

EXPRESSION PATTERN OF MRPS18 FAMILY GENES IN GLIOMAS

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Aim: To assess expression patterns of MRPS18 family genes in glioblastoma tissues and glioma cell lines. **Materials and Methods:** Expression of MRPS18 family genes was analyzed by quantitative polymerase chain reaction in glioma cell lines and glioblastoma specimens. A bioinformatic analysis of the publicly available data on the expression of these genes was also provided. **Results:** The genes of MRPS18 family show different expression patterns in glioblastomas and glioma cell lines. The highest levels of expression were found for *MRPS18-2* at mRNA and protein levels in both glioblastomas and glioma cell lines; the lowest — for *MRPS18-1* at mRNA level. **Conclusions:** The elevated levels of relative expression of the *MRPS18-2* gene are characteristic for glioma tumor tissues and cell lines.

Key Words: MRPS18 family, MRPS18-1, MRPS18-2, MRPS18-3, glioblastoma, glioma cell lines, expression pattern, bioinformatic analysis.

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A family of the mitochondrial ribosomal proteins S18 (MRPS18) consists of the three members — the MRPS18-1 (S18-1, MRPS18-C), MRPS18-2 (S18-2, MRPS18-B), and MRPS18-3 (S18-3, MRPS18-A). All three proteins show high homology to one bacterial protein-ancestor. Only one *MRPS18* gene is found in the bacterial genome, while three *S18* genes are encoded by the human genome [1]. Earlier, we have shown that the S18-2 protein is expressed at high levels in several tumor tissues, compared to their normal counterpart, such as endometrial [2], breast [3], prostate [4] cancers, hepatocellular carcinomas [5] and in Hodgkin's lymphomas [6]. Moreover, S18-3 is also overexpressed in breast cancer [7] and in Hodgkin's lymphomas [6].

Yet several works are published that describe experimental data on expression of the S18 family genes. Taking into consideration the importance of S18-2 protein and, perhaps, other member of S18/2 family in carcinogenesis it is logical to study an expression pattern of these genes in other types of tumors. In the present work, we assessed an expression pattern of S18 genes in sera and cancer tissues of glioblastoma patients, and in glioma cell lines as well.

MATERIALS AND METHODS

Patients. The use of glioma tumor tissue and patient sera was approved by the Ethical Committee of A.P. Romodanov Neurosurgery Institute of the National Academy of Medical Sciences of Ukraine and by the Institutional Review Board and Research Ethics Committee of R.E. Kavetsky Institute of Experimental

Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, according to the Declaration of Helsinki. All experimental work was performed according to the approved protocols. The diagnostics was performed at A.P. Romodanov Neurosurgery Institute of the National Academy of Medical Sciences of Ukraine. In this study, samples (tissue and serum) of 9 patients with glioblastoma were evaluated (see Table 1 and Fig. 1). The median age of patients was 61.4 ± 5.2 , with a male: female ratio of 1.25. Serum of 4 healthy donors was used as the control.

Table. Characteristics of glioblastoma patients

N	Sex	Age	Location	Description
1	M	48	Cerebellum	Signs of increased permeability of the blood–brain barrier
2	M	62	Ventricle	
3	M	57	Sin pariet	
4	M	61	Dex temp	Highly invasive
5	M	70	Dex temp	Highly invasive
6	F	75	Dex front	Signs of angioproliferation
7	F	62	Dex front	Highly invasive
8	F	60	Dex temp	Signs of angioproliferation
9	F	58	Dex temp	

Cell lines. The following glioma cell lines were used: A172, U87, produced from glioblastoma; U251, produced from astrocytoma and U343, sub-clone of U251, selected *in vitro*; NCH89 and NCH92, low passage glioblastoma culture; TE671, rhabdomyosarcoma. All cell lines were obtained from the Bank of Cell Lines from Human and Animal Tissues of the Institute of Experimental Pathology, Oncology and Radiobiology (Ukraine). Cells were grown in IMDM medium containing 10% FBS, 2 mM L-glutamine, and appropriate antibiotics. Cell lines used in this study were screened for mycoplasma infection and confirmed to be negative.

RNA isolation, cDNA synthesis and quantitative polymerase chain reaction (q-PCR). The total

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Abbreviation used: MRPS18 – mitochondrial ribosomal proteins (S18).

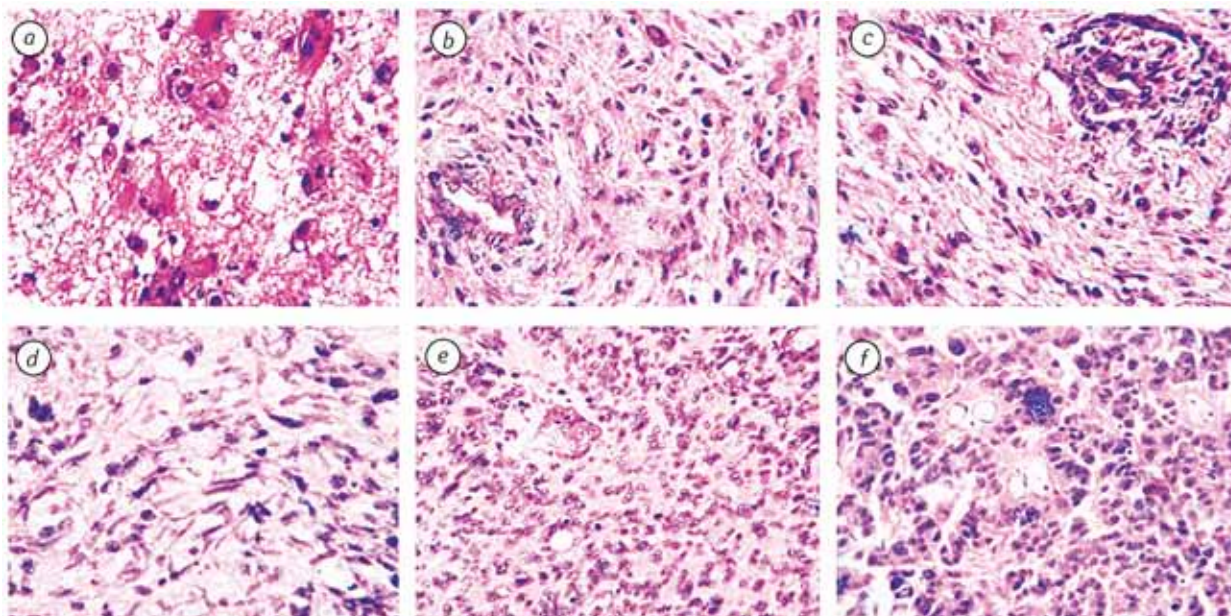


Fig. 1. Selected images of glioblastoma tissue staining with hematoxylin and eosin for a histological analysis: *a* — highly invasive glioblastoma, $\times 800$; *b*, *c* — highly invasive glioblastomas, $\times 400$; *d* — glioblastoma with signs of angio-proliferation, $\times 450$; *e* — glioblastoma with signs of angio-proliferation, $\times 250$; *f* — glioblastoma with signs of increased permeability of the blood–brain barrier

RNA was isolated from serum, using the RNeasy Mini Kit (Qiagen Inc., Germany), according to the manufacturer's instructions. The fresh tissue samples were frozen in the liquid nitrogen immediately after excision and kept at -80°C prior to use. To isolate RNA using TRIzol (Thermo Fisher Scientific Inc., USA), tissues were mashed in a marble mortar after freezing in liquid nitrogen. The cDNAs were synthesized, using $2\ \mu\text{g}$ of total RNA, M-MLV Reverse Transcriptase and RNase inhibitor (Invitrogen, USA), according to the manufacturer's protocol. Quantitative PCR (q-PCR) was performed, using $2\ \mu\text{g}$ cDNA and the HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne, Estonia), on the PCR System 7500 (Applied Biosystems, USA). Primers were the following: for *MRPS18-1* (NM_016067) forward — $5'\text{-CAGGTATCCAGCAATGAGGACC-3'}$, reverse $5'\text{-GCA TCCAGTAAATGGA GA AACAAAC-3'}$; for *MRPS18-2* (NM_014046) — forward $5'\text{-CACAGCGG ACTCGGAAGACATG-3'}$, reverse $5'\text{-GCGCAGACAAATT-GCTCCAAGAG-3'}$; for *MRPS18-3* (NM_018135) — forward $5'\text{-CATCTGCCGTGGAACCTTGAAG-3'}$, reverse $5'\text{-CTTGCGGTGTT CTTCCTGG CAT-3'}$. As an internal control for standardization, a gene encoding TATA-binding protein (*TBP*, NM_003194) was used: forward primer — $5'\text{-TTTCTTGCCA GTCTGGAC-3'}$, reverse $5'\text{-CACGAACC ACGGCACTGATT-3'}$. Relative quantification (comparative $\Delta\Delta\text{Ct}$) method) was used to compare expression levels of the S18 family genes with the internal control. Two or three reactions (each in triplicate) were run for each gene, so the standard deviation might be calculated.

Bioinformatic data analysis. To analyze expression of genes at the mRNA level, a publicly available data bases Oncomine and Protein Atlas were used. The Oncomine data base contains published data that have been collected, standardized, annotated, and analyzed by Compendia Bioscience (www.oncomine.com,

March 2021, Thermo Fisher Scientific, Ann-Arbor, MI, USA). Human Protein Atlas is available from www.proteinatlas.org [8–10].

Statistical analysis. GraphPad Prism software (version 8, GraphPad Software, USA) was used to determine the means of the *MRPS18* gene expression. The Kruskal — Wallis test for non-parametric criteria for the groups was performed for each gene.

RESULTS AND DISCUSSION

In the present study, we compared expression of the S18 family genes in glioblastoma tissue samples and in sera of glioblastoma patients. Although we had no possibility to compare mRNA expression in normal brain tissue, expression of these genes was assessed in sera of four healthy donors, and the values were below an experimental detection. This is in line with our earlier data: we observed that the S18-2 protein

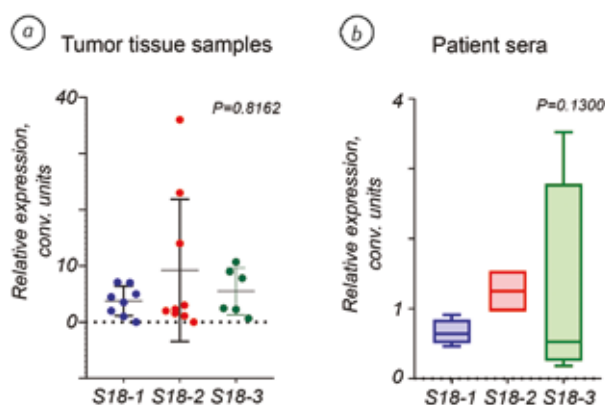


Fig. 2. An expression pattern of the S18 family genes at mRNA levels: *a* — expression of *S18* genes in tissue samples; *b* — expression of *S18* genes in blood sera. Figure was prepared with the help of GraphPrism software; the Kruskal — Wallis test for non-parametric values in groups was applied for each gene. No significant difference in expression levels was found

is expressed ubiquitously, but at very low levels in normal cells [4, 5].

We found that *S18-2* gene was expressed at high levels in glioblastoma tissue samples (Fig. 2, *a*), while *S18-1* showed the lowest level of expression. In sera, the highest expression levels were detected for *S18-3*, and the lowest — also for *S18-1* (Fig. 2, *b*). Noteworthy, the expression levels were 10-fold lower compared to those in tissues. The decreased mRNA in human sera in comparison with tumor tissues could be explained by the low quantity of extracellular RNA in serum, which represents a mixture of various RNAs, protected from degradation by lipid or proteins, or being incorporated in exosomes (reviewed in [11]).

To corroborate our experimental data, a bioinformatic analysis on the expression of S18 family genes

was performed, using the data deposited to a portal Oncomine (Fig. 3). The highest expression was found for *S18-2* and *S18-3* genes, while *S18-1* was almost undetectable. Importantly, the data, calculated for *S18-2* (Fig. 3, *c*) by the authors of [12], were in concordance with statistics described in [13] (Fig. 3, *d*).

The bioinformatic analysis of expression of S18 family genes at mRNA and protein levels on a platform Protein Atlas revealed that S18-2 and S18-3 are highly expressed in gliomas, according to the data of RNA sequencing (RNA seq). Thus, *S18-2* median expression value is over 35 FPKM (Fragments Per Kilobase of transcript, per Million mapped reads), *S18-3* — approximately 20 FPKM, while for *S18-1* this value was only about 3 FKPM (Fig. 4, *a*). A Protein Atlas team produced few antibodies for S18 family proteins and stained several gliomas. Only 2 out of 8 gliomas

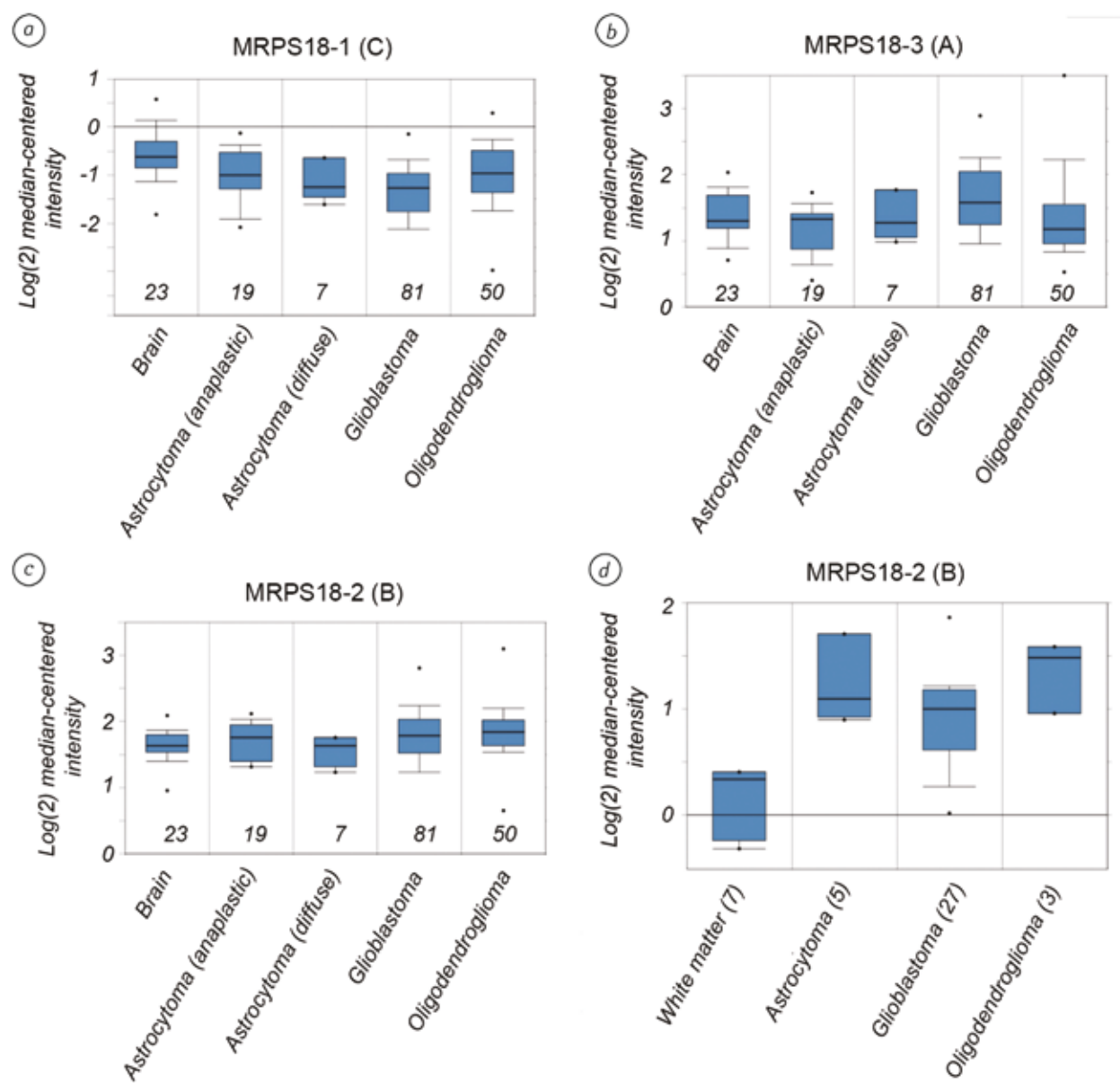


Fig. 3. Expression pattern of S18 family genes in normal and cancer tissue of brain: *a* — expression of S18-1, according to [12]; *b* — S18-3 expression, as reported by [12]; *c* — S18-2 expression, according to [12] and *d* — as reported by [13]. Number of samples is depicted in each column. Results of a study, using the Human Genome U133 Plus 2.0 array, altogether 19 574 measured genes and 180 samples, are described in [12]. The work on the 495-Av2 array that included 42 samples and measured 8603 genes were reported by [13]

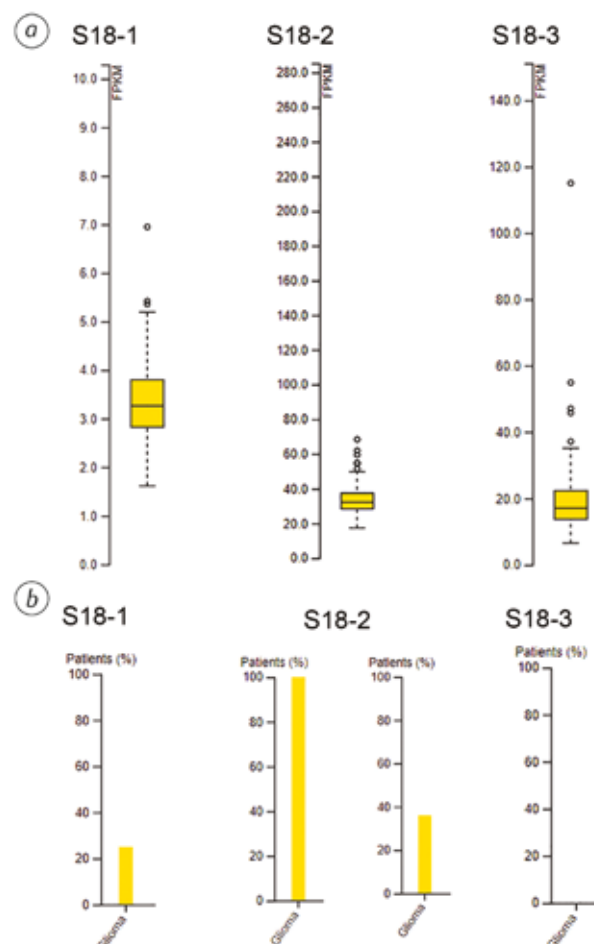


Fig. 4. Expression levels of the S18 family genes in gliomas on a platform Protein Atlas: *a* — expression of S18 family genes at mRNA levels, according to the data of RNA seq. FPKM — Fragments Per Kilobase of transcript, per Million mapped reads; *b* — expression of S18 family proteins, stained with antibodies, produced by the Protein Atlas team. S18-1 was assessed with the HPA050404 antibody; S18-2 protein with the HPA043485 and the HPA050334 antibodies; S18-3 — with the HPA035451 and the HPA035450 antibodies

studied (25%) showed the S18-1 signal with the HPA050404 antibody (Fig. 4, *b*, the left panel). No S18-3 signal was detected with the use of the HPA035451, nor with the HPA035450 antibodies in 11 gliomas (Fig. 4, *b*, the right panel). The highest expression

was demonstrated for S18-2 protein — all 11 gliomas were stained positively with the HPA043485 antibody, while only 4 out of 11 (36%) were positive for the HPA050334 antibody.

Next, we assessed expression of the S18 family genes in glioma cell lines. As was mentioned in Materials and Methods, cell lines A172 and U87 were obtained from glioblastoma samples; NCH89 and NCH92 are the low passage primary cells from glioblastoma [14]; U251 was produced from astrocytoma, and U343 is a sub-line of U251, selected *in vitro* [15]. It was revealed that TE671 represents rhabdomyosarcoma (www.dsmz.de/collection/catalogue/details/culture/ACC-263). The high relative expression was detected for S18-2, and its level was the highest in glioblastoma cells A172, U87 and in one of primary cell line — NCH92 (Fig. 5). Relative expression of S18-1 was quite low in all lines, except U87. Interestingly, in one of primary cell lines, NCH89, S18-1 showed the highest expression level compared with other two members of S18 family (Fig. 5). Noteworthy, in U343 subline selected for the fast growth *in vitro*, all genes were upregulated compared to primary astrocytoma line U251 (Fig. 5).

CONCLUSIONS

We have found that genes of the MRPS18 family (1–3) show different expression patterns in both glioblastomas and glioma cell lines. The S18-2 gene demonstrated the highest expression levels in tumor tissues and glioma cell lines compared to other members of the family. Once again, we demonstrated various properties of the S18-2 family genes. The further studies on a larger patient cohort of glioma patients would strengthen our experimental data. It is important to continue studying the expression pattern of the S18 family genes in glioma tissues and in patient sera, due to low number of published studies. Based on differential expression of the S18-2 and S18-3 genes in normal and cancer cells they might be considered in the future as the putative tumor markers.

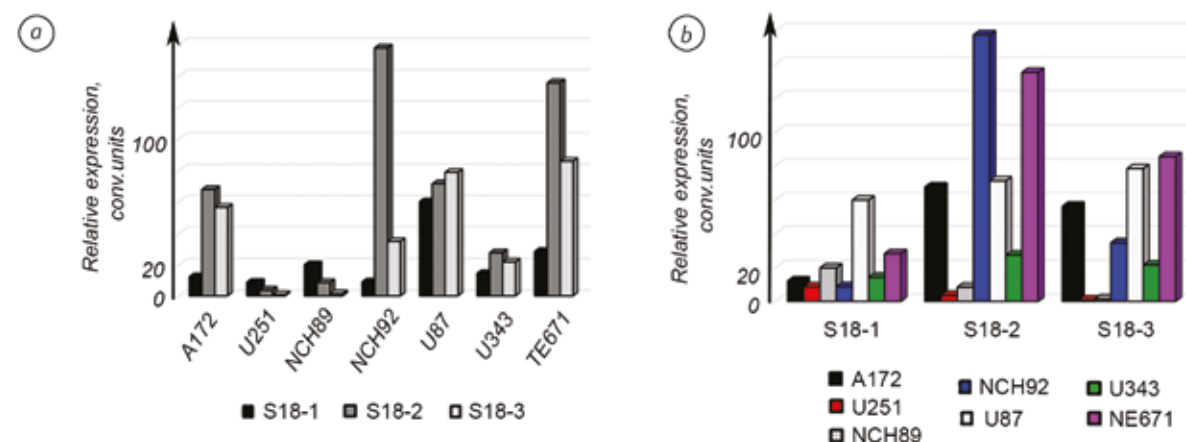


Fig. 5. Expression levels of the S18 family genes in glioma cell lines on the mRNA levels assessed by qPCR: *a* — relative expression sorted by cell lines; *b* — relative expression sorted by genes

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ЕКСПРЕСІЯ ГЕНІВ РОДИНИ MRPS18 В ГЛІОМАХ

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Мета: Визначити характер експресії генів родини MRPS18 в тканині гліобластом та в клітинних лініях гліом. **Матеріали та методи:** Експресію генів родини MRPS18 визначали кількісним ПЛР. Крім того, проводили біоінформатичний аналіз експресії цих генів за даними відкритого доступу. **Результати:** Характер експресії генів родини MRPS18 в тканині гліобластом та в клітинних лініях гліом різниться. Найвищі рівні експресії на рівні мРНК та білка було визначено для MRPS18-2, найнижчі — на рівні мРНК для MRPS18-1. **Висновки:** Тканина гліом та клітинні лінії гліом характеризуються підвищеними рівнями експресії гена MRPS18-2.

Ключові слова: родина MRPS18, MRPS18-1, MRPS18-2, MRPS18-3, гліобластома, клітинні лінії гліом, експресія, біоінформатичний аналіз.