DETERMINATION OF MOLECULAR GENETIC MARKERS IN PROGNOSIS OF THE EFFECTIVENESS OF TREATMENT OF MALIGNANT INTRACEREBRAL BRAIN TUMORS

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Abstract
Intracerebral malignant brain tumors remain one of the most complex problems of neuro-oncology. Today, promising results of the use of targeted drugs have been received, which determine the important diagnostic and predictive value of molecular genetic markers of glial and metastatic brain tumors.

Aim: The study of the prevalence of *MGMT* (O6-methylguanine-DNA methyltransferase) and *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) gene expression by real time polymerase chain reaction in tumor tissue of gliomas and brain metastases.

Materials and methods: From thirty patients were received tumor material (29 cases of glioma III–IV degree of anaplasia and one case of metastatic brain lesion of adenocarcinoma). The normalized expression of *MGMT* and *PTEN* genes was determined by real-time polymerase chain reaction.

Results: In all 30 (100 %) patients with tumor fragments, we determined normalized expression of *MGMT* and *PTEN* genes. In most cases, 53 % of the observations (16 out of 30 patients) showed a low normalized expression of *MGMT* gene (<40 c. u.) and a low normalized *PTEN* expression rate of 73 % (22 out of 30 patients) (<40 c. u.). The average expression level of the MGMT gene in the range from 40 to 100 c. u. (6/20 % of patients) was considered prognostic favourable for the response to temozolomide chemotherapy.

Conclusions: The study of *MGMT* gene expression, a chemotherapy marker for temozolomide, indicates a trend toward correlation between expression levels and therapeutic efficacy. The study of the expression of the *PTEN* gene, the blocker of the PI3K/AKT signal pathway, indicates a different degree of expression of this enzyme in the tumour samples studied. The predictive value of the indicator for target therapy is appropriate in comparison with the EGFR mutation. Further profound analysis of the results is required with increasing number of sampling and observation period.

Keywords: malignant brain tumors, glioblastoma, *MGMT* gene, *PTEN* gene, EGFR, personalized medicine.

1. Introduction

Intracerebral malignant brain tumors, regardless of their genesis, primary or metastatic, remain the most painful and unresolved issue of neuro-oncology [1, 2]. Since the 70s of the last century, surgery along with radiotherapy (RT) remains the main methods of treating patients with malignant intracerebral tumors. The combination of these two treatments allowed doubling the survival rate [3, 4]. The survival of patients with malignant gliomas progressed with the development of therapeutic protocols, especially in the introduction of the Stupp protocol of chemotherapy (CT) in 2005 in the clinical practice. According to the Stupp protocol, the temozolomide cytotoxic effect (TMZ) is added to the postoperative RT, followed by TMZ CT for six cycles. This allowed a two-year overall survival rate to be increased to 26.5 % compared with only 10.4 % for post-operative RT in mono-regimen [5]. It is known that TMZ CT in the complex treatment of patients with glioblastoma (GBM) in 39 % of cases increases the average life expectancy of some categories of patients up to 24 months [6]. However, TMZ CT is also widely known, and is associated, first of all, with resistance to this drug. Despite the fact that TMZ CT has been used for more than ten years in the treatment of malignant glial tumors, the causes and mechanism of TMZ resistance development are not fully understood today and are the subject of scientific research [7, 8].

Independent risk factors associated with less long-term survival in malignant glial tumors are age of the patient (≥60 years), non-radical tumour removal, low preoperative functional status of the patient (KPS <70), absence of post-operative RT and CT, reduction of postoperative TMZ CT (<4 years) [10, 11]. In the case of a more favourable prognosis at the molecular level, the presence of methylation of the promoter of the O^6-methylguanine gene of DNA-methyltransferase (*MGMT*) or the presence of mutations in the isocyte dehydrogenase (*IDH*) gene (Hartmann et al. 2013; Reifenberger et al., 2014) may be indicated. It is with the differences in the molecular biology of the tumors that the same histotype tumors are different in their response to therapy. Therefore, in modern neuro-oncology of special diagnostic and prognostic significance acquire molecular genetic markers of tumors, as an essential component of the selection of treatment tactics [12].

The development of molecular biology and the orientation towards personalized medicine have led to a fundamentally new approach in the treatment of cancer patients with the use of molecular targeting drugs that block the proliferation of tumour cells by selective inhibition of its major signalling pathways - ligands, membrane receptors, intracellular proteins, etc. [6].

CT is an important component of the integrated therapy of cancer patients. Heterogeneity of tumors of one histotype according to the molecular mechanism of their development and mechanisms of chemo-resistance induced to personification of therapy. Today, along with tradition-

al cytotoxic chemotherapy, targeted therapy is becoming increasingly important. The essence of the approach lies in the preliminary study of key genetic mutations that trigger uncontrolled cell proliferation and inhibition of apoptosis. Therefore, targeting therapy is based on the blocking of processes that are abnormally activated by mutation [13, 14].

When assigning cytostatics, it is important to determine the expression of the corresponding genes of chemoreceptor. Studies are conducted using biopsy or postoperative tumour tissue. Thus, the use of an antibacterial CT is based on the need for the prescription of chemotherapy, either traditional or targeted, from the standpoint of the current level of knowledge about molecular genetic prediction. The deepening of the search for molecular genetic markers, which allows predicting the effectiveness of therapy of intracerebral tumors, remains relevant in neuro-oncology.

2. Aim of research

The study of the prevalence of *MGMT* (O^6-methylguanine-DNA methyltransferase) and *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) gene expression by real time polymerase chain reaction in glioma tumour tissue and brain metastases.

3. Materials and methods

The research was conducted at the State Institution "Institute of Neurosurgery named after A.P. Romodanova NAMS of Ukraine" (certificate on quality management is registered in the Register of Certification Systems of UkrSEPRO № UA.2.166/0909-15 dated June 10, 2015, valid until June 09, 2020), in the Department of Neurobiology (Certificate of Attestation No. PT-221/14 dated 04.07.2014 till 03.07.2019) which certifies that the department is certified on the basis of the Law of Ukraine "On Metrology and Metrological Activity", meets the criteria for attestation of measuring laboratories in accordance with the Rules of Authorization and Certification in the State Metrology System. The department is certified for carrying out measurements of the indicators of the objects according to the industry, which is given in the appendix to the certificate and is an integral part of it.

All patients included in the study were treated at the State Institution "Institute of Neurosurgery named after A.P. Romodanova NAMS of Ukraine".

The study included patients with different tumour brain lesions: both primary and metastatic. Surveyed and operated to obtain tumour material for further studies of 30 patients from 04.2017 to 09.2018: 1 case of anaplastic astrocytomas (AA); 26 cases of GBM IV grade anaplasia, of which 22 cases was first-time GBM diagnosis; 3 cases of continued growth (recurrent) GBM; 1 case of secondary GBM; 1 case of prolonged growth of anaplastic oligodendroglioma (AOG); 1 case of anaplastic ganglioglioma; 1 case of metastatic adenocarcinoma in the brain. Two of the 30 patients with GBM, were dropped out of observation, the observations of the remaining 28 patients are known.

All patients were informed about the conduct of research and informed consent to their implementation. The research was carried out in accordance with the ethical norms adopted by the Ukrainian legislation.

Polymerase chain reaction (PCR) method in real time

Material for the study was obtained from fragments of brain tumour tissue extracted during surgical intervention. Tissue immediately after sterile removal was treated as follows: the fragments of blood vessels and necrotized fragments were removed, the purified tissue was placed in sterile cryotubes and stored in liquid nitrogen at a temperature of – 196 °C until the time of use in the experiment.

Isolation of total RNA

The total RNA was isolated from samples of tissues frozen in liquid nitrogen using the commercially available PureLink RNA Mini Kit Kit (Applied Biosystems, USA), respectively,

the kit instructions. Selection steps consisted of lysis of cells, RNA binding to the membrane, purification of RNA and elution. Guanidine isothiocyanate is included in the prevention of degradation of RNA into the lysis and washing buffers. The isolated RNA was placed on ice, checked for quality and used immediately for the synthesis of cDNA in the reverse transcription reaction.

Determination of the quality of the isolated RNA

The absorption spectrum of the total RNA solution was roughly estimated by the degree of purification of RNA from proteins and guanidine. The ratio of 260 nm/280 nm should be between 1.9 and 2.1. Samples that had lower or higher values were not included in the experiment. The RNA was evaluated by absorbance at 260 nm on a spectrophotometer.

Synthesis of the first chain of cDNA

The cDNA was obtained by kit of Tag Man reverse transcription reagents (Applied Biosystems, USA) using the reverse transcription reaction, respectively, with the kit instructions. The reaction was carried out in a volume of 20 μl. OligodT primers were used as primers.

Determination of the level of expression of genes by real-time PCR

The level of expression of *MGMT* (Hs01037698_m1) and *PTEN* (Hs02621230_s1) genes was analyzed on a real-time PCR fluorescence detection system of the SFX96 Touch (BioRad, USA) using Tag Man Universal PCR Master Mix (Applied Biosystems, USA) and Tag Man Gene Expression Assays (Applied Biosystems, USA) on the following protocol:

Primary denaturation 95 °C – 10 minutes (1 cycle).

Amplification cycle (×40):

– denaturation 95 °C – 15 sec;

– annealing of primers, elongation and fixation of fluorescence signal 60 °C – 1 minute.

All reactions were carried out in separate test tubes. The volume of the reaction mixture was 20 μl.

To evaluate the reproducibility of the threshold cycle values, all samples with each pair of primers were amplified in double repetition. The difference between the samples was no more than 0.5 cycle.

Accumulation of amplification products was determined using oligonucleotide samples of the TagMan type (probes), complementary to the central portion of the amplified transcript fragment. The FAM is attached to the 5 'end of the probe, while the 3' end carries the MGB shield. Upon amplification of the transcript there is a separation of the fluorophore and the absorber and the device fixes the fluorescence signal. The oligonucleotide samples that were used to determine the expression of the genes studied are shown in **Table 1**.

To check the absence of amplification of transcript regions of the genes studied on possible impurities of genomic DNA in the sample, parallel reactions of amplification of the RNA specimen without reverse transcription and cDNA were pre-performed.

Table 1

Nucleotide sequence of fluorescence probes used to determine the level of gene expression

Gene	Sequence of the oligonucleotide test
MGMT	5'gcaanccngnccccatcctcatccc 3'
PTEN	5'atgctgcacagaaattttcaatttg3'
GUSB	5'gctactacttgaagatggtgatcgc3'

For the real time PCR method, the gene of glucuronidase *GUSB* (Hs00939627_m1), which belongs to the family of "housekeeping genes", has been selected as the reference

gene for the gene reference *MGMT* and *PTEN,* which is characterized by a stable expression level among various specimens. It is assumed that the change in the amount of its cDNA depends only on the influence of additional factors that determine the kinetics of reverse transcription. This allows comparing the level of gene expression between different samples.

The data analysis was performed at the threshold fluorescence, which is the main criterion for evaluating the results. It describes a certain cycle of PCR (Ct), which shows a significant increase in fluorescence compared with the background level and begins the exponential phase of the PCR cycle.

The absolute number of copies of the mRNAs of the *MGMT* and *PTEN* genes in tumour samples was determined using the tenfold dilutions of a fragment of the *MGMT* gene enclosed in the plasmid and constructed on the basis of these data of the standard curve. GeneArt Gene Synthesis (Invitrogen, Thermo Fisher Scientific, Germany) was used for this purpose. The calculations used the equation of the curve $y=-0.3415x+13.275$. The difference in the level of gene expression between the specimens was evaluated at the normalized number of copies of the mRNAs of the *MGMT* and *PTEN* genes relative to the number of copies of the housekeeping gene, *GUSB*.

4. Results

In all 30 (100 %) patients with tumour fragments, we determined normalized expression of *MGMT* and *PTEN* genes. Table 2 presents the results of the determination of normalized expression of *MGMT* and *PTEN* genes in the tissue of glial tumour fragments and brain metastases extracted during surgical removal, further adjuvant treatment was traced. In 18 (69 %) of 26 patients with GBM, adjuvant radiotherapy (RT) was conducted with concomitant TMZ CT. In 14 (54 %) of 26 patients with GBM after completion of postoperative adjuvant RT, TMZ CT was performed.

In 16 (53 %) out of 30 examined patients, a low normalized expression of *MGMT* gene (<40 c. u.) was observed. In 6 (20 %) patients, this indicator was in the range of 40 c. u. to 100 c. u. The use of TMZ in such cases is considered appropriate and effective, as evidenced by overall survival (OS) in the study group of patients (Table 2).

In one case, in a patient with a recurrent GBM (observation No. 27, Table 2), the normalized expression of *MGMT* gene significantly exceeded this indicator and amounted to 2,292.8 c. u. in the remaining patients, which is a prognostic negative factor. In this observation, the recurrence-free period (RFP) was 18 months, overall survival (OS) was 25 months; the patient died 7 months after the onset of relapse.

In the case of secondary GBM (observation № 25, Table 2), the normalized expression of the *MGMT* gene was 10.3 c. u., RT was performed with concomitant TMZ CT, in the subsequent treatment of 6 courses of adjuvant TMZ CT. This patient is alive, the observation lasts 22 months.

In our observation group, in the majority of patients, a low normalized expression of the *PTEN* gene (<40 c. u.) was detected in 22 (73 %) cases. In the remaining 8 (27 %) patients, the normalized expression of the *PTEN* gene exceeded 40 c. u. and ranged from 61.6 c. u. to 21390.5 c. u. In this case, the correlation between normalized expression of *PTEN* gene and the survival rate of patients, nor the level of normalized expression of *MGMT* gene has not been established. The highest normalized index of *PTEN* gene expression is 21390.5 c. u. was observed in a patient with GBM, which is still alive, is observed for 10 months, data for relapse of the tumour has not been received (observation № 19б **Table 2**). Two patients with GBM with high OS 22 months, who are still alive (observation № 1 and 8, **Table 2**), had the same normalized expression of *PTEN* gene – 0.001 c. u. At the same time, they differed in the level of expression of the gene *MGMT* – 3.1 c. u. and 22.5 c. u. in accordance. Both cases belong to a group that has a low normalized expression of *MGMT* gene (<40 c. u.) and accounts for most observations (53 %).

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Table 2

Expression of *MGMT* and *PTEN* genes in the tissue of glial and metastatic brain tumor fragments in RT-treated patients using TMZ CT in concomitant and / or adjuvant regimens

No.	Date of operation	Sex	Age, years	Diagnosis	Localization/ Lesion side	Radicality of extraction	RT (Dose, Gy) +/– TMZ	ACT	Overall survival, OS (months)	Non-recurrence period, NRP (months)	Normalized expression of MGMT gene (c. u.)	Normalized expression of PTEN gene (c. u.)	Disposals
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	13.07.17	male	56	GBM	parieto-occipital/ left	subtotal	60+TMZ	TMZ 6 courses	22	12	**3.1**	**0.001**	alive (radiosurgery 07/2018)
2	21.06.18	female	48	GBM	frontoparietal/ left	frac-tional	60+TMZ	TMZ 2 courses	11	11	**3.3**	**0.1**	alive
3	13.06.18	female	50	GBM	fronto-callous/ right	frac-tional	63+TMZ	CCNU 1 course	11	11	**7.3**	**0.2**	alive
4	27.07.17	female	68	GBM	two side parietal-callous	frac-tional	–	–	9	6	**13.5**	**61.6**	died
5	26.06.17	male	39	GBM	posterior-frontal/right	subtotal	60+TMZ	TMZ 3 courses	13	7	**17.4**	**0.1**	died
6	04.07.17	female	36	GBM	frontal-callous/ left	frac-tional	60+TMZ	TMZ 3 courses	6	6	**17.9**	**2.0**	died as a result of pulmonary artery thrombosis, death that is not related to the underlying disease
7	08.08.18	male	80	GBM	temporoparietal/ left	subtotal	40+TMZ	–	9	3	**19.8**	0.3	alive
8	27.07.17	male	64	GBM	subcortical frontal/right	subtotal	60+TMZ	–	22	15	**22.5**	**0.001**	alive
9	18.12.17	male	56	GBM	corpus callosum/ left	frac-tional	60+TMZ	TMZ 6 courses	18	18	**26.30**	**1.7**	alive
10	20.11.17	male	61	GBM	frontotemporal/ left	frac-tional	–	–	–	–	**32.8**	**229.0**	unknown (the occupied regions of the Donetsk region)
11	05.07.17	male	55	GBM	frontal medial/ right	subtotal	60+TMZ	CCNU 5 courses	22	22	**39.6**	1.3	alive
12	03.04.17	male	37	GBM	frontal medial occipital/right	subtotal	60+TMZ	TMZ 6 courses	11	8	**40.6**	**0.5**	died
13	30.05.18	male	43	GBM	temporal/right	total	60+TMZ	TMZ 3 courses	12	12	**48.6**	**0.2**	alive
14	13.04.17	male	53	GBM	frontotemporal/ right	subtotal	60+TMZ	TMZ 5 courses	25	12	**49.8**	**0.4**	alive
15	12.04.17	male	40	GBM	frontal/right	subtotal-frac-tional	60+TMZ	TMZ 6 courses	25	17	**70.4**	1.45	alive
16	18.12.17	male	47	GBM	temporal/right	total	60–TMZ	TMZ 3 courses	17	17	**89.8**	**259.7**	alive

Continuation of Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
17	20.06.18	male	70	GBM	frontal medial/right	subtotal	60+TMZ	TMZ 2 courses	11	11	**90.5**	**24.6**	alive
18	12.12.17	female	37	GBM	frontal/left	total	–	–	–	–	**105.9**	**2747**	unknown
19	30.07.18	female	49	GBM	frontal medial/right	subtotal	60+TMZ	–	10	10	**118.3**	21390.5	alive
20	27.11.17	male	67	GBM	frontotemporal parietal/right	subtotal	60–TMZ	–	18	5	**125.6**	**651.6**	alive
21	23.04.18	female	62	GBM	partial temporal subcortical/left	subtotal	60+TMZ	TMZ 4 courses	13	13	**192.6**	**13.1**	alive
22	27.04.17	female	66	GBM	temple/right	total	60+TMZ	TMZ 3 courses	13	12	**251.6**	**2.7**	died
23	13.06.18	female	65	GBM with oligodendroglial component	temporal subcortical/left	fractional	–	–	–	–	**9.0**	**0.8**	died in the postoperative period, RT and ACT were not conducted
24	29.06.17	male	52	AA	frontotemporal/right	subtotal	–	–	–	–	**125.0**	**8.6**	died in the postoperative period, RT and ACT were not conducted
25	24.07.17	female	50	secondary GBM	frontal/right	total	60+TMZ	TMZ 6 courses	22	22	**10.3**	**0.2**	alive
26	11.05.18*	male	52	cont. growth. GBM	temple/right	total	–	–	5**	5**	**20.5**	**390.8**	died
27	26.02.18*	female	58	cont. growth. GBM	temple/left	fractional	–	–	7**	7**	**2292.8**	**164.6**	died (RFP after the first operation 18 months)
28	02.04.18*	female	42	cont. growth. (AOD)	temple/right	total	60–TMZ	PCV	13	13	**205.1**	**9.6**	alive
29	30.05.18	female	62	Adenocarcinoma metastasis	sincipital/right	total	60–TMZ	CCNU	12	12	**5.3**	**0.001**	alive
30	14.09.18	female	43	Anaplastic ganglioglioma	parietomedial/right	fractional	60–TMZ	PCV	8	**8**	**6.8**	**0.1**	alive

*Note: * – date of surgery for tumour recurrence (re-operation); ** – survival in the post recessional period; MGMT (O^6-methylguanine-DNA-methyltransferase); PTEN (phosphatase and tensin homologue deleted on chromosome 10); RT (radiotherapy); CT (chemotherapy); TMZ (temozolomide); OS (overall survival); RFP (recurrence-free period); ACT (adjuvant chemotherapy); CCNU (lombustine); Dose, Gy – total dose of RT in Gray*

5. Discussion

As a result of our work, the prevalence of expression of *MGMT* and *PTEN* genes by real-time PCR in glandular tumor tissue and brain metastases has been studied. In all 30 (100 %) patients with tumor fragments, we determined normalized expression of *MGMT* and *PTEN* genes. In most cases, 53 % of the observations (16 out of 30 patients) showed a low normalized expression of *MGMT* gene (<40 c. u.) and a low normalized expression of *PTEN* gene – 73 % (22 out of 30 patients) (<40 c. u.). In this case, correlation of normalized expression of *PTEN* gene with survival of patients and level of normalized expression of *MGMT* gene has not been established. In 6 (20 %) patients, normalized expression of the gene *MGMT* varied in the range of 40 c. u. up to 100 c. u. Such cases were considered prognostic favorable for the response to TMZ CT, which is confirmed

by survival rates in the study group. Our results indicate a correlation between the level of expression of the *MGMT* gene and the efficacy of TMZ CT.

Unfortunately, in our work, due to the small sample size, we were unable to analyze the statistical significance of the results obtained, as well as to estimate the predictive significance of the expressions of the *MGMT* gene and the *PTEN* gene. This is a weak link in our study and requires further analysis of the results with an increase in the number of samples and the period of observation.

It would also be interesting to investigate the mutations in the *EGFR* gene (epidermal growth factor receptor, ErbB-1) in the samples presented, the correlation of this mutation with the level of expression of the *PTEN* gene, and to analyze their prognostic value for assessing the effectiveness of *EGFR* targeting therapy in neuro-oncology patients.

Thus, there is a need to continue such a study, deepen the analysis and implement the results in clinical practice.

The subject of our research is relevant in neuro-oncology and corresponds to the demands of modern scientific research. To date, molecular biology has identified several genetic loci in the human genome, whose functioning determines the effectiveness of antitumor effects of CT and RT. Deepening understanding of the molecular-biological properties of brain tumors leads to the expansion of therapeutic options, primarily targeted therapy. In recent years, the number of publications devoted precisely to the clinical aspects of molecular biology has increased.

Yes, Brandes A.A. and co-authors (2017) studied the role of MGMT methylation status at various stages of GBM pathomorphism. It has been determined that one of the key factors to be taken into account when treating malignant glioma is the functioning of the gene of the reparative enzyme *MGMT*. The gene of the reagent enzyme, *MGMT*, rapidly resumes damage to the DNA of cells by glioma (and all other proliferating cells of the patient's body) after the effects of the alkylating agent. The presence of *MGMT* gene expression depends on the functioning of other genes (for example, DNA gene promoter of methylation), some gene ensembles that affect apoptotic processes, etc. [15].

Published in 2018, the work of Nguyen, H. S., and co-authors is devoted to the study of molecular markers of resistant GBM and ways to overcome therapeutic resistance [16]. The authors emphasize that the study of expression of the gene *MGMT*, a product that reduces the effectiveness of alkylating cytostatics, is necessary for the administration of TMZ CT.

The main modern trend in neuro-oncology is the person-oriented approach in the appointment of antibacterial chemotherapies. First of all, this is due to the heterogeneity of the tumors of one histotype by the molecular mechanism of their development and mechanisms of chemo-resistance. The basis of targeted therapy, unlike traditional cytotoxic chemotherapy, is the blocking of processes that are abnormally activated due to mutations.

In the cells of many tumors, EGFR abnormal receptors are detected as a consequence of the mutation of this gene. In cells with mutation activation of the signaling pathway of EGFR occurs, which, in turn, initiates the processes of malignant transformation in most tumors. The presence of gene mutations in the EGFR allows the selection of a group of patients who may have a positive response to the inhibitor of the tyrosine kinase of the epidermal factor receptor. The work of Kwatra M. M. and co-authors (2017) is devoted to the analysis of EGFR targeting GBM therapy [17]. The EGFR transmembrane receptor binds to extracellular ligands belongs to the family of transmembrane receptors: EGFR (ErbB-1), HER2 / c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4), the intracellular part of which exhibits tyrosine kinase activity. The amplification of the *EGFR* gene is found at 60 % GBM, in the half of GBM, hyperfragmentation of *EGFR* is observed. Studies with blockers and antibodies to *EGFR* (Erlotinib, Gefitinib, Nimotuzumab) are currently ongoing and require more advanced scientific research [17, 18].

Eskilsson E. and co-authors in 2018 presented a work concerned with *EGFR* heterogeneity GBM and therapeutic aspects of this problem. Such studies provide an opportunity for the proper administration of a specific inhibitor of tyrosine kinase - erlotinib and hefitinib [19].

Elsamadicy A. A. and co-authors (2017) show positive results of the use of the EGFR vIII (Rindopepimut (CDX-110)) protein in the vaccine against line I therapy in patients who did not receive TMZ. At this time, a separate type of *EGFR-EGFR* variation-*EGFR* version III mutation, which occurs in 20 % GBM, is highlighted. It should be emphasized that the best results in this study were obtained in patients with unmethylated *MGMT* [20].

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Recent studies have shown the expediency of molecular genetic prediction of the effectiveness of target-therapy of the brain tumors with the detection of mutations in the gene of *EGFR* (abnormal splicing and mutations in 18–21 exons, only 19 mutations) and expression of the *PTEN* gene. Recent work has been published that thoroughly the role of the PTEN protein in neuro-oncogenesis has been determined. In articles by Kang Y. J. and Benitez J. A., the molecular-biological significance of the PTEN protein and mutations in the *PTEN* gene [21, 22] is analyzed in detail. The PTEN protein encoded by the *PTEN* gene plays a role in the regulation of the cell cycle, is a tumor suppressor and functions as phosphatase. The main function of PTEN is the inhibition of the signal path controlled by phosphoinositol-3-kinase - one of the main pathways responsible for proliferation and cell survival. PTEN is part of a signaling pathway that stops cell division and induces apoptosis. Apoptosis is that of uncontrolled cell growth and progression of the oncological process. With malignant transformation, the cells accumulate mutations that ensure the progression of tumors. Mutations in the *PTEN* gene lead to the development of many tumors. The genetic inactivation of PTEN is indicated for GBM, uterine cancer, prostate cancer, and a decrease in PTEN activity is observed in lung cancer, breast cancer, and others.

In 2014, a meta-analysis was conducted on the study of the *PTEN* gene and its predictive value in gliomas [14]. It has been shown that the *PTEN* gene study is needed to evaluate the efficacy of EGFR targeting therapy, which is one of the few negative PI3K/AKT/mTOR signaling pathways and plays an important role in cellular proliferation, apoptosis and tumor invasion regulation. The sensitivity to therapy of brain tumors with EGFR inhibitors is significantly higher when coexpressed by the EGFRvIII oncogene and the PTEN tumor suppressor. In this case, the loss or mutations of the PTEN gene make the tumor resistant to EGFR inhibitors, as the PI3K/AKT/mTOR signaling pathway is not excluded. It has been established that a decrease in *PTEN* expression is a negative predictive marker for GBM survival, as well as a therapy response marker.

All this confirms the relevance of our research and the feasibility of further scientific research, aimed at studying the molecular genetic features of intracerebral tumors.

The authors report that there is no conflict of interest.

6. Conclusions

1. A study of MGMT gene expression, a marker of temozolomide chemotherapy sensitivity, indicates a trend towards correlation between expression levels and therapeutic efficacy. To determine statistically significant prognostic effects and informativeness of MGMT expression, it is necessary to expand the statistical sample and further studies with a larger number and observation period.

2. A study of the expression of the PTEN gene, the PI3K/AKT signalling pathway blocker, indicates a different degree of expression of this enzyme in the tumour samples studied. The predictive value of this indicator for targeting chemotherapy is possible in comparison with the EGFR mutation in these tumour tissue samples.

3. The study of molecular biological characteristics of intracerebral tumors and the implementation of these data in clinical practice provides the opportunity to develop more effective therapeutic protocols based on a personified approach to the treatment of neuro-oncology patients.

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