samples were separated by SDS-polyacrylamide gel electrophoresis followed by electro transfer to a nitrocellulose membrane (Bio-Rad) [15]. The membranes were incubated with specific primary antibodies (anti-Surf1 (Abcam; ab110256, 1 : 1000), anti-MTCO1 (Abcam; ab14705, 1 : 1000) followed by incubation with secondary anti-species HRP-linked antibodies. The signals were analyzed by enhanced chemiluminescence assay using the Novex™ ECL Chemiluminescent Substrate Reagent Kit (INVITROGEN, USA) according to the manufacturer's instructions. The signal from the proteins of interest was normalized to the total protein content determined by the staining intensity using Ponceau's dye (0.5% in 1% acetic acid).

Target Genes Expression Assessment in Fibroblasts Lysates after Transfection

Gene expression was assessed by real-time reverse transcription using polymerase chain reaction (PCR) with transcript-specific primers (Table 1). RNA isolation and purity analysis were performed as described above. For reverse transcription (RT), 2 µl of 20 µM random hexaprimer were added to 0.5 µg of total RNA and incubated for 2 min at 70 °C. The tube was then transferred to ice, and 4 µl of 5x RT buffer, 2 µl of 10 mM dNTP mixture, 2 µl of 20 mM DTT, and 1 µl of MMLV reverse transcriptase (50 U/µl) (Eurogen, Russia) were added. The mixture was incubated for 40 min at 40 °C and the reaction was stopped by heating for 10 min at 70 °C. Subsequent real-time PCR was carried out according to the following scheme: 50 ng of template cDNA, 0.4 µl of each forward and reverse primer (100 pmol), 2 µl of 5xqPCRmixHS SYBR. Amplification was monitored by electrophoresis in 3% agarose gel. Statistical data processing was performed using the QGENE software (2-ΔCt method). The expression of each target gene was normalized to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. In the course of the study, primers specific to the transcripts of the corresponding genes were selected (Table 2).

RESULTS

The relative content of SURF1 and MTCO1 proteins in venous blood cells of 20 pediatric patients (3–11 years old) without mitochondrial pathologies (control group) was determined using Western blot analysis. Data for reference values are presented in Fig. 1. It was shown that the relative content of the studied proteins is reduced in patients with mutations in the *SURF1* gene compared to the control group.

To determine the safety of viral construction, a dose of 104 per cell was found to be optimal based on the results of a study examining the relationship between cell viability and the number of viral particles used for transfection (Fig. 2). Next,

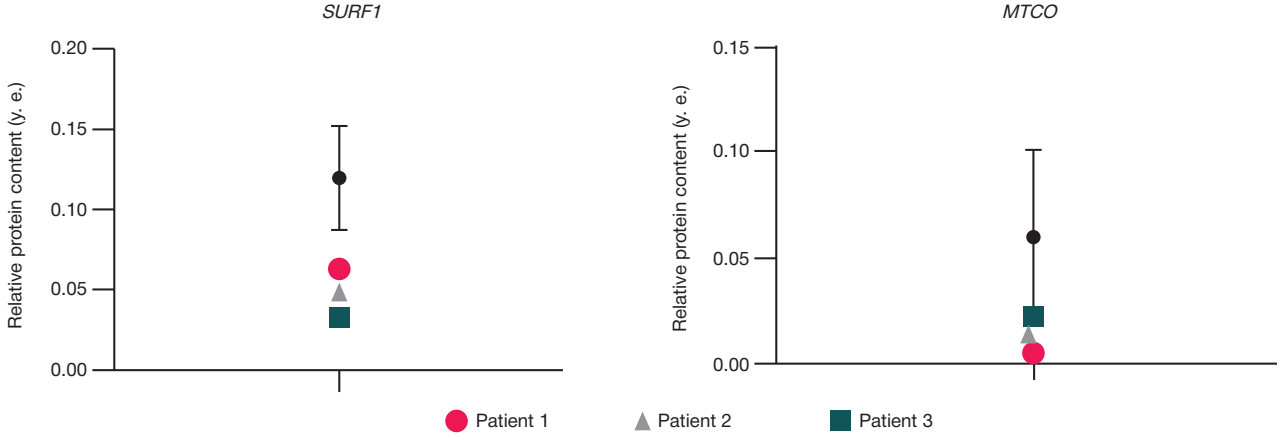

Fig. 1. Relative protein content in whole blood cells obtained for a samples (*n* = 20, *black circles*) of healthy patients (3–11 years) and patients with Leigh syndrome (*colored symbols*) are presented as mean with 95% percentiles in deviations

an adenoviral construct with a normal *SURF1* gene analogue was added to fibroblasts from patients with a mutation in the *SURF1* gene and to control fibroblasts at a dose optimized to ensure no reduction in cell viability in the MTT assay.

Expression of the *SURF1* and *MTCO1* genes in fibroblast lysates after infection with AAV9-SURF1

We used quantitative PCR to evaluate the expression levels of target genes in fibroblasts treated with the gene therapy construct (Fig. 3). First, we found that fibroblasts from patients without SURF1 mutations had significantly higher expression levels than fibroblasts from patients with Leigh syndrome and *SURF1* mutations (Fig.3, left panel). Furthermore, MTCO1 expression levels were not significantly different between fibroblasts from patients and controls (Fig.3, right panel). Second, SURF1 expression levels significantly increased in fibroblasts from patients with mutations and did not significantly differ from the expression levels of this gene in control fibroblasts treated with the gene therapy construct.

We observed a similar pattern for the *MTCO1* gene — a significant increase following transfection in diseased fibroblasts compared to pre-transfection levels. Interestingly, the *MTCO1* gene expression level after transfection was several times higher than baseline for both diseased and healthy cells (Fig. 3).

Western blot analysis of SURF1 and MTCO1 content in fibroblasts

When analyzing the levels of target proteins after exposure to the gene therapy construct, we identified significant differences between control cells and subcutaneous fibroblasts from a patient with the *SURF1* mutation. Using Western blot analysis, we assessed changes in the relative amounts of the studied proteins and showed that 24 hours after virus introduction into the culture medium, both wild-type cells and SURF1 mutants showed a significant increase in protein levels ($p < 0.05$), but this increase was more pronounced for fibroblasts from patients with the mutation (Fig. 4, left panel). We also demonstrated that the baseline SURF1 protein level in wild-type cells was significantly higher than in mutant cells. As for the MTCO1 protein, which is initially significantly more abundant in wild-type cells, no increase in its expression under the influence

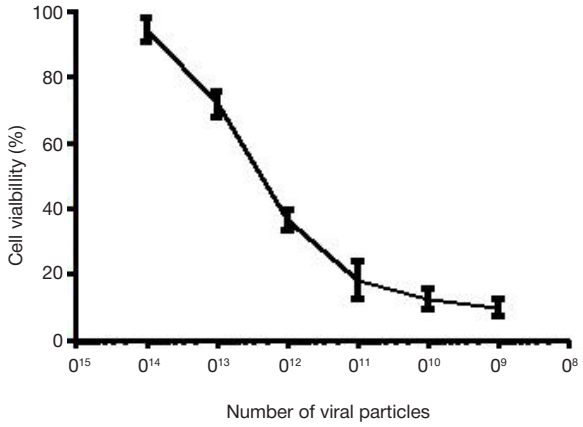

Fig. 2. Cytotoxic effect of the virus on control fibroblasts. The assay was performed three times to calculate the mean half-maximal inhibitory concentration (IC_{50}) and standard deviation

of the virus is observed in control fibroblasts, while for cells from patients with the SURF1 mutation, the MTCO1 level is significantly increased 24 hours after adding the virus to the cells (Fig.4, right panel) and even exceeds the protein level in wild-type cells ($p < 0.05$).

DISCUSSION

In frame of the generally accepted concept that SURF1 protein plays a critical role in the assembly of the cytochrome oxidase holoenzyme, from a mechanistic point of view it can be proposed that the loss of SURF1 protein affects COX activity by stabilizing the structure of the multiprotein complex in the mitochondrial membrane [15]. When Leigh syndrome manifests in patients with mutations in the *SURF1* gene, usually no more than 20% of COX activity is retained, while in model animals with a knockout of this gene, at least 50% of the enzyme activity remains, which leads to such a significant difference between the phenotypes of patients with Leigh syndrome and mice deficient in the SURF1 protein [12]. On the other hand, although the use of such models does not seem promising due to the observed phenotypic differences, gene therapy using an AAV9-SURF1-based vector resulted in a significant (up to 30%) [12] improvement in COX activity in the brain, muscle, and liver of SURF1-deficient mice.

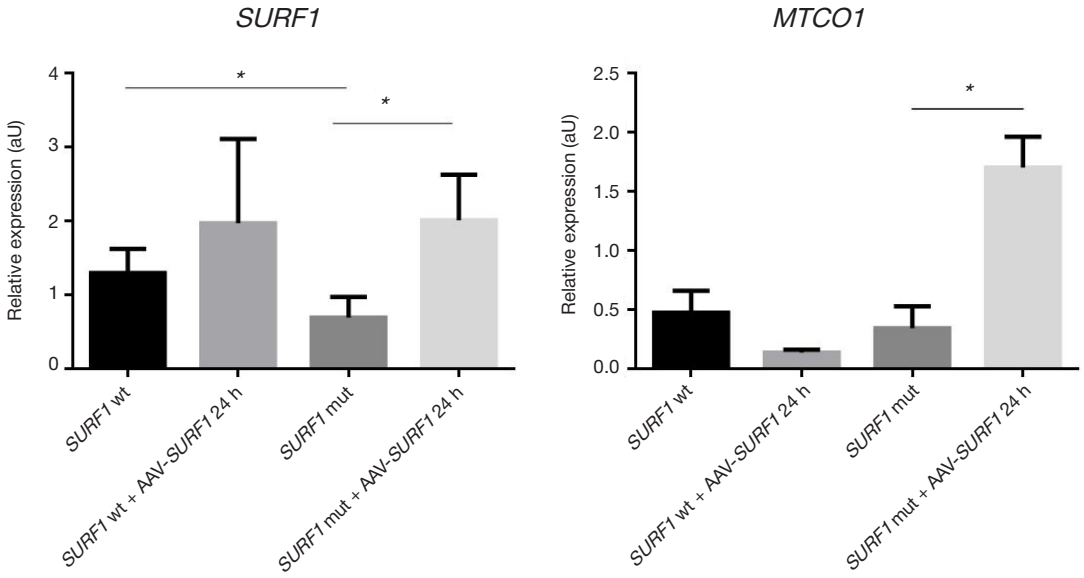

Fig. 3. Expression of the *SURF1* and *MTCO1* genes in lysates of subcutaneous fibroblasts from patients without the mutation (wt — wild type in the graphs) and with a mutation in the *SURF1* gene (SURF1 mut.) 24 hours after infection with AAV9-SURF1; * — $p < 0.05$ (Mann–Whitney)

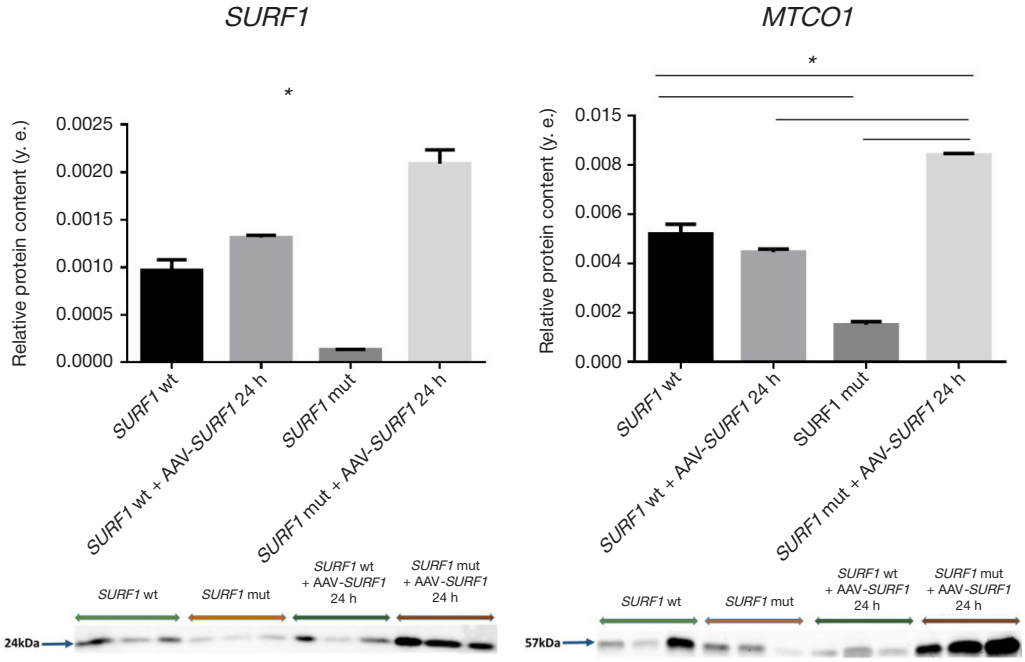

Fig. 4. Relative amount of SURF1 and MTCO1 proteins in lysates of subcutaneous fibroblast from patients without the *SURF1* mutation (wt — wild type in the graphs) and with the mutation in the *SURF1* gene (SURF1 mut.) 24 hours after infection with AAV9-SURF1 (upper panel); * — $p < 0.05$ (Mann–Whitney). The lower panel shows representative electrophoregrams illustrating changes in the levels of target proteins under the influence of the viral construct

In our model, in which we compared the effect of AAV9-SURF1 transfection of fibroblasts from patients with clinically diagnosed Leigh syndrome and fibroblasts from healthy children, we aimed to determine not only the expression level of SURF1 protein but also MTCO1, which is commonly used as a marker for the level and native structure of the entire cytochrome oxidase. The observed synchronous increase in the expression levels of both the *SURF1* and *MTCO1* genes and proteins in fibroblasts from patients, along with the previously observed improvements in the condition of model animals with similar changes in response to gene therapy, suggest the possibility of increased cytochrome oxidase activity. Measuring this activity is our goal for next study on cells from patients with Leigh syndrome to further substantiate the need to continue preclinical trials of the proposed adenoviral-based drug.

The proposed approach to studying the effectiveness of gene therapy in patient cells avoids the shortcomings of the mouse model, where, according to the researchers, a more severe phenotype must be created to detect an effect due to the absence of behavioral defects and neuromotor impairments. It has previously been shown that in existing model animals, the manifestation of the disease in a mild form is due to the activation of compensatory mechanisms in surviving individuals during natural selection, in contrast to humans, where medical care leads to survival even when a severe form of the disease manifests. [10].

CONCLUSIONS

The resulting AAV9-hSURF1 construct allows for safe transfection at a dose that, after just 24 hours, leads to the possibility of recording a reliable multiple increase in the expression of both genes and proteins *SURF1* and *MTCO1* in cells from patients with Leigh syndrome with an initially low content of these proteins compared to cells from healthy individuals.

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