

microRNA interaction with MAPK and AKT pathways in paediatric brain tumours – preliminary results and review of the literature

Renata Gruszka¹, Krzysztof Zakrzewski², Paweł P. Liberski¹, Magdalena Zakrzewska¹

¹Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Lodz, Poland, ²Department of Neurosurgery, Polish Mother Memorial Hospital Research Institute in Lodz, Lodz, Poland

Folia Neuropathol 2020; 58 (2): 123-132

DOI: <https://doi.org/10.5114/fn.2020.96734>

Abstract

Nowadays molecular investigations have a significant impact on the understanding of primary brain tumour biology, as well as on their classification and progress in the treatment modalities. Among novel type of biomarkers with potential therapeutic value, microRNAs (miRNAs) are considered in some cases. miRNAs are small molecules regulating gene expression, including genes encoding key proteins involved in signalling pathways responsible for growth and cell survival during tumour formation. Incorrectly hyperactivated pathways implicated in brain tumour development are inter alia the PI3K/AKT/mTOR and RAS/MAPK/ERK cascades associated with worse prognosis and decreased patient survival. This work presents relationships between changes in the expression of individual miRNAs and the genes involved in the regulation of PI3K/AKT/mTOR and RAS/MAPK/ERK signalling pathways in primary brain tumours. Herein we present the preliminary results of miR-17-5p and miR-20a (key representatives of the miR-17-92 oncogenic cluster) expression analysis and their connection with signalling pathway activation in two of the most frequent paediatric tumours: medulloblastoma and ependymoma. Our study was performed using the microarray and qPCR techniques and showed PI3K/AKT/mTOR and RAS/MAPK/ERK among the forefront of the list of pathways with the largest number of genes involved in their activation compared to the control. Predicted target analysis indicated the agents from miR-17-92 cluster within miRNAs regulating activity of PI3K/AKT/mTOR and RAS/MAPK/ERK deregulated genes. The expression level of key representatives of the oncogenic cluster, miR-17-5p, and miR-20a, increased with the WHO grade of the analysed cases; the highest levels were found in medulloblastomas.

Key words: brain tumour, children, ependymoma, expression, medulloblastoma, miRNA, PI3K/AKT/mTOR, RAS/MAPK/ERK.

Introduction

Primary brain tumours are a histopathologically diverse group of lesions classified mainly due to their clinical features, radiological image, and histological appearance. Currently, due to the novel World Health Organisation (WHO) guidelines, several molecular

changes are incorporated into diagnostics as often as possible [3,21,25]. Until now different types of molecular markers have been recognised, but despite that, more information about further genomic alterations is still needed to improve patient's treatment and overall survival. Here we report our preliminary data

Communicating author

Renata Gruszka, Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Pomorska 251, 92-213 Lodz, Poland, e-mail: renata.gruszka1@stud.umed.lodz.pl

showing the correlation between microRNAs (miRNAs) and the genes involved in the RAS/MAPK/ERK and PI3K/AKT/mTOR pathways with an in-depth literature review on a specific topic.

miRNA in primary brain tumours

miRNAs comprise a group of endogenous, non-coding, single-stranded sequences that influence the activity of genes and pathways as well as many cellular processes [25]. The miRNA inhibits translation by blocking specific mRNA sequences, which does not cause permanent modification of the DNA; therefore, it is considered to be an integral part of the epigenetic machinery [7]. Many studies have underlined miRNA alterations in brain tumours and the possibility of developing an miRNA-based therapy. However, the complexity of the disease has limited the diagnostic and prognostic power of miRNAs so far.

It has been noticed that miRNAs could be involved in canonical signalling pathway activation. One member of the oncogenic miRNA family that was previously validated was the miR-17-92 cluster, known also as OncomiR-1 due to its strong link to oncogenesis. It was found to be deregulated in some solid tumours [5]. Recent analyses showed that the components of the cluster were involved in the modulation of RAS/MAPK/ERK and PI3K/AKT/mTOR signalling pathways activated in many types of neoplasms including brain tumours [9,15,32].

RAS/MAPK/ERK and PI3K/AKT/mTOR pathways in primary brain tumours

The RAS/MAPK/ERK and PI3K/AKT/mTOR are critical molecular signalling pathways involved in key cellular processes, which play a fundamental role in the pathogenesis of brain tumours [11,12,17,30,31,35]. Both pathways are activated by receptor tyrosine kinases (RTKs), and a lot of defined genomic alterations stimulate both pathways simultaneously [14]. Hence, dual inhibition of the pathways is promising for the development of novel therapeutics [14].

The RAS/MAPK/ERK pathway is a signal transduction mediated by a cascade of mitogen-activated protein kinases (MAPK) [6]. The cascade stimulus is an extracellular factor that is a growth factor that binds to a transmembrane receptor with tyrosine kinase activity. In brief, RAS-GTPases activates RAF serine-threonine kinases, which activates mitogen-activated protein kinases 1/2 (MAP2K1/2 or MEK1/2).

MEK1 and MEK2 phosphorylate their two known substrates, ERK1 and ERK2, respectively. Active ERK proteins translocate to the nucleus, where they are responsible for activating the transcription factors and inducing expression of specific genes involved in the cell cycle, apoptosis, differentiation, and migration [6,28].

The PI3K/AKT/mTOR pathway plays a fundamental role in cellular processes that promote proliferation, growth, angiogenesis, cell survival, and metabolism in response to extracellular signals [14]. PI3K is a lipid kinase that catalyses the formation of PIP3, the key protein, which interacts with AKT on the cell membrane, where it undergoes phosphorylation and activation. After activation, AKT regulates function by activating phosphorylation or suppressing a wide range of proteins involved in cellular processes. One of the molecules regulated by AKT is serine-threonine kinase mTOR [8,10]. Stimulation of the PI3K pathway includes growth receptors with an intracellular tyrosine kinase domain, G-related receptors (G protein coupled receptors – GPCRs), proteins responsible for cell adhesion (e.g. integrins), and oncogenes such as RAS [10]. The PI3K/AKT/mTOR pathway is highly complex with multiple levels of feedback inhibition, and it shows significant permeation with the RAS/MAPK/ERK cascade [10].

Functioning RAS/MAPK/ERK and PI3K/AKT/mTOR pathways ensure the preservation of basic physiological processes. Many studies have suggested that both pathways are activated in parallel, have many common points, and affect each other at various stages of signal transmission, both negatively and positively, resulting in a dynamic and complex cross-talk [1]. Both cascades integrate the signals responsible for brain development, maintenance, memory, repair, and cellular plasticity in adulthood [27,33].

miRNA and signalling pathway interactions in primary brain tumours

On the basis of molecular analyses and *in vitro* studies, miRNA has been indicated as one of the important factors modulating cellular signalling pathway activity. The majority of the scientific data concern adult patients and tumours of glial origin (Fig. 1).

Analyses of glioblastoma (GBM) cell lines showed that suppression of miR-92b inhibits cell viability, migration, and invasion and causes an increased apoptosis rate. The bioinformatics analysis indi-

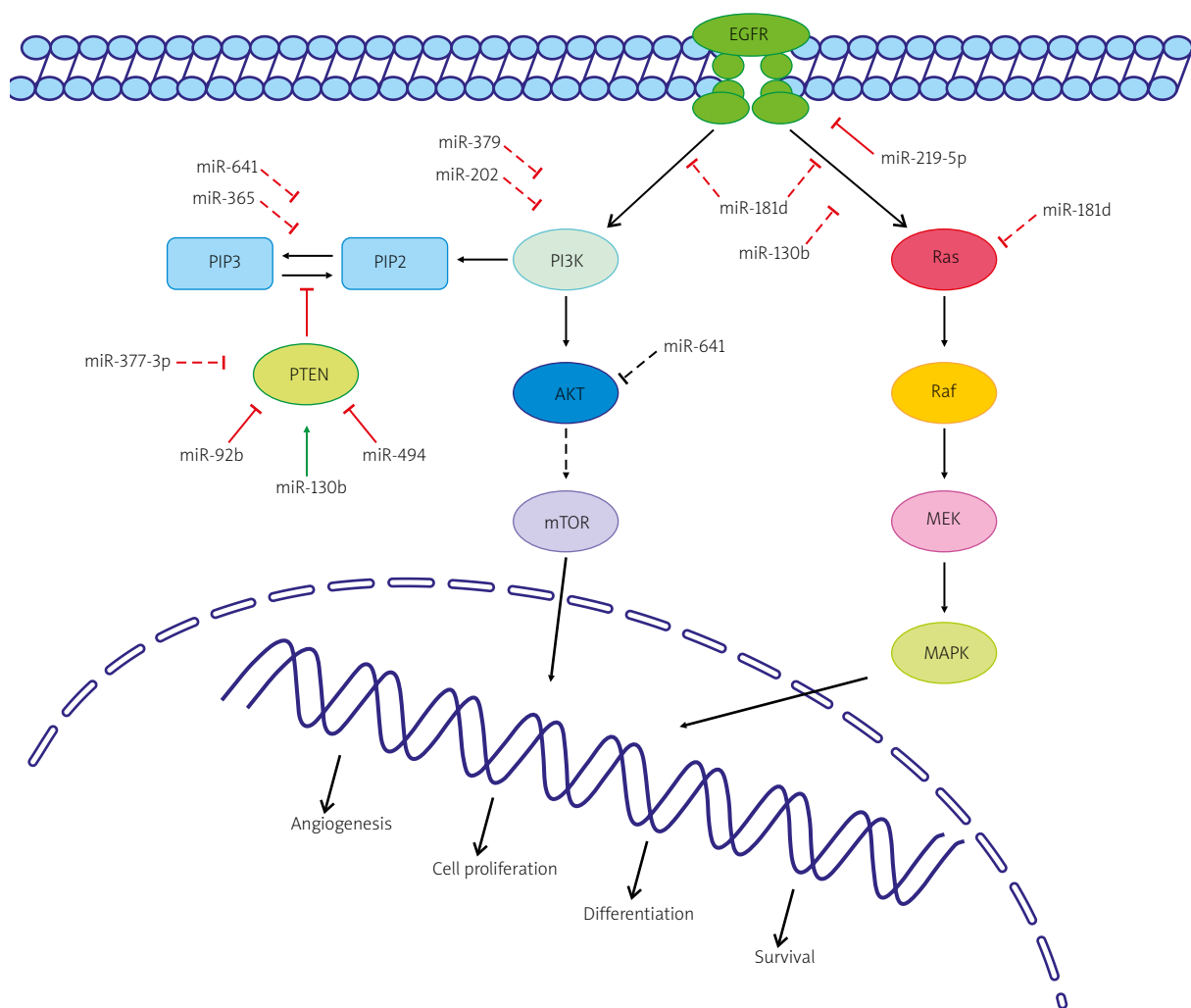


Fig. 1. Representation of miRNA-signal pathway interactions in gliomas. Solid line arrows – direct action, dashed line – indirect action, green arrow – stimulatory effect, red arrow – inhibitory effect (created based on cited literature).

cated *PTEN* as a potential target for miR-92b [31]. It turned out that *PTEN* is also the predicted target for miR-494, for which increased levels in the serum of patients with GBM were correlated with decreased survival [12]. *PTEN* negatively regulates the PI3K/AKT/mTOR pathway by phosphorylation of PIP3 in favour of PIP2, which consequently inhibits the phosphorylation of AKT. These data suggested that both miR-494 and miR-92b could bind to *PTEN* and downregulate the signal transduction [12,31].

Gu *et al.* demonstrated that expression of miR-130b in GBM samples and cell lines was significantly increased compared to controls. Inhibition of miR-130b caused suppression of proliferation and migra-

tion of tumour cells *in vitro* and inhibition of tumour growth *in vivo* [11]. In addition, reduction of its expression increased the level of *PTEN* and inhibited PI3K/AKT/mTOR pathway [11].

Modification of PI3K/AKT/mTOR activity was also observed for the *LASP1* gene. Liu *et al.* focused on the role of miR-377-3p during glioma progression and identified *LASP1* as its direct target [19]. While overexpression of *LASP1* results in activation of the pathway, its knockdown inhibits glioma cell proliferation. The latter could be achieved by miR-377-3p, recognised as a potential therapeutic target [19].

Zhu *et al.* indicated the role of miR-365, which was linked to the initiation and development of

many cancers, as a therapeutic target also for glioma. The molecule suppressed glioma cell proliferation, migration, and invasion. The immediate target for miR-365 is the *PIK3R3* gene, expression of which is inversely correlated to the miRNA level. The overexpression of miR-365 significantly reduces the expression of the *PIK3R3* protein *in vitro* and suppresses tumour growth *in vivo*. Thus, miR-365 mediates the regulation of the PI3K/AKT/mTOR by direct regulation of the *PIK3R3* expression [43].

miR-641 also regulates the expression of *PIK3R3* and additionally *MAPKAP1*. In glioma samples decreased levels of miR-641 were accompanied by increased levels of *AKT2*. However, the *AKT2* gene did not transpire to be the direct target for miR-641. Studies of kinases known to phosphorylate and activate *AKT2* showed that overexpression of miR-641 leads to a reduction in the expression levels of *PIK3R3*, *PDK2*, and *MAPKAP1*, whereas expression of mTOR and RICTOR remains constant [13].

A similar negative feedback loop was noted for miR-29a and *TRAF4* [20]. With the increase in malignancy of gliomas, the expression of miR-29a decreases. The overexpression of miR-29a in cell cultures leads to a reduction in cell proliferation and migration, while inhibition of miR-29a was linked with increased invasive activity of cells. miR-29a could directly bind to *TRAF4* to inhibit gene and protein expression, thereby suppressing PI3K/AKT/mTOR signalling in GBM [20].

Other molecules with documented role in brain tumours are miR-379 and miR-202, which are significantly reduced in GBM cell lines and tissues [18,38]. Their target gene *AEG-1/MTDH*, was overexpressed in a large number of brain tumours, in which they were shown to promote tumour growth and invasion [18,38]. miR-379 attenuates glioma progression by direct targeting of *AEG-1/MTDH* and by indirectly regulating the PI3K/AKT/mTOR pathway [18].

Comprehensive studies of pAKT expression in GBM showed linking of miR-181d to both PI3K/AKT/mTOR and RAS/MAPK/ERK pathways associated with KRAS [35]. Similarly, miR-181b expression was altered in GBMs [30]. Studies of biological functions of miR-181b have shown that overexpression of miR-181b *in vitro* reduces cell growth, decreases the ability to migration, and inhibits normally strong invasive cell capability. After a bioinformatics search for potential targets of miR-181b, insulin-like growth factor-1 receptor (IGF-1R) was selected for further

analysis. It was shown that miR-181b overexpressing cells have low levels of IGF-1R protein, which is inversely correlated with miR-181b activity [30]. Studies focused on the effect of miR-181b on the suppression of the PI3K/AKT/mTOR and RAS/MAPK/ERK pathways showed that the levels of pAKT and pERK1/2 were reduced compared to control cells, while no significant reduction in AKT or ERK levels was detected. These data suggest that miR-181b suppressive activity in GBM can be regulated by both pathways [30].

In the current literature there is a lot of further research indicating the presence of potential miRNAs that may be useful as therapeutic agents related to the cell cycle [17,22]. Reduction of the level of miR-130b causes the inhibition of the cell cycle in the G0-G1 phase and the reduction of the process in the G2-S phase. Moreover, transfection with anti-miR-130b promotes apoptosis in glioma cell lines. Analysis of the regulation of the RAS/MAPK/ERK pathway showed that the level of phosphorylated MEK1/2, ERK1/2, MAPK, and JNK1/2/3 decreased significantly in cells transfected with miR-130b inhibitor as compared to control cells, which suggests that miR-130b may play a role in glioma formation *via* the pathway activation [17].

Recent studies have indicated simultaneous regulation of both pathways by altered expression of miRNAs. Rao *et al.* confirmed that increased expression of miR-219-5p reduces proliferation, anchorage, independent growth, and migration due to binding site for miR-219-5p in 3' in-UTR of *EGFR*. The luciferase assay confirmed the direct interaction of miR-219-5p with *EGFR*, which activates PI3K/AKT/mTOR and RAS/MAPK/ERK pathways. Such results indicate that overexpression of miR-219-5p inhibits the pathways' activity; downregulation of miR-219-5p could increase their activity and thereby accelerate the tumour growth [26].

There is evidence showing that the molecules of the miR-17-92 cluster could act by targeting cellular pathways during oncogenesis also in brain tumours [5].

Material and methods

Patients and tissue samples

The analysed group comprised 43 cases of brain tumours classified as WHO grade II infratentorial ependymoma (16), WHO grade III infratentorial ependymoma (13), and medulloblastoma (14) [21].

All patients were under 18 years of age. All tumour tissues stabilised in RNAlater were stored at -80°C . Control material consisted of Human Brain Total RNA (Invitrogen, cat. no. AM7962), which is certified to contain also small RNAs (miRNA, siRNA, and snRNA). The protocol of the study was approved by the Bioethical Committee at the Medical University of Lodz (permit no. RNN/122/17/KE).

RNA isolation and reverse transcription

Total RNA was isolated according to the manufacturer's instructions using a commercially available miRNeasy Mini Kit (Qiagen, Germany). The quantity and purity of RNA were analysed quantitatively and qualitatively. 500 ng of total RNA was used for cDNA synthesis with 5xHiFlex Buffer. For selective conversion of mature miRNA a reverse-transcription reaction mix with 5xHiSpec Buffer was prepared (miScript II RT Kit, Qiagen, Germany).

Microarray profiling

Microarray gene expression analysis was conducted using the Human Genome U133+ PM Array Strip (901569, Affimetrix) on 16 brain tumours (WHO grade II ependymoma – 6, WHO grade III ependymoma – 5, medulloblastoma – 5) and the control Human Brain Total RNA. cDNA was synthesised using reverse transcriptase and an oligo-dT primer. The cDNA was used as a template in the *in vitro* transcription, during which biotin labelled antisense mRNA (cRNA) was synthesised and amplified. Before hybridisation, cRNA was fragmented into 50 to 200 bases. After hybridisation, the GeneChip matrix was washed, stained, and scanned (GeneAtlas™ System, Affymetrix). The results were analysed using the designed software (Transcriptome Analysis Console, Applied Biosystems).

Quantification of miRNA levels

Quantitative real time polymerase chain reaction PCR (qPCR) for miRNA expression analysis was performed using miScript SYBR Green PCR Kit and miScript Primer Assays for the most prominent members of OncomiR-1, miR-17-5p (MS00029274, Qiagen) and miR-20a (MS0003199, Qiagen), in an independent group, which consisted of 16 cases of WHO grade II ependymomas, 13 cases of WHO grade III ependymomas, and 14 cases of medulloblastomas. Mature miRNA levels were normalised to RNU6B

(MS00033740, Qiagen) and SNORD95 (MS00033726, Qiagen). All experiments were performed on the CFX96™ Touch Real-Time PCR Detection System (Bio-Rad, Germany). The PCR reactions for each assay were run in duplicate, and the results were averaged over those analyses. The normalised relative expression level was calculated according to the ddCt method.

Bioinformatics analysis of miRNA predicted targets

The potential miRNAs regulating these genes were examined using the TargetScan 7.2 database (http://www.targetscan.org/vert_72/).

Results

The PI3K/AKT/mTOR and RAS/MAPK/ERK pathway genes are most activated in paediatric brain tumours

Microarray analysis identified deregulated genes, and PI3K/AKT/mTOR and RAS/MAPK/ERK signalling pathways showed the highest differences in the number of genes with altered expression compared to the control. Expression analysis showed that medulloblastoma were the most disturbed group in terms of gene expression relative to control (Fig. 2B). In all examined groups, the PI3K/AKT/mTOR and RAS/MAPK/ERK signalling pathways were at the forefront of the list of pathways with the largest number of activated genes compared to the control (Fig. 2C, D). A list of the genes involved in pathways, their functions, and associated miRNAs are presented in Table I.

Expression of miR-17-5p and miR-20a increase with tumour grade

The statistical analysis revealed that the lowest expression levels of the most prominent members of the miR-17-92 cluster were found in WHO grade II ependymomas, in which 53% and 50% of cases showed an increased level of miR-17-5p and miR-20a, respectively (Fig. 2A). In contrast, the highest expression of miR-17-5p and miR-20a was revealed for medulloblastoma samples, at 100% and 97%, respectively (Fig. 2A). Therefore, the increased miRNA expression level is correlated with tumour type and grade, and, together with data confirming sig-

A

miRNA	Histology	WHO grade	FC	Average	Median	Up	Down
17-5p	EP	II	-0.81 to 1.33	0.23	1.01	53%	47%
	EP	III	-0.40 to 4.00	0.40	1.40	92%	8%
	MB	IV	1.63 to 8.47	4.28	3.58	100%	0%
20a	EP	II	-0.68 to 0.81	0.05	1.00	50%	50%
	EP	III	-0.36 to 5.31	1.06	1.35	71%	29%
	MB	IV	0.99 to 5.16	2.61	2.19	93%	7%

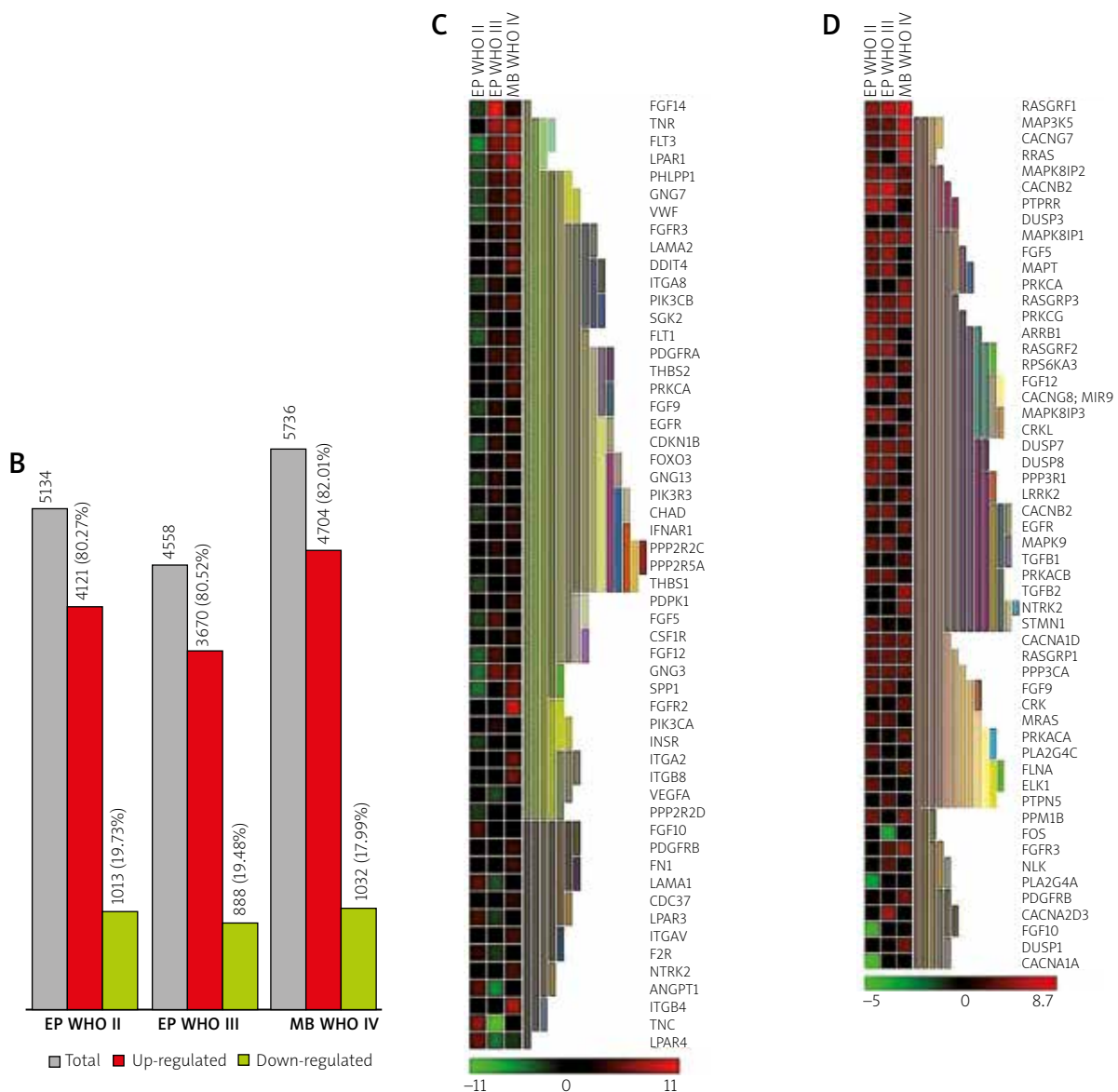


Fig. 2. MiRNA and gene expression analysis. **A)** Analysis of miR-17-5p and miR-20a expression. FC – fold change, Up – percentage of upregulated samples, Down – percentage of downregulated samples. The results are presented as the average of the values obtained in the study group. **B)** Number of differentially expressed genes in paediatric tumours compared to control. Results obtained during microarray analysis. Fold change < -2 – > 2 ; p -value < 0.05 . **C)** Heatmap showing genes with altered expression within PI3K/AKT/mTOR signaling pathway. **D)** Heatmap showing genes with altered expression within RAS/MAPK/ERK pathway. The genes with increased normalised expression are marked in red; the genes with the reduced level of normalised expression are marked in green. Fold change > 3 – < -3 ; p -value < 0.05 . EP WHO II – WHO grade II ependymoma, EP WHO III – WHO grade III ependymoma, MB WHO IV – medulloblastoma.

Table I. List of selected differentiating genes involved in PI3K/AKT/mTOR and RAS/MAPK/ERK signalling pathways detected during microarray analysis. Potential miRNAs involved in gene regulation were generated using the TargetScan database

Gene	Function	miRNA
PI3K/AKT/mTOR		
<i>LAMA2</i>	Associated with outcome of ependymoma	miR-29c-3p, miR-29a-3p
<i>EGFR*</i>	Overexpression occurs in various brain tumours, activation is necessary for the survival, migration, and differentiation of immature cells	miR-133b, miR-133a-3p
<i>FGFR3*</i>	Regulates progenitor cell proliferation and apoptosis during embryonic development	miR-181d, miR-181a, miR-181b, miR-181c, miR-4262
<i>FLT1</i>	Shows tyrosine protein kinase activity, upregulated in brain tumour vasculature	miR-200b-3p, miR-200c-3p, miR-429
<i>PDGFRA</i>	Frequently mutated/amplified in paediatric gliomas, amplification connected with worse prognosis in adults	miR-140-5p
<i>GNG13</i>	Significantly associated with overall survival in astrocytoma patients	miR-5590-3p, miR-142-5p
<i>FGF5*</i>	Gene with oncogenic activity in astrocytic brain tumours	miR-802, miR-5195-3p, miR-145-5p
<i>FGF9*</i>	Exhibits mitogenic activity in gliomas	miR-140-5p
<i>FGF10*</i>	Crucial for brain development	miR-425-5p
<i>VEGFA</i>	Growth factor specific for vascular endothelial cells	miR-205-5p
<i>FOXO3</i>	Overexpression is associated with GB progression	miR-223-3p
<i>PIK3CA</i>	Missense mutations promote glioblastoma	miR-152-3p, miR-148a-3p, miR-148b-3p
<i>PIK3R3</i>	Overexpressed in glioblastoma and ovarian cancers	miR-365b-3p, miR-365a-3p
<i>ITGB4</i>	Upregulated in GB, potential therapeutic target for glioma	miR-9-5p
<i>PIK3CB</i>	Correlated with high incidence and poor survival of glioblastoma recurrences	miR-19b-3p, miR-19a-3p
RAS/MAPK/ERK		
<i>RASGRF1</i>	Highly expressed in the brain, linked to the control of cell growth and neurite outgrowth	miR-139-5p
<i>MAP3K5</i>	Essential role in apoptosis, cell differentiation, and immune responses	miR-20b-5p, miR-17-5p, miR-106a-5p, miR-93-5p, miR-20a-5p, miR-106b-5p
<i>RASGRP3</i>	Highly expressed in glioblastoma	miR-100-5p, miR-99a-5p, miR-99b-5p
<i>DUSP1</i>	Modulates cytokine levels	miR-25-3p, miR-363-3p, miR-92a-3p, miR-92b-3p, miR-367-3p, miR-32-5p
<i>RPS6KA3</i>	Upregulated in GB, high expression correlates with poor survival	miR-218-5p
<i>MAPK8IP2</i>	Downregulated in GB, involved with antiapoptosis	miR-4319, miR-125b-5p, miR-125a-5p
<i>NLK</i>	Plays a role in tumour restriction through WNT pathway and mesenchymal activity in GB	miR-199a-3p, miR-199b-3p, miR-3129-5p
<i>PRKACB</i>	Involved in cell proliferation, apoptosis, gene transcription, metabolism, and differentiation	miR-19a-3p, miR-19b-3p
<i>PRKCG</i>	Potential prognostic factor for GB	miR-338-3p

*Indicated gene also applies to the RAS/MAPK/ERK pathway.

nalling pathway activation, indicates its plausible role in paediatric brain tumours.

miRNAs targets belong to the OncomiR-1 cluster or its paralogues

Deregulated genes belonging to the PI3K/AKT/mTOR and RAS/MAPK/ERK signalling pathways detected during microarray analysis were screened for plausible correlations with miRNAs using the

TargetScan tool. Genes with known functions the origins of brain tumours or brain development and their predicted miRNA targets are listed in Table I. Selected molecules comprised miRNAs from the miR-17-92, miR-106b-25, and miR-106a-363 clusters: miR-19b-3p, miR-19a-3p (*PIK3CB*, *PRKACB*), miR-20b-5p, miR-17-5p, miR-106a-5p, miR-93-5p, miR-20a-5p, miR-106b-5p (*MAP3K5*), miR-25-3p, miR-363-3p, miR-92a-3p, and miR-92b-3p (*DUSP1*).

Discussion

The role of miRNAs as essential regulators of gene expression in development and disease, including cancer, is now intensively studied. There is also evidence confirming that miRNA molecules target cellular cascades and accordingly affect cell proliferation, differentiation, and migration, as well as cancer development, progression, and aggressiveness. Among such factors the miRNA-17-92 cluster was mentioned with its leading members, miR-17-5p and miR-20a.

MiR-17-5p acts as an oncogene, and the analysis of various types of cancers revealed its upregulation in solid tumours [34]. Elevated miR-17-5p levels in adult glioblastoma samples were found to be negatively associated with patient survival [23]. MiR-20a is also commonly upregulated in different types of cancer and has a regulating function in proliferation, differentiation and apoptosis also in brain tumours. The molecule is also regarded as a potential disease biomarker [2,42].

Previous studies have shown a correlation between the expression level of miR-17-5p, 19a-3p, 106b-5p, and tumour malignancy observed in WHO grade II and III infratentorial ependymomas [40]. Continuing this idea in the current study, we analysed a group of paediatric brain tumours comprising WHO grade II infratentorial ependymoma, WHO grade III infratentorial ependymoma, and medulloblastoma (WHO grade IV). We show that PI3K/AKT/mTOR and RAS/MAPK/ERK are among the most activated pathways in the studied group. We also examined the expression levels of miR-17-5p and miR-20a belonging to cluster 17-92, and we found that miRNA levels increased with the WHO grade.

The literature review presented here shows that childhood brain tumours have been less studied in terms of the cellular pathways and miRNA relationship. There are data showing the PI3K/AKT/mTOR and RAS/MAPK/ERK pathways as being the most likely 'targets' of dysregulated miRNA in paediatric low-grade gliomas [4,16]. It was confirmed that miR-139-5p may contribute to the development of childhood gliomas by suppressing *PIK3CA* expression and promoting activation of the PI3K/AKT/mTOR pathway. The overexpression of miR-139-5p in cell cultures resulted in significantly reduced proliferation and caused a decreased level of pAKT and p70 S6K, the mTORC1 activation trait [4].

The miRNA profiling analyses of medulloblastomas showed plausible connection between miRNAs

and signalling pathways associated with cancer, such as RAS/MAPK/ERK [41]. Subsequent studies revealed increased expression of *MYCC* with a simultaneous decrease of miR-494 expression in medulloblastomas [23]. The authors showed that *MYCC* is the target gene of miR-494, which, when overexpressed, reduces the *MYCC* level and inhibits activation of the MAPK signalling pathway [37].

Analysis of the predicted targets for genes selected during microarray profiling showed that several genes involved in the PI3K/AKT/mTOR and RAS/MAPK/ERK pathways are probably regulated by sequences of the miR-17-92 cluster. Up-regulated *MAP3K5* gene proved to be the target gene for miR-17-5p, miR-20a, and also miR-106a-5p, miR-93-5p, miR-20b-5p, and 106b-5p from 106b-25 paralogous molecules from clusters miR-106b-25 and 106a-363. Such an observation suggests the possibility of pathway regulation by the entire OncomiR-1 family.

Another gene regulated by miRNAs, including the miR-92 family (miR-25-3p, miR-363-3p, miR-92a-3p) from a sister cluster, is the *DUSP1* gene. It has been proven that the increased *DUSP1* expression contributes to carcinogenesis in prostate, breasts, lungs, and thyroid cancers. On the other hand, studies on human head and neck squamous cell carcinoma and hepatocellular carcinoma showed its reduced level [29].

The role of miR-17-5p and miR-20a as oncomiRs is proposed here due to the observation of elevated expression in higher grade of the analysed tumours. Such an observation indicates that miRNA molecules should be considered as biomarkers in brain tumours, especially when considering minimally invasive liquid biopsies that can influence treatment. Recent data suggest that anti-miRs targeting miR-17-92 and/or its paralogues may have therapeutic application for the treatment of medulloblastomas [24]. Moreover, recently anti-miR-17 treatment reducing mTOR signalling was shown for genetic diseases, which confirmed the possibility of using the miR-17-92 cluster as a therapeutic target [39].

Further studies concerning miRNA activity in paediatric brain tumours are needed, especially when miRNA strengthens its position as a diagnostic, prognostic, and therapeutic marker.

The work was supported by National Science Centre grant no. 2017/25/N/NZ4/01545 and Medical University of Lodz grant no. 502-03/1-034-04/502-14-375-18.

Disclosure

The authors report no conflict of interest.

References

- Aksamitiene E, Kiyatkin A, Kholodenko BN. Cross-talk between mitogenic Ras/MAPK and survival PI3K/Akt pathways: a fine balance. *Biochem Soc Trans* 2012; 40: 139-146.
- Babu KR, Muckenthaler MU. miR-20a regulates expression of the iron exporter ferroportin in lung cancer. *J Mol Med* 2016; 94: 347-359.
- Bächli H, Ecker J, van Tilburg C, Sturm D, Selt F, Sahm F, Koelsche C, Grund K, Sutter C, Pietsch T, Witt H, Herold-Mende C, von Deimling A, Jones D, Pfister S, Witt O, Milde T. Molecular diagnostics in pediatric brain tumors: impact on diagnosis and clinical decision-making – a selected case series. *Klin Padiatr* 2018; 230: 305-313.
- Catanzaro G, Besharat ZM, Miele E, Chiacchiarini M, Po A, Carai A, Marras CE, Antonelli M, Badiali M, Raso A, Mascelli S, Schimpf D, Stichel D, Tartaglia M, Capper D, von Deimling A, Giangaspero F, Mastronuzzi A, Locatelli F, Ferretti E. The miR-139-5p regulates proliferation of supratentorial paediatric low-grade gliomas by targeting the PI3K/AKT/mTORC1 signalling. *Neuropathol Appl Neurobiol* 2018; 44: 687-706.
- Chakraborty C, Sharma AR, Patra BC, Bhattacharya M, Sharma G, Lee SS. MicroRNAs mediated regulation of MAPK signaling pathways in chronic myeloid leukemia. *Oncotarget* 2016; 7: 42683-42697.
- Cowan KJ, Storey KB. Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress. *J Exp Biol* 2003; 206: 1107-1115.
- Dubuc AM, Mack S, Unterberger A, Northcott PA, Taylor MD. The epigenetics of brain tumors. *Methods Mol Biol* 2012; 863: 139-153.
- Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009; 9: 550-562.
- Fiedler J, Thum T. New insights into miR-17-92 cluster regulation and angiogenesis. *Circ Res* 2016; 118: 9-11.
- Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell* 2017; 170: 605-635.
- Gu JJ, Fan KC, Zhang JH, Chen HJ, Wang SS. Suppression of microRNA-130b inhibits glioma cell proliferation and invasion, and induces apoptosis by PTEN/AKT signaling. *Int J Mol Med* 2018; 41: 284-292.
- Han K, Li Z, Sun P. MicroRNA-494 promotes the proliferation and migration of human glioma cancer cells through the protein kinase B/mechanistic target of rapamycin pathway by phosphatase and tensin homolog expression. *Oncol Rep* 2019; 41: 351-360.
- Hinske LC, Heyn J, Hübner M, Rink J, Hirschberger S, Kreth S. Intronic miRNA-641 controls its host Gene's pathway PI3K/AKT and this relationship is dysfunctional in glioblastoma multiforme. *Biochem Biophys Res Commun* 2017; 489: 477-483.
- Jahangiri A, Weiss WA. It takes two to tango: dual inhibition of PI3K and MAPK in rhabdomyosarcoma. *Clin Cancer Res* 2013; 19: 5811-5813.
- Jiang L, Wang C, Lei F, Zhang L, Zhang X, Liu A, Wu G, Zhu J, Song L. miR-93 promotes cell proliferation in gliomas through activation of PI3K/Akt signaling pathway. *Oncotarget* 2015; 6: 8286-8299.
- Jones TA, Jeyapalan JN, Forsheew T, Tatevossian RG, Lawson AR, Patel SN, Doctor GT, Mumin MA, Picker SR, Phipps KP, Michalski A, Jacques TS, Sheer D. Molecular analysis of pediatric brain tumors identifies microRNAs in pilocytic astrocytomas that target the MAPK and NF- κ B pathways. *Acta Neuropathol Commun* 2015; 3: 86.
- Li B, Liu YH, Sun AG, Huan LC, Li HD, Liu DM. MiR-130b functions as a tumor promoter in glioma via regulation of ERK/MAPK pathway. *Eur Rev Med Pharmacol Sci* 2017; 21: 2840-2846.
- Li L, Zhang H. MicroRNA-379 inhibits cell proliferation and invasion in glioma via targeting metadherin and regulating PTEN/AKT pathway. *Mol Med Rep* 2018; 17: 4049-4056.
- Liu Y, Duan N, Duan S. MiR-29a inhibits glioma tumorigenesis through a negative feedback loop of TRAF4/Akt signaling. *Biomed Res Int* 2018; 2018: 8.
- Liu Y, Gao Y, Li D, He L, Iw L, Hao B, Chen X, Cao Y. LASP1 promotes glioma cell proliferation and migration and is negatively regulated by miR-377-3p. *Biomed Pharmacother* 2018; 108: 845-851.
- Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 world health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol* 2016; 131: 803-820.
- Ma XL, Shang F, Ni W, Zhu J, Luo B, Zhang YQ. MicroRNA-338-5p plays a tumor suppressor role in glioma through inhibition of the MAPK-signaling pathway by binding to FOXD1. *J Cancer Res Clin Oncol* 2018; 144: 2351-2366.
- Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stühler K, Meyer HE, Reifenberger G. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol* 2010; 20: 539-550.
- Murphy BL, Obad S, Bihannic L, Ayrault Q, Zindy F, Kauppinen S, Roussel MF. Silencing of the miR-17-92 cluster family inhibits medulloblastoma progression. *Cancer Res* 2013; 73: 7068-7078.
- Oliveto S, Mancino M, Manfrini N, Biffo S. Role of microRNAs in translation regulation and cancer. *World J Biol Chem* 2017; 8: 45-56.
- Rao SA, Arimappamagan A, Pandey P, Santosh V, Hegde AS, Chandramouli BA, Somasundaram K. miR-219-5p inhibits receptor tyrosine kinase pathway by targeting EGFR in glioblastoma. *PLoS One* 2013; 8: e63164.
- Sánchez-Alegría K, Flores-León M, Avila-Muñoz E, Rodríguez-Corona N, Arias C. PI3K signaling in neurons: a central node for the control of multiple functions. *Int J Mol Sci* 2018; 19: 3725.
- Santaripa L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. *Expert Opin Ther Targets* 2012; 16: 103-119.
- Shen J, Zhang Y, Yu H, Shen B, Liang Y, Jin R, Liu X, Shi L, Cai X. Role of DUSP1/MKP1 in tumorigenesis, tumor progression and therapy. *Cancer Med* 2016; 5: 2061-2068.
- Shi ZM, Wang XF, Qian X, Tao T, Wang L, Chen QD, Wang XR, Cao L, Wang YY, Zhang JX, Jiang T, Kang CS, Jiang BH, Liu N,

- You YP. MiRNA-181b suppresses IGF-1R and functions as a tumor suppressor gene in gliomas. *RNA* 2013; 19: 552-560.
31. Song H, Zhang Y, Liu N, Wan C, Zhang D, Zhao S, Kong Y, Yuan L. miR-92b regulates glioma cells proliferation, migration, invasion, and apoptosis via PTEN/Akt signaling pathway. *J Physiol Biochem* 2016; 72: 201-211.
 32. Sun G, SiMa G, Wu C, Fan Y, Tan Y, Wang Z, Cheng G, Li J. Decreased MiR-17 in glioma cells increased cell viability and migration by increasing the expression of cyclin D1, p-Akt and Akt. *PLoS One* 2018; 13: e0190515.
 33. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal transduction system subserving synaptic plasticity and memory. *J Neurochem* 2001; 76: 1-10.
 34. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006; 103: 2257-2261.
 35. Wang XF, Shi ZM, Wang XR, Cao L, Wang YY, Zhang JX, Yin Y, Luo H, Kang CS, Liu N, Jiang T, You YP. MiR-181d acts as a tumor suppressor in glioma by targeting K-ras and Bcl-2. *J Cancer Res Clin Oncol* 2012; 138: 573-584.
 36. Wood MD, Halfpenny AM, Moore SR. Applications of molecular neuro-oncology – a review of diffuse glioma integrated diagnosis and emerging molecular entities. *Diagn Pathol* 2019; 14: 29.
 37. Xu XH, Zhang SJ, Hu QB, Song XY, Pan W. Effects of microRNA-494 on proliferation, migration, invasion, and apoptosis of medulloblastoma cells by mediating c-myc through the p38 MAPK signaling pathway. *J Cell Biochem* 2019; 120: 2594-2606.
 38. Yang J, Fan B, Zhao Y, Fang J. MicroRNA-202 inhibits cell proliferation, migration and invasion of glioma by directly targeting metadherin. *Oncol Rep* 2017; 38: 1670-1678.
 39. Yheskel M, Lakhia R, Cobo-Stark P, Flaten A, Patel V. Anti-microRNA screen uncovers miR-17 family within miR-17~92 cluster as the primary driver of kidney cyst growth. *Sci Rep* 2019; 9: 1920.
 40. Zakrzewska M, Fendler W, Zakrzewski K, Sikorska B, Grajkowska W, Dembowska-Bagińska B, Filipek I, Stefańczyk Ł, Liberski PP. Altered microRNA expression is associated with tumor grade, molecular background and outcome in childhood infratentorial ependymoma. *PLoS One* 2016; 11: e0158464.
 41. Zhang Y, Li L, Liang P, Zhai X, Li Y, Zhou Y. Differential expression of microRNAs in medulloblastoma and the potential functional consequences. *Turk Neurosurg* 2018; 28: 179-185.
 42. Zhou Q, Dong J, Luo R, Zhou X, Wang J, Chen F. MicroRNA-20a regulates cell proliferation, apoptosis and autophagy by targeting thrombospondin 2 in cervical cancer. *Eur J Pharmacol* 2019; 844: 102-109.
 43. Zhu Y, Zhao H, Rao M, Xu S. MicroRNA-365 inhibits proliferation, migration and invasion of glioma by targeting PIK3R3. *Oncol Rep* 2017; 37: 2185-2192.