Clinical Neurology and Neurosurgery 186 (2019) 105488

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

# Pediatric ependymoma: GNAO1, ASAH1, IMMT and IPO7 protein expression and 5-year prognosis correlation

Monserrat Pérez-Ramírez[a], Antonio García-Méndez[b], Alicia Georgina Siordia-Reyes[c], Anahí Chavarría[d], Celedonio Gómez[e], Normand García-Hernández[a,*]

[a] Unidad de Investigación Médica en Genética Humana, Hospital de Pediatría "Dr. Silvestre Frenk Freud", Centro Médico Nacional Siglo XXI, IMSS. Av. Cuauhtémoc 330, Col. Doctores, Del. Cuauhtémoc, 06720, Mexico
[b] Servicio de Neurocirugía Pediátrica, Hospital General "Dr. Gaudencio González Garza", Centro Médico Nacional "La Raza", IMSS, Calzada Vallejo y Jacarandas S/N, Col. La Raza, Del. Azcapotzalco, 02980, Ciudad de México, Mexico
[c] Servicio de Patología, Hospital de Pediatría "Dr. Silvestre Frenk Freud", Centro Médico Nacional Siglo XXI, IMSS. Av. Cuauhtémoc 330, Col. Doctores, Del. Cuauhtémoc, 06720, Ciudad de México, Mexico
[d] Unidad de Investigación en Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510, Ciudad de México, Mexico
[e] Facultad de Química, Universidad Nacional Autónoma de México, Avenida Ciudad Universitaria 3000, Coyoacán, 04360, Ciudad de México, Mexico

ARTICLE INFO

*Keywords:*
Pediatric ependymoma
Infratentorial ependymoma
Protein expression and survival
Protein expression and progression-free survival
Protein expression and clinical features

ABSTRACT

*Objective:* The aim of this work was to evaluate a pediatric ependymoma protein expression that may be useful as a molecular biomarker candidate for prognosis, correlated with clinical features such as age, gender, histopathological grade, ependymal tumor recurrence and patient survival.
*Patients and methods:* Immunohistochemistry assays were performed for GNAO1, ASAH1, IMMT, IPO7, Cyclin D1, P53 and Ki-67 proteins. Kaplan-Meier and Cox analysis were performed for age, gender, histopathological grade, relapse and survival correlation.
*Results:* We found that three proteins correlate with histopathological grade and relapse; two proteins correlate with survival; one protein does not correlate with any clinical feature.
*Conclusion:* Our results suggest that, out of the proteins analyzed, five may be considered suitable prognostic biomarkers and one may be considered a predictive biomarker for response to treatment of pediatric ependymoma.

## 1. Introduction

There has been considerable improvement in the treatment and prognosis of many pediatric brain tumors, which are currently the primary cause of mortality, as well as long-term morbidity in oncology being the Central nervous system (CNS) neoplasms remain the most common solid tumors in children [1]. Ependymomas (EP) nowadays are treated by surgical resection followed by radiotherapy [2], but despite the advances in multidisciplinary treatments of brain tumors, the EP are relatively resistant to chemotherapy, and patients with surgery-refractory tumors still have a poor prognosis [3]. EP exhibit heterogeneous clinical courses that cannot be predicted accurately by current clinical, pathologic or molecular markers [4]. Consequently, new targeted therapies are needed for these patients [5] and studies aiming at the identification of molecular markers of clinical value [6].

The identification of genetic abnormalities responsible for the generation and maintenance of the malignant phenotype in EP are crucial in postulating biomarker characteristics; several studies have proposed to molecular changes and clinical features as prognostic markers [7–9]; so that molecular classification has become critical for diagnosis, but molecularly-guided trials are complicated due to the apparent molecular EP diversity [10,11]; thus, it is necessary to determine specific molecular markers. Previously, we proposed the genes *GNAO1*, *ASAH1*, *IMMT*, and *IPO7* as candidate molecular biomarkers for prognosis [12].

In the current study had following objectives: 1) The protein expression of P53, KI67, Cyclin D1, GNAO1, ASAH1, IPO7 and IMMT was evaluated, 2) Correlated protein expression with the clinical features: age, gender, histopathological grade, 3) Correlated protein expression

* Corresponding author.
*E-mail address:* normandgarcia@gmail.com (N. García-Hernández).

https://doi.org/10.1016/j.clineuro.2019.105488
Received 6 June 2019; Received in revised form 18 July 2019; Accepted 11 August 2019
Available online 12 August 2019
0303-8467/ 

with progression-free survival and 4) Correlate protein expression and overall survival.

## 2. Patients and methods

### 2.1. Samples collection

The ependymal tumors were collected from formalin-fixed paraffin-embedded (FFPE) tissue from the Pathology Service at the Hospital de Pediatría "Dr. Silvestre Frenk Freud", from the Centro Médico Nacional "Siglo XXI", IMSS. The histopathological records from 2010 to 2017 were reviewed. The samples were collected from pediatric patients aged 0–16 with a confirmed diagnosis of grade II and grade III ependymoma; cerebellar tissue adjacent and located in periphery to the tumor was used as control tissue.

### 2.2. Review of clinical records

Clinical records of patients were consulted in the database and the written archives of the hospital. The following clinical data were obtained: histopathological grade, anatomical location, age, gender, recidivism, and survival.

### 2.3. Immunohistochemistry (IHC) assays

Five-µm tissue sections were made and placed on adhesive lamellae (Santa Cruz Biotechnology). Tissue sections were incubated in a wet chamber for 20 min with the primary antibodies GNAO1 (1:100, GTX114439, GeneTex), ASAH1 (1:100, GTX114267, GeneTex), IMMT (1:100, GTX81949, GeneTex), IPO7 (1:100, GTX106408), Cyclin-D1 (sc-718, Santa Cruz Biotechnology), P53 (sc-126, Santa Cruz Biotechnology), and Ki-67 (1:50, DAKO Corporation, Carpinteria Ca), following the manufacturer recommendations. The primary antibody was detected with a biotinylated secondary antibody (sc-471863, Santa Cruz Biotechnology). Tissue was dehydrated with alcohols of lower to higher concentration (70%, 90% and 100%) and xylol. Preparations were mounted with entellan (107961, Merck Millipore).

A positive control was included for each antibody: for GNAO1 and ASAH1 pancreas tissue, for IPO7 and IMMT esophageal tissue, and for Ki-67, Cyclin D1 and P53 glioma tissue. The quality of the tissue was evaluated with the expression of Ki-67, a positive marker for all EP.

### 2.4. Image capture

IHC images were taken with the 40X objective of a 6 × 31 photomicroscope (Olympus Life Sciences). These images were stored in TIFF format. Protein expression quantified using ImageJ software [13].

### 2.5. Analysis of results

Protein expression was qualitatively quantitated according to the percentage of cells expressing a given protein. The following values were established: negative (< 1%), weak staining (1–15%), moderate staining (15–30%), and strong staining (> 30%). The sample size was calculated for the hypothesis test according to Camacho-Sandoval [14] and de Bekker-Grob et al. [15] The number and distribution of samples was considered. The statistical analysis (Kaplan-Meier and Cox analysis) was performed using the IBM SPSS Statistics 24 Software. We considered a P-value of $< 0.05$ statistically significant.

## 3. Results

### 3.1. Collected samples

The total number of samples collected was 30. All the samples analyzed had a *De Novo* diagnosis and infratentorial localization (Table 1). These samples came from pediatric patients diagnosed with grade II or grade III EP (Fig S1A). 13 patients were female, and 17 patients male. 20% of patients were 7 years old, and 16% were 5 years at the time of diagnosis.

**Table 1**
Samples feature. Show the histopathological grade (Grade), age (Age), gender (M.- Male or F.- Female), tumor location, relapse, and survival.

| SAMPLE | GRADE | GENDER | AGE | LOCALIZATION | RESIDIVISM | SURVIVAL |
|---|---|---|---|---|---|---|
| 1 | II | M | 13 | Intracranial | Yes | Yes |
| 2 | II | F | 1 | Intracranial | Yes | Yes |
| 3 | III | F | 2 | Intracranial | Yes | No |
| 4 | III | M | 5 | Intracranial | No | Yes |
| 5 | II | F | 14 | Intracranial | Yes | Yes |
| 6 | III | M | 1 | Intracranial | Yes | Yes |
| 7 | II | M | 7 | Intracranial | Yes | Yes |
| 8 | III | M | 5 | Intracranial | No | No |
| 9 | III | M | 5 | Intracranial | Yes | Yes |
| 10 | III | F | 12 | Intracranial | No | No |
| 11 | II | F | 4 | Intracranial | No | No |
| 12 | II | F | 12 | Intracranial | No | No |
| 13 | II | M | 15 | Intracranial | No data | No data |
| 14 | II | F | 2 | Intracranial | No data | No data |
| 15 | III | F | 4 | Intracranial | No data | No data |
| 16 | III | F | 7 | Intracranial | No data | No data |
| 17 | II | M | 7 | Intracranial | No data | No data |
| 18 | II | M | 3 | Intracranial | No data | No data |
| 19 | II | M | 8 | Intracranial | No data | No data |
| 20 | II | M | 5 | Intracranial | No data | No data |
| 21 | III | M | 14 | Intracranial | No data | No data |
| 22 | III | F | 3 | Intracranial | No data | No data |
| 23 | II | M | 7 | Intracranial | No data | No data |
| 24 | II | M | 7 | Intracranial | No data | No data |
| 25 | II | M | 1 | Intracranial | No data | No data |
| 26 | II | F | 8 | Intracranial | No data | No data |
| 27 | III | M | 5 | Intracranial | No data | No data |
| 28 | III | F | 14 | Intracranial | No data | No data |
| 29 | III | F | 5 | Intracranial | No data | No data |
| 30 | II | M | 0 | Intracranial | No data | No data |

### 3.2. Clinical data

We analyzed a cohort of samples between the years 2010 and 2013 to study the rates of relapse and survival in patients after a five-year period. Twelve complete clinical files were obtained. Table 1 shows 7 patients relapsed and 7 patients survived.

### 3.3. Protein expression

We performed IHC assays for the following proteins: GNAO1, ASAH1, IMMT, IPO7, Cyclin D1, P53, and Ki-67; on the following tissue: cerebellum, ependymal tumor, and as positive control tissues according to methods (Fig. S1-B). Through qualitative analysis, we found different percentages of protein expression. We observed negative expression in the following proteins: GNAO1 (60%), IMMT (73.3%), IPO7 (90%), Cyclin D1 (60%) and P53 (76.6%); positive expression in the proteins ASAH1 (73%) and Ki-67 (70%) (Fig. 1 and Table 2).

### 3.4. Correlation analysis between protein expression and age, gender, or histopathological grade

The correlation analysis between age and protein expression showed the following significant Chi-square values Cyclin D1 = 0.011 (Fig. 2A), P53 = 0.037 (Fig. 2B) and the no significative values: GNAO1 = 0.554, ASAH1 = 0.204, IMMT = 3.033, IPO7 = 6.269, and Ki67 = 1.725.

The correlation analysis between gender and protein expression exhibited the following significant values of Chi-square: Cyclin D1 = 0.088 (Fig. 2C), Ki-67 = 0.019 (Fig. 2D) and the no significative values: GNAO1 = 0.252, ASAH1 = 0.587, IMMT = 0.156, IPO7 = 0.107, P53 = 0.172.

The values of Chi-square results from correlation analysis between histopathological grade and protein expression were the following significative values: Cyclin D1 = 0.019 (Fig. 2E), P53 = 0.001 (Fig. 2F), Ki67 = 0.085 (Fig. 2G) and no significative values: GNAO1 = 0.347, ASAH1 = 0.223, IMMT = 0.118, IPO7 = 0.205.

### 3.5. Correlation analysis between protein expression and recidivism or survival

The correlation analysis between relapse and protein expression showed the following significative Chi-square: Cyclin D1 = 0.094 (Fig. 3A), P53 = 0.013 (Fig. 3B), Ki67 = 0.021 (Fig. 3C) and no significative values: GNAO1 = 0.974, ASAH1 = 1.017, IMMT = 0.389, IPO7 = 0.345.

To accomplish the fourth objective. The correlation analysis between survival and protein expression showed the following significative values of Chi-square: IMMT = 0.007 (Fig. 3D), IPO7 = 0.019 (Fig. 3E) and no significative values: GNAO1 = 0.103, ASAH1 = 1.220, Cyclin D1 = 0.371, P53 = 1.069, and Ki-67 = 1.816.

## 4. Discussion

In a previous work related to pediatric EP, we found *ASAH1*, *IMMT*, and *IPO7* genes overexpressed, and the *GNAO1* gene underexpressed [12]. In worldwide papers, we found expression changes in *Cyclin D1*, *P53*, and *Ki-67* genes in EP [16,17]. According to the reports, these genes participate in the EP tumorigenesis process; therefore, we determined, through IHC assays, whether these changes were reflected in protein expression and what their behavior in EP is in the Mexican population.

We found P53 protein underexpression in 76.6% of analyzed tumors, concordant to the findings published by Wu et al. [18], reported gene inactivation due to *P53* in more than 50% of tumors. We found that P53 correlates with the age; probably at a patient's early age, P53 is one of the main deregulated proteins that favor cell proliferation. We did not find correlation between P53 expression and patients' gender. We found correlation of P53 underexpression with the histopathological grade III. In the samples collected from Mexican patients, we observed that the P53 protein decreases in anaplastic EP; contrariwise, Alexiou et al. reported that in Caucasian patients the high P53 expression correlated with anaplastic EP [16]; these differences are possibly due to polymorphisms, mutations, or epigenetic factors that influence protein expression among races. The P53 found correlates with the biological function of P53. It is known that P53 regulates several apoptosis and cell proliferation and promotes DNA repair [15,18,19]. This suggests that the P53 absence favors the anaplastic development of EP. We found that P53 underexpression delay the relapse; it has been reported that P53 is related to the patients' survival and the level of aggressive biological behavior of tumors [16]. No correlation between protein expression and survival was found. This suggests that the P53 expression is a candidate for prognosis biomarker in pediatric EP. Correlation between P53 expression and histopathological grade or relapse was also found; therefore, P53 may permit us to distinguish the anaplastic grade of tumor and patient prognosis.

Infratentorial EP displayed Cyclin D1 underexpression in 83.4% and overexpression in 16.6% of the samples, contrary to Rogers et al. [17], who reported that the Cyclin D1 expression was significantly higher in supratentorial EP. The above shows that, in Mexican patients, Cyclin D1 can be overexpressed in infratentorial EP. The nuclear Cyclin D1 overexpression can inactivate P53 through PRMT5-dependent P53 methylation, thereby allowing tumor progression [21]. Even though we did not observe any correlation between Cyclin D1 and P53 expression ($X^2 = 0.776$), we did find Cyclin D1 overexpression and P53 underexpression in 10 samples. Cyclin D1 overexpression correlated with grade III EP, favors neoplastic transformation. It is known that overall accumulation of Cyclin D1 in tumor cells is associated to neoplastic transformation [21]. We found a tendency to overexpression in patients less than 7 years old and male patients; this may have been influenced by clinical data and types and number of samples. We found that Cyclin D1 correlates with relapse; Cyclin D1 overexpression in infratentorial EP delays relapse, therefore it is important to know the treatment prognosis; de Andrade et al. reported that in patients with chemotherapy treatment Cyclin D1 may play a more important mediator function in residual EP than in *De Novo* EP in facilitating tumoral DNA repair and promoting residual re-growth [22,23]. Cyclin D1 loss correlates with increased phosphorylation of the ROCK II substrates, favoring the cell migration [21]. Cyclin D1 possibly plays the same role in EP. We did not find correlation between Cyclin D1 expression and survival; we observed that Cyclin D1 underexpression possibly does not allow a correlation between Cyclin D1 and survival; it is important to increase the sample number and check if Cyclin D1 correlates with survival.

It was reported that the Ki67 index in intracranial EP is an independent prognostic factor and accurate predictor of outcome in EP patients [24,25]; the Ki67 protein is associated with the proliferative activity of intrinsic cell populations in malignant tumors, and can therefore be used as a marker of tumor aggressiveness [26], thus we evaluate the Ki-67 expression in EP. We observed Ki-67 overexpressed in all samples, the expression was higher in grade III compared to grade II. We found that Ki-67 expression correlates with the histopathological grade, in concordance with the findings of Suri et al. [27] and Alexiou et al. [16]. We did not find correlation between Ki-67 expression and age or survival, but the high Ki-67 expression favors early relapse; this suggests that Ki-67 expression favors the anaplastic phenotype through the deregulation of cell cycle. It is known that Ki-67 affects cell cycle progression in p21 checkpoint; in addition, it has been reported that the P53 expression correlates with Ki67 in several cancers, including oral squamous cell cancer and breast cancer. It has been proposed that P53 inhibits Ki67 promoter activity via P53-dependent and Sp1-dependent pathways that affect the transcriptional repression of the Ki67 promoter

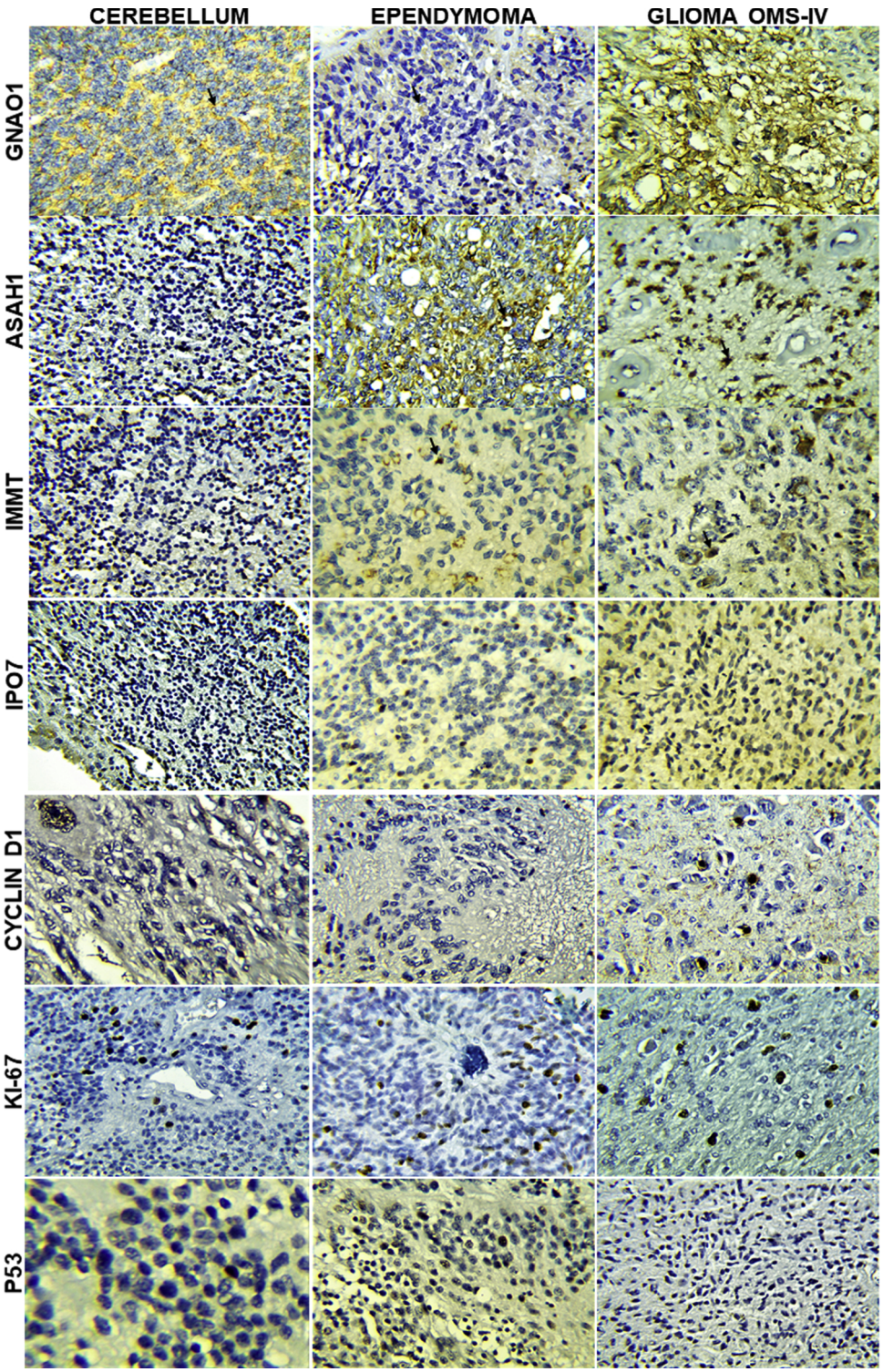


**Fig. 1.** Protein expression by immunochemistry assay. Representative micrographs showing the protein staining in the analyzed tissue. The expression of *IPO7*, *Ki-67*, and *p53* was nuclear; the expression of *GNAO1*, *ASAH1*, and *Cyclin D1* was cytoplasmic; *IMMT* expression was cytoplasmic and mitochondrial.

[28,26]; Ki67 has been shown to be a tool for cancer diagnosis (judging the cutoff level of 10–14% positive staining as high risk of prognosis), thus the inhibition of Ki67 might be considered when designing novel strategies for cancer therapy and Ki67 is proposed as an attractive therapeutic target for cancer because it is highly expressed in most malignant cells [29]; this suggests that Ki-67 is a candidate for prognostic and therapeutic biomarker.

In a previous work, we reported the *ASAH1* gene is overexpressed in pediatric EP [12], therefore we analyzed the protein and we found ASAH1 was overexpressed in 86.6% of the samples; it has been shown that it is overexpressed in various human cancers, such as melanoma and breast cancer [25]. We found that ASAH1 does not correlate with age, gender, histopathological grade, relapse, or survival. It is known that ASAH1 participates in cell survival, inflammation and angiogenesis through the sphingolipid pathway that metabolizes ceramide into sphingosine and free fatty acid [30,32]. We think that ASAH1 may have

**Table 2**
Staining intensity of proteins. The percentage of the samples showing the staining intensity for *GNAO1*, *ASAH1*, *IMMT*, *IPO7*, *CYCLIN D1*, *Ki67*, and *p53*.

| STAINING TYPE | GNAO1 | ASAH1 | IMMT | IPO7 | CYCLIN D1 | KI67 | P53 |
|---|---|---|---|---|---|---|---|
| **Negative** | 60% (18/30) | 13.3% (4/30) | 73.3% (22/30) | 90% (27/30) | 60% (18/30) | 6.6% (2/30) | 76.6% (23/30) |
| **Weak** | 10% (3/30) | 73.3% (22/30) | 3.3% (1/30) | 6.6% (2/30) | 13.3% (4/30) | 70% (21/30) | 10% (3/30) |
| **Moderate** | 16.6% (5/30) | 13.3% (4/30) | 16.6% (5/30) | 3.3% (1/30) | 10% (3/30) | 16.6% (5/30) | 6.6% (2/30) |
| **Strong** | 13.3% (4/30) | 0% (0/30) | 6.6% (2/30) | 0% (0/30) | 16.6% (5/30) | 6.6% (2/30) | 6.6% (2/30) |

other clinical functionality; ASAH1 overexpression confers resistance to apoptosis and stimulates proliferation and invasiveness of cancer cell. Lai et al. [30] reported that ASAH1 overexpression confers resistance to radiation, impacting the treatment through altering the sphingolipid metabolism pathway. Doan et al. [33] reported that in the treatment of glioblastoma ASAH1 is excellent drug target (e.g. dacarbazine, anthracyclines) depend on the ability of these agents to increase the intracellular levels of ASAH1 [31]. It is suggested that the protein overexpression may be important in the treatment response at EP. It has been suggested that cells that express a high level of ASAH1 could survive radiation through the complex c-Jun/AP-1 and transcription factors that have been implicated in the DNA-repair pathway [30,34]. Therefore, it is important to know the expression of other proteins involved in the sphingolipid pathway and other ceramides or proteins that participate in the DNA-repair pathway— to determine the role of *ASAH1* in EP, as well as the post-translational modifications involved.

It has known that GNAO1 is abundantly expressed in neuronal tissue [35]; we found GNAO1 protein underexpression in 60% of the samples in accordance and a previous work in which the *GNAO1* gene was underexpressed in pediatric EP [12], and Zupancic et al. [36] reported GNAO1 underexpression in GBM tissue; this may suggest that the absence of GNAO1 is characteristic in EP and other gliomas. We did not find any correlation between GNAO1 expression and age, gender, histopathology, relapse or survival. We thought that methylation played an important role in the regulation of GNAO1 expression; Xu et al. [35] and Pei et al. [38] reported that the change of GNAO1 expression in Hepatocelluar carcinoma cells might be regulated by it is methylation status. In accordance to what we previously reported, this GNAO1 underexpression is the consequence of a chromosomal deletion and/or methylation [12]. According to our results, we may have observed a tendency to correlate the GNAO1 underexpression with increased survival; it is suggested that the GNAO1 could be important for knowing the prognosis of patients. The importance of GNAO1 in prognosis is probably due to their participation in cell proliferation by affecting cell apoptosis through the accumulation of apoptotic proteins, including PARP, Puma and Bim [39]. It has been reported that GNAO1 promotes oncogenic transformation through STAT3 signaling [37]; therefore, it will be interesting to know how this signaling pathway participates in the tumorigenesis of EP. We think that this process deserves a detailed analysis based on functional assays.

We found the *IPO7* gene was overexpressed in a previous work in pediatric EP [12]. At protein level, we found underexpression of IPO7 on 90% of analyzed samples. This contrasting behavior may possibly be due to post-translational or post-transcriptional changes that actively participate in IPO7 protein regulation. Xue et al. [40] reported that the FOXM1 protein stimulates the transcription of IPO7 by binding directly to its promoter at three sites in humans, thus, the FOXM1 overexpression can markedly increase IPO7 expression. This regulatory feedback may represent critical proliferation and invasion mechanisms in human brain tumors [40]. The protein underexpression may be explained through the participation of miRNAs, a role reported by. Szczyrba et al [41] shows *IPO7* as miR-22 target. We did not find any correlation of protein expression with age, gender, histhopathological grade or relapse. IPO7 overexpression favors survival; we think this may related through P53, according to Golomb et al., [42], who described that IPO7 transcription is repressed by P53 and IPO7 depletion triggers P53 activation. However, we did not find significant any correlation between IPO7 expression and P53 expression ($X^2 = 0.640$). Golomb et al. [42] mention that this feedback occurs in non-stressed cells; this depletion of IPO7 results in P53 activation and subsequent growth inhibition. It is suggested that IPO7 is a good prognosis biomarker through the regulation of proliferation and metastasis. It is important to know the signaling pathways in which IPO7 participates during tumorigenesis.

We found *IMMT* gene overexpressed in 100% of samples of pediatric EP [12] but the IMMT protein we found underexpression in 73.3%. This contrasting behavior may possibly be due to post-translational or post-transcriptional changes, but it has only been identified that Yme1L regulates the stability of IMMT [43]. This protein is implicated in a variety of processes, including energy or free radical generation, regulation of apoptosis, modulation of various signaling pathways, and regulation of mitochondrial cristae morphology [43,44]. We think that IMMT is important in mitochondrial regulation. Madungwe et al. [45] reported that IMMT is indispensable for normal mitochondrial function and has a functional impact on cellular activity. We did not find any correlation between clinical characteristics (age, gender, histopathological grade) and relapse. We observed that IMMT underexpression promotes increased overall survival. Sotgia et al. [46] reported that, in cases gastric cancer, IMMT is associated with significantly reduced time to first progression. It is suggested that IMMT is important during tumorigenesis; however, functional assays are needed for known the IMMT regulation during tumorigenesis in EP.

## 5. Conclusion

We confirm a consistent expression behavior from mRNA to protein expression in favor of ASAH1 and GNAO1 on ependymal tumors. Our results suggest some of the analyzed proteins may be considered prognostic biomarkers: P53 (underexpression), Cyclin D1 (overexpression), and Ki67 (overexpression), since their quantity correlates with histopathological grade and relapse; IPO7 (overexpression) and IMMT (underexpression) since their quantity correlates with survival. We thought that IMMT and IPO7 deserve functional assays to know their regulation role during tumorigenesis on cancer and that ASAH1 (overexpression) and Ki-67 (overexpression) may be candidates for treatment response. Finally, we found that GNAO1 did not correlate with any feature in the present research.

## Declaration of Competing Interest

The authors declare that they have no competing interest.

## Acknowledgments

We thank the FIS (Fondo de Investigacion en Salud) from IMSS (Instituto Mexicano del Seguro Social), Mexico for their support: FIS/

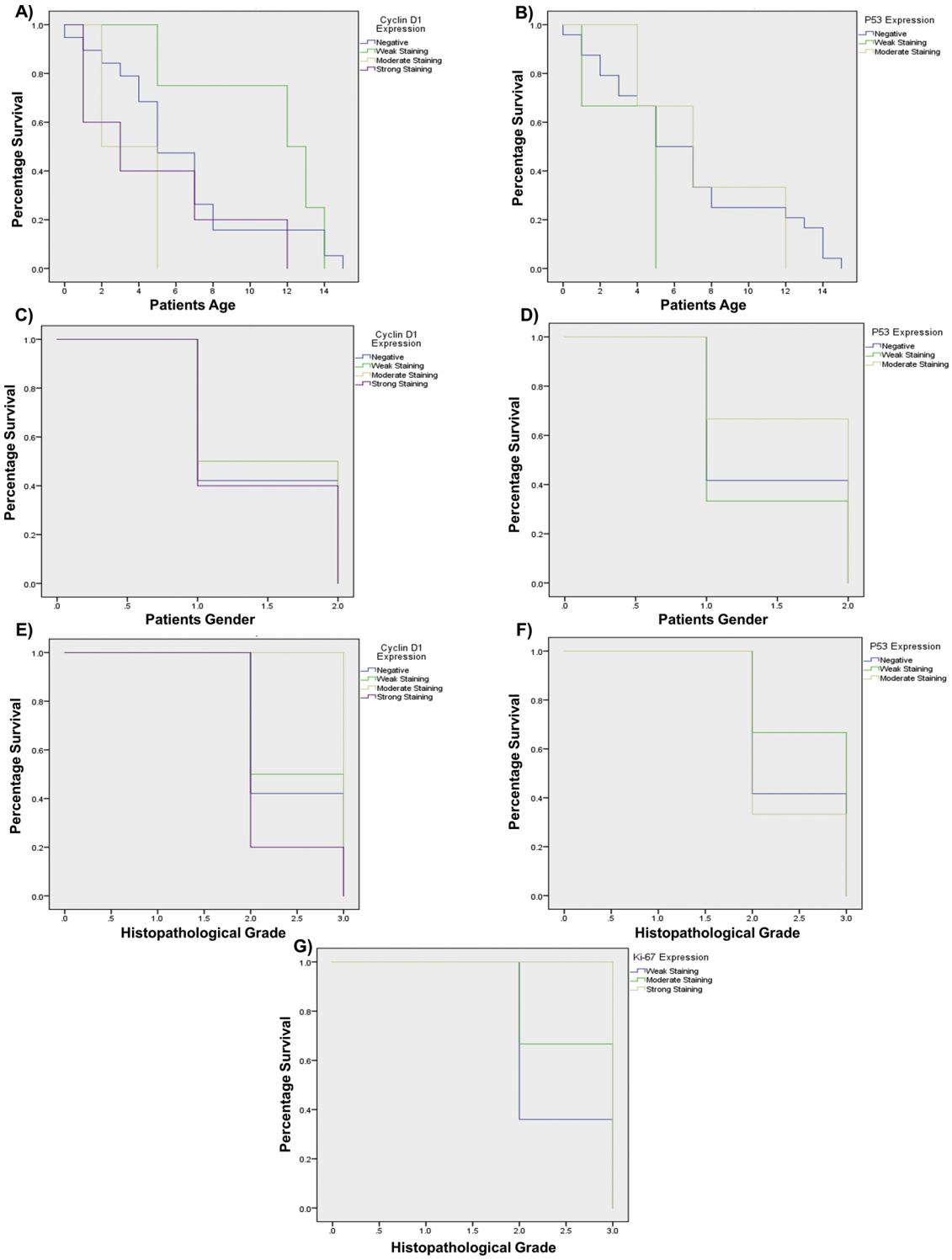


**Fig. 2.** Clinical feature correlation for Kaplan Meier analysis. The line color refers to the staining type. The ordinates axis shows the patient's percentage of survival. **A**). Cyclin D1 expression and age. The abscissa axis shows the patient's age. **B**) P53 expression and age. The abscissa axis shows the age of patients. **C**) Cyclin D1 expression and gender. The abscissa axis shows the gender of patients. 1.-Male, 2.- Female. **D**) Ki-67 expression and gender. The abscissa axis shows the gender of patients. 1.-Male, 2.- Female. **E**) Cyclin D1 expression and histopathological grade. The abscissa axis shows the histopathological grade. 2.-EP grade II. 3.-EP grade III. **F**) p53 expression and histopathological grade.

IMSS/PROT/949, 2009-785-042, and R-2014-785-094, and the scholarship CVU 404762 granted by CONACYT (Consejo Nacional de Ciencia y Tecnología), Mexico and IMSS 011-2013 for the development of the student and the postgrad program of Ciencias Biológicas de la Universidad Nacional Autónoma de México. We thank the research scholarship received from IMSS Foundation A. C. given to PhD. Normand García Hernández. We thank the given support and material to PhD Alejandro M. García-Carrancá from Instituto Nacional de Cancerología; to Dr. Fernando Chico Ponce de Léon from Hospital Infantil "Federico Gómez" and to Dr. Fabio A. Salamanca Gómez from Hospital de Pediatría "Dr. Silvestre Frenk Freund", IMSS.

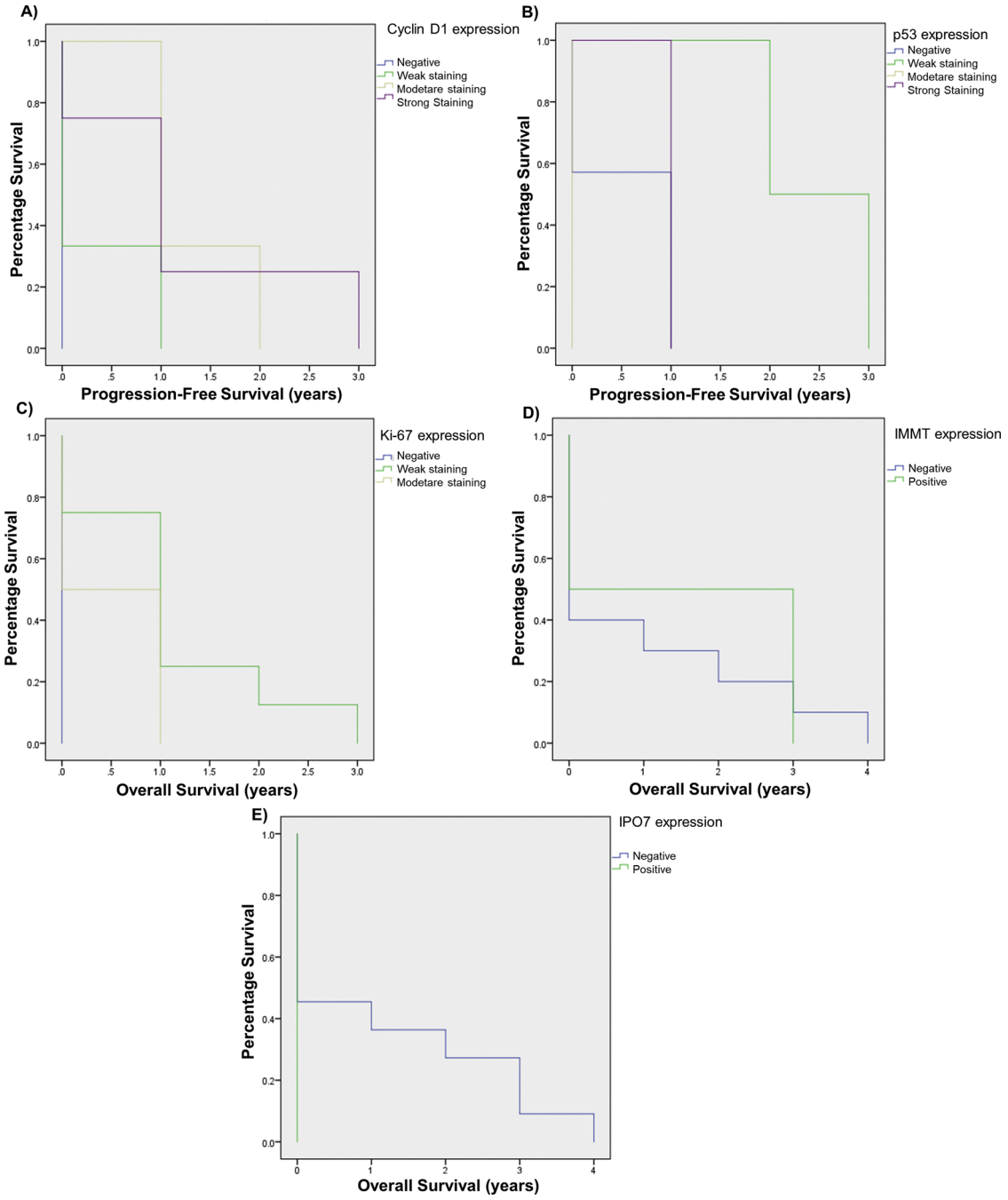


**Fig. 3.** Prognosis correlation with the Kaplan Meier analysis. The line color refers to the staining type. The ordinates axis shows the patient's percentage of survival. **A**) Cyclin D1 expression and relapse. The abscissa axis shows the relapse**. B**) p53 expression and relapse. The abscissa axis shows the relapse. **C**) Ki-67 expression and relapse. The abscissa axis shows the relapse. **D**) IMMT expression and survival. The abscissa axis shows the survival. **E**) IPO7 expression and survival. The abscissa axis shows the survival.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.clineuro.2019.105488.

## References

[1] V.C.H. Wong, A. Morrison, U. Tabori, C.E. Hawkins, Telomerase inhibition as a novel therapy for pediatric ependymoma, Brain Pathol. 20 (2010) 780–786, https://doi.org/10.1111/j.1750-3639.2010.00372.x.

[2] H.A. Rogers, J.-P. Kilday, C. Mayne, J. Ward, M. Adamowicz-Brice, E.C. Schwalbe, S.C. Clifford, B. Coyle, R.G. Grundy, Supratentorial and spinal pediatric ependymomas display a hypermethylated phenotype which includes the loss of tumor suppressor genes involved in the control of cell growth and death, Acta Neuropathol. 123 (2012) 711–725, https://doi.org/10.1007/s00401-011-0904-1.

[3] T. Tsuruta, Y. Aihara, H. Kanno, M. Funase, T. Murayama, M. Osawa, H. Fujii, O. Kubo, Y. Okada, Shared molecular targets in pediatric gliomas and ependymomas, Pediatr. Blood Cancer 57 (2011) 1117–1123, https://doi.org/10.1002/pbc.23009.

[4] P. Modena, F.R. Buttarelli, R. Miceli, E. Piccinin, C. Baldi, M. Antonelli, I. Morra, L. Lauriola, C. Di Rocco, I. Sardi, L. Genitori, R. Maestro, L. Gandola, F. Facchinetti, P. Collini, G. Sozzi, F. Giangaspero, M. Massimino, P. Gemelli, S. Cuore, G.P. Hospital, Predictors of outcome in an AIEOP series of childhood ependymomas: a multifactorial analysis, Neuro Oncol. 14 (2012) 1346–1356.

[5] L. Moreno, S. Popov, A. Jury, S. Al Sarraj, C. Jones, S. Zacharoulis, Role of platelet derived growth factor receptor (PDGFR) over-expression and angiogenesis in ependymoma, J. Neurooncol. 111 (2013) 169–176, https://doi.org/10.1007/s11060-012-0996-z.

[6] F.F. Costa, J.M. Bischof, E.F. Vanin, R.R. Lulla, M. Wang, S.T. Sredni, V. Rajaram,

M. de F. Bonaldo, D. Wang, S. Goldman, T. Tomita, M.B. Soares, Identification of microRNAs as potential prognostic markers in ependymoma, PLoS One 6 (2011) e25114, , https://doi.org/10.1371/journal.pone.0025114.
[7] I. Lukashova-v, S. Zangen, C.M. Kneitz, S. Monoranu, B. Rutkowski, G.H. Hinkes, B. Vince, W. Huang, Roggendorf, Ependymoma gene expression profiles associated with histological subtype, proliferation, and patient survival, Acta Neuropathol. 113 (2007) 325–337, https://doi.org/10.1007/s00401-006-0190-5.
[8] K. Wani, T.S. Armstrong, E. Vera-Bolanos, A. Raghunathan, D. Ellison, R. Gilbertson, B. Vaillant, S. Goldman, R.J. Packer, M. Fouladi, I. Pollack, T. Mikkelsen, M. Prados, A. Omuro, R. Soffietti, A. Ledoux, C. Wilson, L. Long, M.R. Gilbert, K. Aldape, Collaborative Ependymoma Research Network, A prognostic gene expression signature in infratentorial ependymoma, Acta Neuropathol. 123 (2012) 727–738, https://doi.org/10.1007/s00401-012-0941-4.
[9] K. Sowar, J. Straessle, A.M. Donson, M. Handler, N.K. Foreman, Predicting which children are at risk for ependymoma relapse, J. Neurooncol. 78 (2006) 41–46, https://doi.org/10.1007/s11060-005-9072-2.
[10] S.P. Ferris, J.W. Hofmann, D.A. Solomon, A. Perry, Characterization of gliomas: from morphology to molecules, Virchows Arch. 471 (2017) 257–269, https://doi.org/10.1007/s00428-017-2181-4.
[11] H.-G. Wirsching, M. Weller, The role of molecular diagnostics in the management of patients with gliomas, Curr. Treat. Options Oncol. 17 (2016) 51, https://doi.org/10.1007/s11864-016-0430-4.
[12] M. Pérez-Ramírez, A.J. Hernández-Jiménez, A. Guerrero-Guerrero, E. Benadón-Darszon, M. Pérezpeña-Díazconti, A.G. Siordia-Reyes, A. García-Méndez, F.C.P. De León, F.A. Salamanca-Gómez, N. García-Hernández, Genomics and epigenetics: a study of ependymomas in pediatric patients, Clin. Neurol. Neurosurg. 144 (2016) 53–58, https://doi.org/10.1016/j.clineuro.2016.02.041.
[13] M.D. Abràmoff, P.J. Magalhães, S.J. Ram, Image processing with imageJ, Biophotonics Int. 11 (2004) 36–41, https://doi.org/10.1117/1.3589100.
[14] J. Camacho-Sandoval, Tamaño de muestra en estudios clínicos, Acta Med. Costarric. 50 (2008) 20–21 (Accessed 13 June 2019), https://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0001-60022008000100004.
[15] E.W. de Bekker-Grob, B. Donkers, M.F. Jonker, E.A. Stolk, Sample size requirements for discrete-choice experiments in healthcare: a practical guide, Patient 8 (2015) 373–384, https://doi.org/10.1007/s40271-015-0118-z.
[16] G.A. Alexiou, K. Stefanaki, M. Moschovi, A. Patereli, N. Prodromou, O. Karentzou, Immunohistochemical expression of cell cycle/apoptosis regulators and epidermal growth factor receptor in pediatric intracranial ependymomas, J. Child. Neurol. 26 (2011) 195–198, https://doi.org/10.1177/0883073810378750.
[17] H.A. Rogers, C. Mayne, R.J. Chapman, J.P. Kilday, B. Coyle, R.G. Grundy, PI3K pathway activation provides a novel therapeutic target for pediatric ependymoma and is an independent marker of progression-free survival, Clin. Cancer Res. 19 (2013) 6450–6460, https://doi.org/10.1158/1078-0432.CCR-13-0222.
[18] H. Wu, S.L. Pomeroy, M. Ferreira, N. Teider, J. Mariani, K.I. Nakayama, S. Hatakeyama, V.A. Tron, L.F. Saltibus, L. Spyracopoulos, R.P. Leng, UBE4B promotes Hdm2-mediated degradation of the tumor suppressor p53, Nat. Med. 17 (2011) 347–355, https://doi.org/10.1038/nm.2283.
[19] M. Dave, A.B.M.M.K. Islam, R.V. Jensen, A. Rostagno, J. Ghiso, A.R. Amin, Proteomic analysis shows constitutive secretion of MIF and p53-associated activity of COX-2−/−lung fibroblasts, genomics, Proteom. Bioinf. 15 (2017) 339–351, https://doi.org/10.1016/j.gpb.2017.03.005.
[21] S. Qie, J.A. Diehl, D. Cyclin, Cancer progression, and opportunities in cancer treatment, J. Mol. Med. 94 (2016) 1313–1326, https://doi.org/10.1007/s00109-016-1475-3.
[22] F.G. de Andrade, S.K.N. Marie, M. Uno, H. Matushita, M.A. Taricco, M.J. Teixeira, S. Rosemberg, S.M. Oba-Shinjo, Immunohistochemical expression of cyclin D1 is higher in supratentorial ependymomas and predicts relapses in gross total resection cases, Neuropathology 35 (2015) 312–323, https://doi.org/10.1111/neup.12195.
[23] M.-L. Liang, T.-H. Hsieh, Y.-R. Liu, Y.-W. Chen, Y.-Y. Lee, F.-C. Chang, S.-C. Lin, M.-C. Huang, D.M.-T. Ho, T.-T. Wong, Y. Yen, M.-H. Yang, Significance of cyclin D1 overexpression in progression and radio-resistance of pediatric ependymomas, Oncotarget 9 (2018) 2527–2542, https://doi.org/10.18632/oncotarget.23509.
[24] M. Preusser, H. Heinzl, E. Gelpi, R. Höftberger, I. Fischer, I. Pipp, I. Milenkovic, A. Wöhrer, F. Popovici, S. Wolfsberger, J.A. Hainfellner, Ki67 index in intracranial ependymoma: a promising histopathological candidate biomarker, Histopathology 53 (2008) 39–47, https://doi.org/10.1111/j.1365-2559.2008.03065.x.
[25] R.S. D'Amico, M. Praver, G.J. Zanazzi, Z.K. Englander, J.S. Sims, J.L. Samanamud, A.T. Ogden, P.C. McCormick, N.A. Feldstein, G.M. McKhann, M.B. Sisti, P. Canoll, J.N. Bruce, Subependymomas are low-grade heterogeneous glial neoplasms defined by subventricular zone lineage markers, World Neurosurg. 107 (2017) 451–463, https://doi.org/10.1016/j.wneu.2017.08.009.
[26] L.T. Li, G. Jiang, Q. Chen, J.N. Zheng, Predic Ki67 is a promising molecular target in the diagnosis of cancer (review), Mol. Med. Rep. 11 (2015) 1566–1572, https://doi.org/10.3892/mmr.2014.2914.
[27] V.S. Suri, M. Tatke, D. Singh, A. Sharma, Histological spectrum of ependymomas and correlation of p53 and Ki-67 expression with ependymoma grade and subtype, Indian J. Cancer 41 (2004) 66–71.
[28] X. Sun, P.D. Kaufman, Ki-67: more than a proliferation marker, Chromosoma 127 (2018) 175–186, https://doi.org/10.1007/s00412-018-0659-8.
[29] C. Yang, J. Zhang, M. Ding, K. Xu, L. Li, L. Mao, J. Zheng, Ki67 targeted strategies for cancer therapy, Clin. Transl. Oncol. 20 (2018) 570–575, https://doi.org/10.1007/s12094-017-1774-3.
[30] N.B. Doan, H.S. Nguyen, M.M. Al-Gizawiy, W.M. Mueller, R.A. Sabbadini, S.D. Rand, J.M. Connelly, C.R. Chitambar, K.M. Schmainda, S.P. Mirza, Acid ceramidase confers radioresistance to glioblastoma cells, Oncol. Rep. 38 (2017) 1932–1940, https://doi.org/10.3892/or.2017.5855.
[31] Y. hong Li, H. ting Liu, J. Xu, A. yan Xing, J. Zhang, Y. wen Wang, G. Yin, P. Gao, The value of detection of S100A8 and ASAH1 in predicting the chemotherapy response for breast cancer patients, Hum. Pathol. 74 (2018) 156–163, https://doi.org/10.1016/j.humpath.2018.01.004.
[32] S.-F. Tan, X. Liu, T.E. Fox, B.M. Barth, A. Sharma, S.D. Turner, A. Awwad, A. Dewey, K. Doi, B. Spitzer, M.V. Shah, S.A.F. Morad, D. Desai, S. Amin, J. Zhu, J. Liao, J. Yun, M. Kester, D.F. Claxton, H.-G. Wang, M.C. Cabot, E.H. Schuchman, R.L. Levine, D.J. Feith, T.P. Loughran, Acid ceramidase is upregulated in AML and represents a novel therapeutic target, Oncotarget 7 (2016) 83208–83222, https://doi.org/10.18632/oncotarget.13079.
[33] N.B. Doan, H. Alhajala, M.M. Al-Gizawiy, W.M. Mueller, S.D. Rand, J.M. Connelly, E.J. Cochran, C.R. Chitambar, P. Clark, J. Kuo, K.M. Schmainda, S.P. Mirza, Acid ceramidase and its inhibitors: a &i&de novo&/i& drug target and a new class of drugs for killing glioblastoma cancer stem cells with high efficiency, Oncotarget 8 (2017) 112662–112674, https://doi.org/10.18632/oncotarget.22637.
[34] M. Lai, N. Realini, M. La Ferla, I. Passalacqua, G. Matteoli, A. Ganesan, M. Pistello, C.M. Mazzanti, D. Piomelli, Complete Acid Ceramidase ablation prevents cancer-initiating cell formation in melanoma cells, Sci. Rep. 7 (2017) 1–14, https://doi.org/10.1038/s41598-017-07606-w.
[35] X. Liu, J. Wang, G. Sun, Identification of key genes and pathways in renal cell carcinoma through expression profiling data, Kidney Blood Press. Res. 40 (2015) 288–297, https://doi.org/10.1159/000368504.
[36] K. Zupancic, A. Blejec, A. Herman, M. Veber, U. Verbovsek, M. Korsic, M. Knezevic, P. Rozman, T.L. Turnsek, K. Gruden, H. Motaln, Identification of plasma biomarker candidates in glioblastoma using an antibody-array-based proteomic approach, Radiol. Oncol. 48 (2014) 257–266, https://doi.org/10.2478/raon-2014-0014.
[37] D. Xu, M. Du, J. Zhang, P. Xiong, W. Li, H. Zhang, W. Xiong, F. Liu, J. Liu, DNMT1 mediated promoter methylation of GNAO1 in hepatoma carcinoma cells, Gene 665 (2018) 67–73, https://doi.org/10.1016/j.gene.2018.04.080.
[38] X. Pei, J. Zhang, L. Wu, B. Lü, X. Zhang, D. Yang, J. Liu, The down-regulation of GNAO1 and its promoting role in hepatocellular carcinoma, Biosci. Rep. (2013) 755–760, https://doi.org/10.1042/BSR20130001.
[39] Z. Liu, J. Zhang, L. Wu, J. Liu, M. Zhang, Overexpression of GNAO1 correlates with poor prognosis in patients with gastric cancer and plays a role in gastric cancer cell proliferation and apoptosis, Int. J. Mol. Med. 33 (2014) 589–596, https://doi.org/10.3892/ijmm.2013.1598.
[40] J. Xue, A. Zhou, C. Tan, Y. Wu, H. Te Lee, W. Li, K. Xie, S. Huang, Forkhead box M1 is essential for nuclear localization of glioma-associated oncogene homolog 1 in glioblastoma multiforme cells by promoting importin-7 expression, J. Biol. Chem. 290 (2015) 18662–18670, https://doi.org/10.1074/jbc.M115.662882.
[41] J. Szczyrba, E. Nolte, M. Hart, C. Döll, S. Wach, H. Taubert, B. Keck, E. Kremmer, R. Stöhr, A. Hartmann, W. Wieland, B. Wullich, F.A. Grässer, Identification of ZNF217, hnRNP-K, VEGF-A and IPO7 as targets for microRNAs that are down-regulated in prostate carcinoma, Int. J. Cancer 132 (2013) 775–784, https://doi.org/10.1002/ijc.27731.
[42] L. Golomb, D.R. Bublik, S. Wilder, R. Nevo, V. Kiss, K. Grabusic, S. Volarevic, M. Oren, Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis, Mol. Cell. 45 (2012) 222–232, https://doi.org/10.1016/j.molcel.2011.11.022.
[43] H. Li, Y. Ruan, K. Zhang, F. Jian, C. Hu, L. Miao, L. Gong, L. Sun, X. Zhang, S. Chen, H. Chen, D. Liu, Z. Song, Mic60/Mitofilin determines MICOS assembly essential for mitochondrial dynamics and mtDNA nucleoid organization, Cell. Death Differ. 23 (2016) 380–392, https://doi.org/10.1038/cdd.2015.102.
[44] V.S. Van Laar, S.B. Berman, T.G. Hastings, Mic60/mitofilin overexpression alters mitochondrial dynamics and attenuates vulnerability of dopaminergic cells to dopamine and rotenone, Neurobiol. Dis. 91 (2016) 247–261, https://doi.org/10.1016/j.nbd.2016.03.015.
[45] N.B. Madungwe, Y. Feng, M. Lie, N. Tombo, L. Liu, F. Kaya, J.C. Bopassa, Mitochondrial inner membrane protein (mitofilin) knockdown induces cell death by apoptosis via an AIF-PARP-dependent mechanism and cell cycle arrest, Am. J. Physiol. Cell. Physiol. 315 (2018) C28–C43, https://doi.org/10.1152/ajpcell.00230.2017.
[46] F. Sotgia, M.P. Lisanti, Mitochondrial biomarkers predict tumor progression and poor overall survival in gastric cancers: companion diagnostics for personalized medicine, Oncotarget 8 (2017) 67117–67128, https://doi.org/10.18632/oncotarget.19962.