Research Article / Araştırma Makalesi
doi: 10.18678/dtfd.1124225

Duzce Med J, 2022;24(2):201-206
Düzce Tıp Fak Derg, 2022;24(2):201-206

STAT3 Expression and Correlation Between Ki-67 and PHH3 in Meningiomas: Is it Possible to Predict Recurrence?

Meningiomlarda STAT3 Ekspresyonu ve Ki-67 ile PHH3 Arasındaki Korelasyon: Rekürrensi Öngörmek Mümkün mü?

Sinem KANTARCIOĞLU COŞKUN[1]
0000-0002-8133-8665
Güven KILIÇ[2]
0000-0001-5050-7908

[1]Department of Pathology,
Düzce University School of Medicine,
Düzce, Türkiye

[2]Department of Neurosurgery,
Düzce University School of Medicine,
Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Sinem KANTARCIOĞLU COŞKUN
sinemcoskun@duzce.edu.tr

Received / Geliş Tarihi : 31.05.2022
Accepted / Kabul Tarihi : 09.08.2022
Available Online /
Çevrimiçi Yayın Tarihi : 16.08.2022

ABSTRACT

Aim: The aim of this study was to investigate the correlation between PHH3 and ki-67 labeling index, and the association of STAT3 expression with mitotic index, grade by World Health Organization 2016 classification, and clinicopathological features of meningioma cases.
Material and Methods: A total of 25 meningioma cases from the archives of the Department of Pathology, Düzce University School of Medicine, diagnosed between 2012 and 2021 were included in the study. The mitotic count from the ten fields with the highest number of mitotic figures was determined. Immunohistochemistry was performed on the formalin-fixed, paraffin-embedded tissue blocks to determine STAT3, ki-67, and PHH3 expression. STAT3 was scored between 0 and 3 points according to staining intensity. Staining percentages for STAT3 were determined using a manual count of stained cells and the total number of tumor cells. The ki-67 labeling index was determined as a percentage by a manual count. For PHH3, the total number of immunostained mitotic figures per 10 high-power fields were evaluated in each case.
Results: A statistically significant difference was found in terms of the percentage of STAT3 staining between the tumor grades (p=0.047). STAT3 expression was significantly higher in cases with high tumor grades. A moderate positive correlation was found between ki-67 and PHH3 when calculated as a percentage in the area with the highest mitotic index by manual counting (r=0.621, p=0.001).
Conclusion: A combination of ki-67, PHH3, and STAT3 will be useful in the grading of meningiomas and predict the recurrence.
Keywords: Meningioma; tumor grade; STAT3; PHH3; ki-67.

ÖZ

Amaç: Bu çalışmanın amacı meningiom vakalarında PHH3 ile ki-67 proliferasyon indeksi arasındaki korelasyonu incelemek ve STAT3 ekspresyonunun mitotik aktivite, Dünya Sağlık Örgütü 2016 sınıflandırmasına göre derece ve klinikopatolojik özellikler ile ilişkisini değerlendirmektir.
Gereç ve Yöntemler: Düzce Üniversitesi Tıp Fakültesi Patoloji Anabilim Dalı arşivlerinden 2012 ve 2021 yılları arasında tanı alan toplam 25 meningiom olgusu çalışmaya dahil edildi. Mitotik figür sayısının en fazla olduğu on büyük büyütme alanından mitotik sayı belirlendi. STAT3, ki-67 ve PHH3 ekspresyonunu belirlemek için formalinle sabitlenmiş, parafine gömülü doku blokları üzerinde immünohistokimyasal çalışma yapıldı. STAT3, boyama yoğunluğuna göre 0 ile 3 puan arasında puanlandı. STAT3 için boyama yüzdeleri, boyanmış hücrelerin manuel sayımı ve toplam tümör hücresi sayısı ile belirlendi. Ki-67 proliferasyon indeksi, manuel sayımla yüzde olarak belirlendi. PHH3 için her bir vakada, 10 büyük büyütme alanından immün boyanmış mitotik figürlerin toplam sayısı değerlendirildi.
Bulgular: STAT3 boyama yüzdesi bakımından tümör dereceleri arasında istatistiksel olarak anlamlı bir farklılık bulundu (p=0,047). Tümör derecesi yüksek olgularda STAT3 ekspresyonu anlamlı olarak daha yüksek idi. Manuel sayım ile en yüksek mitotik indekse sahip olan alanda yüzde olarak hesaplandığında PHH3 ve ki-67 arasında orta derecede pozitif bir korelasyon olduğu bulundu (r=0,621; p=0,001).
Sonuç: Ki-67, PHH3 ve STAT3'ün kombinasyon olarak kullanılması, meningiomların histopatolojik derecelendirilmesinde ve rekürrensi öngörmede faydalı bir yöntem olacaktır.
Anahtar kelimeler: Meningiom; tümör derecesi; STAT3; PHH3; Ki-67.

The results of this study has been presented partially as an oral presentation at the 3rd International TURAZ Academy Forensic Sciences, Forensic Medicine, and Pathology Congress (September 8-12, 2021; Baku, Azerbaijan).

INTRODUCTION

Meningiomas are the most frequent primary tumors of the central nervous system, consisting of 36% of all brain tumors (1). They are mostly benign, slow-growing, low-grade tumors (1). In 2016, the World Health Organization (WHO), defined a grading system to predict the risk of recurrence; benign, atypical, and anaplastic with the recurrence rates of 7-25%, 29-52%, and 50-94%, respectively (1). Mitotic activity is the most dependable prognostic factor for defining the grade in meningiomas. On hematoxylin and eosin (H&E) stained slides, mitotic figure counts per 10 high-power microscope fields (HPFs) (0.16 mm^2) are essential, but additional immunohistochemical staining with proliferation markers may be helpful (2,3).

The ki-67 labeling index (LI) is widely used worldwide, allowing analysis of the ki-67 antigen immunohistochemically, a non-histone cell cycle protein (4,5). Phosphorylated histone H3 (PHH3) is a phosphorylated histone protein that targets serine 10 of histone H3 (6,7). This marker helps to make the differentiation between mitosis and apoptotic nuclei, to define histopathological grade correctly (8,9). Signal transducer and transcription activator 3 (STAT3) is a pro-oncogenic transcription factor and, STAT3 plays an important role in many biological processes like cell life and proliferation, chronic inflammation, the acute phase response, autoimmunity, and cancer progression (10-12). STAT3 activation is increased more in grade I and II meningiomas than in normal dural tissue, and STAT3 expression is enhanced with increasing tumor grade (13).

In this study, we aimed to evaluate the correlation between PHH3 and ki-67 LI and the association of STAT3 expression with mitotic index, WHO classification grades, and clinicopathological features of meningioma cases.

MATERIAL AND METHODS

The study was compatible with the tenets of the Helsinki Declaration and has been approved by the local ethics committee of Düzce University (protocol number 187 of September 6, 2021). A total of 25 meningioma cases diagnosed between 2012 and 2021 were included in the study. The inclusion criteria were i) histopathologic diagnosis of meningioma, ii) clinically sufficient history, and iii) sufficiency of pathologic material for histological and immunohistochemical analysis. The exclusion criteria were i) insufficient tumor tissue for immunohistochemistry, and ii) the patients without clinical follow-up. All of the cases were recruited from the archives of the Pathology Department of Düzce University School of Medicine. Demographic data (gender, age, and recurrence status after diagnosis) were obtained from the patient files and archived reports. The mitotic count from 10 HPFs with the highest number of mitotic figures was determined. The tumor location, histological subtype, and grade due to WHO 2016 (1), local invasion, presence of psammoma bodies, and tumor size were evaluated.

On the formalin-fixed, paraffin-embedded tissue blocks, immunohistochemistry was performed to determine STAT3, ki-67, and PHH3 expression. The pathological material was examined by a pathologist to make sure every paraffin-embedded tissue block has sufficient tumor content for immunohistochemical analysis. Immunohistochemical staining with a fully automated assay was performed on 3-4 μm-thick slices based on manufacturer's instructions. Phospho-STAT3 (Tyr705) [RM261] Conc. 0.1mL (1:1000-10000), rabbit monoclonal anti-Ki67 (Thermo #RM-9106-R7), and rabbit polyclonal pHH3 (Thermo #RB-9425-R7) antibodies were used on the Ventana® Benchmark XT (Ventana-Roche Diagnostics, Meylan, France).

All the slides were examined by a pathologist. STAT3 expression was scored between 0 and 3 points due to staining intensity. No staining was considered as 0, light staining as 1, moderate staining as 2, and strong staining as 3 points. Staining percentage points were determined using a manual count of stained cells and the total number of tumor cells. After the hotspot was identified under low magnification, the ki-67 LI was determined as percentage by a manual count. For PHH3, for which the highest number of mitotic figures were identified, the total number of immunostained mitotic figures per 10 HPFs was evaluated in each case.

Statistical Analysis

The distribution of the data was examined using the Shapiro-Wilk test, and comparisons of the groups were made using the Mann-Whitney U test. The correlation between numerical variables was analyzed using Spearman correlation analysis. Descriptive statistics were given as mean±standard deviation, or median, and minimum-maximum values for numerical variables, and numbers and percentages for categorical variables. Statistical analyses were made with the IBM SPSS v.22 program and the significance level was taken as 0.05.

RESULTS

Ten (40%) of the cases were male and 15 (60%) were female. The age range was 37-82 years, with a mean age of 62.4±11.8 years. The most common site of meningiomas was the brain (88%, n=22), followed by the spinal cord (8%, n=2), and the sphenoid wing (4%, n=1). Eighteen (72%) of the cases were WHO grade I and the other 7 (28%) cases were grade II. Transitional was the most common histological subtype (44%, n=11), followed by atypical (24%, n=6), meningothelial (20%, n=5), fibrous (4%, n=1), angiomatous (4%, n=1), and chordoid (4%, n=1). Tumor size ranged from 2-11 cm and the mean tumor size was 5.0±2.5 cm. Psammoma bodies were seen in 13 (52%) cases. The dural invasion was present in 8 (32%) cases. In cases with dural invasion, six cases were accompanied by bone invasion (Figure 1). Only one of the tumors with dural invasion was grade II, the others were grade I. Brain invasion was detected in 1 (4%) patient with an atypical meningioma.

The percentage of STAT3 staining ranged from 2 to 25%, with no staining in five (20%) cases, focal positivity below 5% in 10 (40%), 10% in five (20%), 15% in four (16%), and 25% in one (4%), respectively (Figure 2). Staining intensity was moderate (score 2) in all cases with immunoexpression. All cases that did not show staining with STAT3 were grade I tumors. The percentage of STAT3 staining was statistically significantly different between tumor grades (p=0.047). STAT3 expression was not related to recurrence rate and other clinicopathologic parameters such as tumor size, dura, and bone invasion.

The ki-67 LI was between 1 and 5%. Mitotic count per 10 HPFs was between 1 and 5. A moderate positive correlation was found between PHH3 and ki-67 when calculated as a percentage in the area with the highest mitotic index by manual counting (r=0.621; p=0.001). There was also a moderate positive correlation between the mitotic count and PHH3 of 10 HPFs (r=0.576; p=0.003). Recurrence developed in two cases in the 2nd and 3rd years of follow-up, and both were grade I tumors. The relevant data are shown in Table 1.

DISCUSSION

Although meningiomas are mostly slow-growing, benign tumors, it is important to diagnose the high-grade variants because of the risk of recurrence and different therapy approaches (14,15). Meningiomas may recur, at a rate of up to 25% even in grade I tumors, and high recurrence rates remain a challenge in clinical management (1). Being able to predict early recurrence in meningiomas will be beneficial in providing sufficient postoperative treatment (16). The benefits of post-operative radiation, extending the resection area, and drug therapies in high-grade meningiomas are

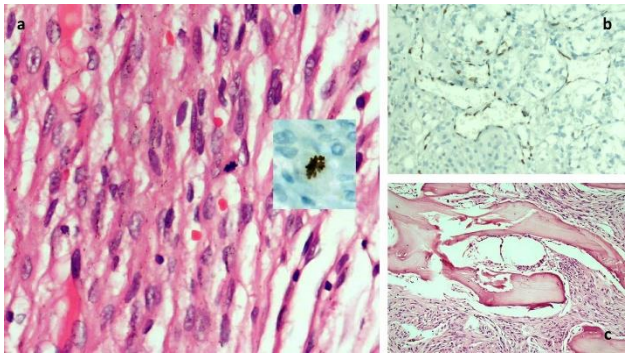

Figure 1. a) Mitotic figure in H&E slide (x40) and the same mitotic figure stained with PHH3 (x40, insert photo), **b)** endothelial staining with PHH3 (x40), **c)** bone invasion in H&E slide (x20)

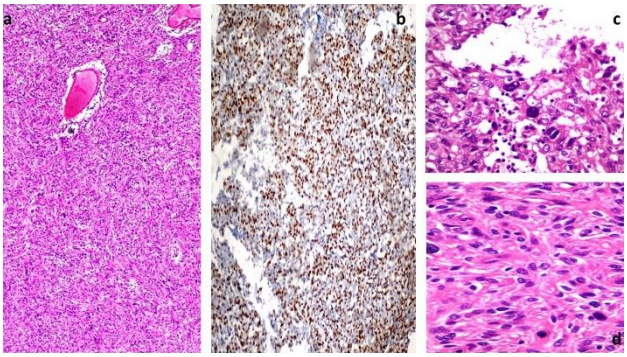

Figure 2. a) Atypical meningioma shows a highly cellular patternless growth pattern (H&E, x4), **b)** STAT3 staining pattern of a hot spot (x10), **c, d)** atypical cells with high nuclear/cytoplasmic ratio (H&E x40)

Table 1. Clinicopathological features of meningiomas

Case	Age	Gender	Localization	Tumor size (cm)	Histological subtype	Grade	STAT3 %	STAT3 score	Ki-67 (%)	Mitotic count (/10 HPF)	PHH3 (/10 HPF)	Bone invasion	Dura invasion	Brain invasion	Recurrence
1	37	M	Left parietal	3	Meningothelial	I	0	0	2	1	4	Yes	Yes	No	No
2	82	F	Right frontal	6	Transitional	I	0	0	1	1	1	No	Yes	No	No
3	56	M	Left frontal	2	Transitional	I	10	2	1	1	6	No	No	No	No
4	75	M	Right frontal	5	Transitional	I	0	0	5	2	6	No	No	No	No
5	59	F	Left frontal	6	Transitional	I	0	0	1	1	3	No	No	No	No
6	66	F	L1	3	Meningothelial	I	0	0	1	1	1	No	No	No	No
7	65	F	Foramen magnum	4	Transitional	I	2	2	1	1	1	No	No	No	No
8	64	F	Left frontal	4	Transitional	I	2	2	2	1	2	No	No	No	No
9	66	M	Right frontoparietal	5	Transitional	I	3	2	1	1	2	Yes	Yes	No	2 years
10	73	M	Left frontal	2	Meningothelial	I	3	2	1	1	1	Yes	Yes	No	No
11	46	M	Right slyvian	5	Angiomatous	I	3	2	2	1	2	No	No	No	No
12	73	F	Right frontoparietal	3	Transitional	I	5	2	2	1	2	No	No	No	No
13	58	M	Left frontal	11	Fibrous	I	5	2	1	1	6	Yes	Yes	No	No
14	59	F	Left slyvian	4	Meningothelial	I	5	2	2	1	4	Yes	Yes	No	No
15	37	F	Right frontal	4.5	Transitional	I	10	2	2	1	5	No	No	No	No
16	56	F	Frontal	2	Transitional	I	10	2	1	1	6	No	No	No	3 years
17	74	M	Right parietal	2	Meningothelial	I	15	2	3	2	30	No	No	No	No
18	79	F	Left frontotemporal	8	Transitional	I	25	2	2	1	5	No	Yes	No	No
19	56	F	Left frontal	4.5	Chordoid	II	10	2	2	1	1	No	No	No	No
20	56	F	T11-12	3.5	Atypical	II	3	2	3	4	12	No	No	No	No
21	66	M	Right frontotemporal	8	Atypical	II	3	2	1	5	7	No	No	No	No
22	50	M	Right parietal	8	Atypical	II	10	2	5	3	8	No	No	No	No
23	66	M	Right sphenoid	10	Atypical	II	15	2	1	1	2	Yes	Yes	No	No
24	69	F	Left frontoparietal	8	Atypical	II	15	2	2	3	3	No	No	Yes	No
25	73	F	Left frontoparietal	4	Atypical	II	15	2	3	2	21	No	No	No	No

STAT3: signal transducer and transcription activator 3, PHH3: phosphorylated histone H3, HPF: high power field, M: male, F: female

controversial (17). Advances in molecular and histopathological studies and advanced imaging techniques are currently promising for meningioma patients with diagnostic challenges (18). Finding new biomarkers and advanced targeted therapies requires a better understanding of meningioma oncogenesis, as predicting the aggressiveness of meningiomas based on histological and genetic criteria is not efficient (15,19).

Histomorphological features, STAT3 activation, NF-2 gene mutation, inactivation of DAL-1, and loss of inhibition of TGFβ may all play a role in recurrence, but it remains unclear which specific changes contribute to the pathway (20).

STAT3 plays a role in the control of mitochondrial function and tumor progression by regulating pro-inflammatory genes and cell survival (21). STAT3 is mutated in numerous human cancers, playing an important role in tumor processes as a critical molecular abnormality (22). Previous studies suggested that elevated STAT3 expression is related to better prognosis in breast cancer; however, carcinomas of the lung, stomach, liver, prostate, pancreas, and osteosarcomas and gliomas show poorer prognosis as STAT3 protein expression levels increase (23).

Johnson et al. (24) suggested that the JAK-STAT3, PI3K-Akt-mTOR, and MEK-1-MAPK pathways are activated in WHO grade II meningiomas, and inhibition of STAT3 activation may be effective in next-generation chemotherapies for high-grade meningiomas. They found significantly higher STAT3 activation in grade II meningiomas than in grade I, but no difference between meningioma subtypes, similarly to our results.

Magrassi et al. (13) suggested that various elements of the STAT family show higher immunoreactivity in meningiomas compared to normal dural tissue. In our study, according to the immunohistochemical results, difference between the percentage of STAT3 staining and tumor grade was statistically significant and, STAT3 expression was significantly higher in cases with high tumor grades. On the contrary, Johnson et al. (16) reported no significant difference in STAT3 immunohistochemical staining between grade I and grade II tumors. Johnson et al. (16) also suggested that the expression of STAT3 is not a sensitive predictor of recurrence in meningiomas. In our study, STAT3 expression was not related to recurrence rate; however, only two patients had a recurrent tumor, so this data is not sufficient to suggest that STAT3 does not impact recurrence.

While evaluating tumor grade, the most important parameter is mitotic count (1). Recognizing the mitoses morphologically is essential to define mitotic count (25). The conventional method used to determine the extent of proliferation of the tumor may not be objective, as finding the area with the highest mitotic activity and mitotic figures may be difficult (25). Distinguishing mitotic figures and other chromatin changes such as apoptotic figures, pyknotic nuclei, and artifacts secondary to crush, may vary with experience. Areas of different cellular densities within the tumor may make it difficult to evaluate these features per area (4). PHH3 is helpful to differentiate apoptosis and mitosis, as there is a direct relationship between H3 phosphorylation and mitotic chromosome condensation that begins during the early prophase, whereas phosphorylation of histone H3 is not observed in apoptosis (26). It is important to decide whether to use a "hotspot" or "mean" counting method because the mitotic activity may show variation in different regions of the tumor (25).

The ki-67 proliferation index helps to identify the most active site and predict recurrence (27). While both the mitotic index and ki-67 can be used to quantify cell proliferation, it should be considered that ki-67 stains positive nuclei in the G1, G2, or S phases, which are generally more variable and longer than the M phase (25). The ki-67 LI is a quantitative indicator used to define proliferation activity, which is the percentage of tumor cell nuclei positively stained for ki-67 (26). In the literature, a ki-67 LI above 4% has been defined as a high-risk factor for recurrence, but there is still no definite cut-off value. Various studies have suggested different values, ranging from 1-10% (27,28). This heterogeneity may be due to interlaboratory differences. The ki-67 LI was 1% in two cases with recurrence in our study. To our knowledge, ki-67 does not seem to be a reliable marker for predicting recurrence and prognosis.

PHH3 specifically detects core protein histone H3 when it is phosphorylated at serine 10 (Ser10) or serine 28 (Ser28). Phosphorylation of histone H3 is a process that occurs almost exclusively during mitosis (26). In their series consisting of 48 meningioma cases, Puripat et al. (29) reported that PHH3 is a fast, sensitive, and easy method for determining mitotic activity. Winther et al. (4) stated that the most reliable method for predicting prognosis in a series of 160 cases was the PHH3 proliferation index.

Ozek et al. (3) found a strong correlation between the PHH3 and ki67 proliferation indices in a series of 104 cases; however, there was no significant correlation between PHH3 and tumor recurrence. Elmaci et al. (27) stated in a review that PHH3 is useful, but more studies are needed to replace this method with the mitosis counting method in conventional H&E slides. In our study, we found a moderate positive correlation between mitotic count and PHH3/10 HPF, and a moderate positive correlation between the ki67 proliferation index and PHH3 index.

The background staining of PHH3 in leukocytes and highly vascular areas, and the lack of a definite cut-off value create an obstacle for the use of PHH3 instead of counting mitotic figures. Additionally, staining of any prophase nuclei may create difficulties in evaluating PHH3 (30). Nonetheless, morphologically distinguishing M-phase from prophase nuclei is essential but not very difficult.

CONCLUSION

A combination of ki-67, PHH3, and STAT3 is useful in the grading of meningiomas and prediction of recurrence. The use of these three methods together may contribute to larger studies that will shed light on the prognosis of these tumors.

Ethics Committee Approval: The study was approved by the Non-interventional Health Researches Ethics Committee of Düzce University (06.09.2021, 187).

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: SKC; Design: SKC; Data Collection/Processing: SKC, GK; Analysis/Interpretation: SKC; Literature Review: SKC; Drafting/Writing: SKC; Critical Review: SKC, GK.

REFERENCES

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO classification of tumours of the central nervous system. WHO Classification of Tumours. 4th ed. Lyon: IARC Press; 2016.
2. Olar A. Phosphohistone H3: implications for clinical practice and risk assessment in meningioma. Neuro Oncol. 2015;17(5):631-3.
3. Ozek E, Akdag H, Tosuner Z, Abdallah A, Hatiboglu MA. The correlation between phosphorylated histone H3 (PHH3) and p-STAT3 in meningiomas. Clin Neurol Neurosurg. 2019;178:46-50.
4. Winther TL, Arnli MB, Salvesen Ø, Torp SH. Phosphohistone-H3 proliferation index is superior to mitotic index and MIB-1 expression as a predictor of recurrence in human meningiomas. Am J Clin Pathol. 2016;146(4):510-20.
5. Liu, N, Song SY, Jiang JB, Wang TJ, Yan CX. The prognostic role of Ki-67/MIB-1 in meningioma: A systematic review with meta-analysis. Medicine (Baltimore). 2020;99(9):e18644.
6. Jin C, Huang Y, Nasim M, Yang Y, Lee L. Gastrointestinal stromal tumors risk stratification utilizing phospho-histone H3 evaluated by manual counting and computer-assisted image analysis. Int J Surg Pathol. 2019;27(7):706-12.
7. Hao Q, Dai C, Deng Y, Xu P, Tian T, Shuai Lin S, et al. Pooling analysis on prognostic value of PHH3 expression in cancer patients. Cancer Manag Res. 2018;10:2279-88.
8. Hacking SM, Sajjan S, Lee L, Ziemba Y, Angert M, Yang Y, et al. Potential pitfalls in diagnostic digital image analysis: experience with ki-67 and PHH3 in gastrointestinal neuroendocrine tumors. Pathol Res Pract. 2020;216(3):152753.
9. Kim MJ, Kwon MJ, Kang HS, Choi KC, Nam ES, Cho SJ, et al. Identification of phosphohistone H3 cutoff values corresponding to original WHO grades but distinguishable in well-differentiated gastrointestinal neuroendocrine tumors. Biomed Res Int. 2018;2018:1013640.
10. Huynh J, Chand A, Gough D, Ernst M. Therapeutically exploiting STAT3 activity in cancer - using tissue repair as a road map. Nat Rev Cancer. 2019;19(2):82-96.
11. Setsu N, Kohashi K, Endo M, Yamamoto H, Tamiya S, Takahashi Y, et al. Phosphorylation of signal transducer and activator of transcription 3 in soft tissue leiomyosarcoma is associated with a better prognosis. Int J Cancer. 2013;132(1):109-15.
12. Lee I, Fox PS, Ferguson SD, Bassett R, Kong LY, Schacherer CW, et al. The expression of p-STAT3 in stage IV melanoma: risk of CNS metastasis and survival. Oncotarget. 2012;3(3):336-44.
13. Magrassi L, De-Fraja C, Conti L, Butti G, Infuso L, Govoni S, et al. Expression of the JAK and STAT superfamilies in human meningiomas. J Neurosurg. 1999;91(3):440-6.
14. Chohan MO, Ryan CT, Singh R, Lanning RM, Reiner AS, Rosenblum MK, et al. Predictors of treatment response and survival outcomes in meningioma recurrence with atypical or anaplastic histology. Neurosurgery. 2018;82(6):824-32.
15. Delgado-López PD, Cubo-Delgado E, González-Bernal JJ, Martín-Alonso J. A practical overview on the molecular biology of meningioma. Curr Neurol Neurosci Rep. 2020;20(12):62.
16. Johnson M, O'Connell M, Walter K. STAT3 activation and risk of recurrence in meningiomas. Oncol Lett. 2017;13(4):2432-6.
17. Paldor I, Awad M, Sufaro YZ, Kaye AH, Shoshan Y. Review of controversies in management of non-benign meningioma. J Clin Neurosci. 2016;31:37-46.
18. Nowosielski M, Galldiks N, Iglseder S, Kickingereder P, von Deimling A, Bendszus M, et al. Diagnostic challenges in meningioma. Neuro Oncol. 2017;19(12):1588-98.
19. Parada CA, Osbun J, Kaur S, Yakkioui Y, Shi M, Pan C, et al. Kinome and phosphoproteome of high-grade meningiomas reveal AKAP12 as a central regulator of aggressiveness and its possible role in progression. Sci Rep. 2018;8(1):2098.
20. Johnson MD, O'Connell M, Facik M, Maurer P, Jahromi B, Pilcher W. Cerebrospinal fluid stimulates leptomeningeal and meningioma cell proliferation and activation of STAT3. J Neurooncol. 2012;107(1):121-31.
21. Valle-Mendiola A, Soto-Cruz I. Energy metabolism in cancer: the roles of STAT3 and STAT5 in the regulation of metabolism-related genes. Cancers (Basel). 2020;12(1):124.
22. Page BD, Ball DP, Gunning PT. Signal transducer and activator of transcription 3 inhibitors: a patent review. Expert Opin Ther Pat. 2011;21(1):65-83.
23. Igelmann S, Neubauer HA, Ferbeyre G. STAT3 and STAT5 activation in solid cancers. Cancers (Basel). 2019;11(10):1428.
24. Johnson MD, O'Connell M, Vito F, Bakos RS. Increased STAT-3 and synchronous activation of Raf-1-MEK-1-MAPK, and phosphatidylinositol 3-Kinase-Akt-mTOR pathways in atypical and anaplastic meningiomas. J Neurooncol. 2009;92(2):129-36.
25. Cree IA, Tan PH, Travis WD, Wesseling P, Yagi Y, White VA, et al. Counting mitoses: SI(ze) matters! Mod Pathol. 2021;34(9):1651-7.
26. Kim YJ, Ketter R, Steudel WI, Feiden W. Prognostic significance of the mitotic index using the mitosis marker anti-phosphohistone H3 in meningiomas. Am J Clin Pathol. 2007;128(1):118-25.
27. Elmaci I, Altinoz MA, Sari R, Bolukbasi FH. Phosphorylated histone H3 (PHH3) as a novel cell proliferation marker and prognosticator for meningeal

tumors: A short review. Appl Immunohistochem Mol Morphol. 2018;26(9):627-31.
28. Nagahama A, Yashiro M, Kawashima T, Nakajo K, Morisako H, Uda T, et al. Combination of p53 and Ki67 as a promising predictor of postoperative recurrence of meningioma. Anticancer Res. 2021;41(1):203-10.
29. Puripat N, Loharamtaweethong K. Phosphohistone H3 (PHH3) as a surrogate of mitotic figure count for grading in meningiomas: a comparison of PHH3 (S10) versus PHH3 (S28) antibodies. Virchows Arch. 2019;474(1):87-96.
30. Fukushima S, Terasaki M, Sakata K, Miyagi N, Kato S, Sugita Y, et al. Sensitivity and usefulness of anti-phosphohistone-H3 antibody immunostaining for counting mitotic figures in meningioma cases. Brain Tumor Pathol. 2009;26(2):51-7.