Original Article
Correlation between CT characteristics and P16 expression in malignant meningioma

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Abstract: Objective: As the second major type of intracranial tumor, meningioma leads to high fatality rates. This study aims to investigate the correlation between computed tomography (CT) characteristics and P16 expression in malignant meningiomas. Methods: A total of 63 patients with malignant meningioma treated in our hospital from May 2011 to November 2016 were randomly enrolled. Postoperative carcinoma tissue and para-carcinoma tissue specimens and CT imaging data were collected. The expression of P16 messenger ribonucleic acid (mRNA) in carcinoma tissues and para-carcinoma tissues was detected via reverse transcription-polymerase chain reaction (RT-PCR), and the level of P16 protein was detected via immunohistochemistry (IHC). The correlation between CT scan and P16 expression was analyzed. Results: The expression of P16 mRNA in meningioma tissues was significantly lower than that in para-carcinoma tissues (P<0.05). The positive rate of P16 protein in meningioma tissues was significantly decreased compared to that in para-carcinoma tissues (P<0.05). Among 63 patients with malignant meningioma, there were no significant differences in the shape, profile and calcification in CT scans between the P16 positive group and P16 negative group (P > 0.05), but evident differences were found in focal necrosis, edema and bone changes. After enhancement scans, the spiral CT (SCT) enhancement value in patients with negative P16 expression was significantly higher than that in patients with positive P16 expression (P<0.05). Conclusion: CT detection is correlated with the level of P16, particularly in non-invasive meningioma, the combined use of CT and P16 shows great promise in the clinical diagnosis of malignant meningioma.

Keywords: Malignant meningioma, P16, CT

Introduction

Meningioma is a common primary intracranial tumor, accounting for 15.31% of all intracranial tumors, second only to glioma [1]. Malignant meningioma, unlike benign meningioma, is characterized by clearly atypical and anaplastic features, which include very complex karyotype, invasiveness and are insensitive to traditional treatment methods, such as surgery, radiotherapy and chemotherapy [2]. It has been indicated that patients with malignant meningioma are commonly younger than those with benign meningioma, and intracranial hypertension is often caused by to the compression of the tumor against the adjacent brain parenchyma and cranial nerves, as well as disorders in cerebral blood and cerebrospinal fluid circulation [3]. In addition, the 5-, 10- and 15-year recurrence rates of malignant meningioma af-ter operation are 33%, 66% and 100%, respectively [4].

The deletion of chromosome 1p resulting in a second common chromosome abnormality is observed in malignant meningioma, and often indicates a high recurrence rate [5]. Previous study has proposed that the loss of heterozygosity (LOH) of 1p, 10q and 9p was also closely related to recurrence [6], in which the 9p abnormality is often accompanied by mutations or deletions in the p14 alternative reading frame (ARF) encoding P14, cyclin-dependent kinase 4 inhibitor B (CDKN2B)/p15ARF encoding P15 and CDKN2A/P16 inhibitor of cyclin-dependent kinase A (INKa) encoding P16, signifying a poor prognosis [7]. The P16 gene, also known as multiple tumor suppressor 1 (MTS1) or cyclin-dependent kinase 4 inhibitor (CDK4I), is located on chromosome 9p21 and encodes a pro-

tein product of 16,000 molecular weight, which, as an inhibiting factor of CDK4, inhibits cell division. Therefore, P16 is considered as an important tumor suppressor gene [8].

Computed tomography (CT) examination represents one of the necessary preoperative examinations, but most tumors cannot be accurately diagnosed by a single CT scan. In this study, changes in P16 gene and protein expression levels in 63 pairs of meningioma tissues and para-carcinoma tissues were analyzed via polymerase chain reaction (PCR) and immunohistochemistry (IHC), and the correlation between P16 protein expression and CT results of the patients was also analyzed, so as to explore an improved method in the molecular diagnosis as well as targeted therapy for malignant meningioma.

Patients and methods

Clinical data

A total of 63 patients pathologically diagnosed with malignant meningioma in our hospital from May 2011 to November 2016 were collected, and they all underwent routine CT scan and enhancement scan before operation. There were 30 males and 33 females aged 28-73 years old with an average age of 54 years old. There were 24 cases of fibrous meningioma, 16 cases of epithelial meningioma, 23 cases of vascular meningioma and 20 cases of transitional meningioma. Study inclusion criteria: any patient who planned to receive an elective primary meningioma resection at our hospital with clinical symptoms of headache, nausea, numbness of limb, dyskinesia and impaired vision. Exclusion criteria: any meningioma patients who had their surgery performed outside our hospital. All subjects signed an informed consent, and this clinical trial was approved by the Ethics Committee of Shanxian Central Hospital.

CT imaging equipment and scanning parameters

All patients underwent CT scan and enhancement scan before operation. The 64-slice spiral CT (SCT) (Siemens, Germany) was used in the CT scan with the slice thickness of 1 mm and slice gap of 1 mm. Ultravist was intravenously injected for the enhancement scan.

Detection of P16 messenger ribonucleic acid (mRNA) expression level via reverse transcription-polymerase chain reaction (RT-PCR)

After tissue homogenization, the total RNA was extracted from tissues using the extraction kit (Qiagen, Shanghai, China) according to instructions of the manufacturer. Then 1 µg total RNA was reversely transcribed, followed by RT-PCR using the SYBR® Premix Ex Taq™ II kit (Takara) (annealing at 55°C for 45 cycles of amplification). The relative quantification of each gene was analyzed by $2^{-\Delta Ct}$, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference. The relative expression level of mRNA was calculated as follows: $2^{-\Delta Ct}$ [ΔCt = Ct (target gene) - Ct (GAPDH)]. Primer sequences are as follows: P16: Forward: 5'-GAAGAAAGAGGAGGGGCTG-3', Reverse: 5'-GCGCTACCTGATTCCAATTC-3'. GAPDH: Forward: 5'-ATTGATGGATGCTAFGAGTATT-3', Reverse: 5'-AGTCTTCTGGGTGGCAGTGAT-3'. This experiment was repeated 3 times.

Detection of P16 protein expression via IHC

Streptavidin-peroxidase (SP) staining: carcinoma tissues and para-carcinoma tissues were prepared in paraffin-embedded blocks and sliced into 4 µm-thick sections. Slices were baked in an oven at 65°C for 3-4 h, followed by dewaxing with xylene, dehydration with gradient ethanol, and antigen retrieval via a microwave using sodium citrate buffer. After the peroxidase was blocked via 3% H_2O_2 blocker and sections were sealed with 10% donkey serum, phosphate buffered saline (PBS) was added as the negative control, and the primary antibody (P16, Abcam, USA, diluted at 1:200) was also added dropwise, followed by incubation in a wet box at 4°C overnight. On the next day, sections were washed with PBS 3 times, and the ready-to-use universal secondary antibody was added for further incubation, followed by color development via diaminobenzidine (DAB) and photography under a microscope. Brown and dark brown nuclei under the microscope indicated the positive cells, and the number of positive cells was counted. Number of positive cells/total number of cells in the visual field > 10% indicated positive expression.

Statistical methods

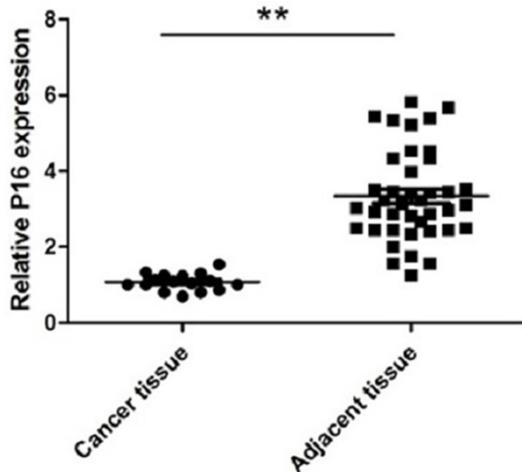MedCalc software (Mariakerke, Belgium) was used for data sorting and processing. Mea-

Figure 1. P16 mRNA expression level in carcinoma tissues and para-carcinoma tissues detected via RT-PCR. Results of RT-PCR show that the P16 mRNA expression level in meningioma tissues is significantly lower than that in para-carcinoma tissues.

surement data were presented as mean ± standard deviation, independent *t*-test was adopted for intergroup comparison, and chi-square test was used for the comparison of enumeration data. $P < 0.05$ suggested that the difference was statistically significant.

Results

P16 mRNA expression level in carcinoma tissues and para-carcinoma tissues detected via RT-PCR

The total mRNA was extracted from 63 pairs of meningioma tissues and para-carcinoma tissues, and the P16 mRNA level in carcinoma tissues and para-carcinoma tissues was detected via RT-PCR. The P16 mRNA expression level in meningioma tissues was significantly lower than that in para-carcinoma tissues with an average fold change of 2.75 ($P < 0.05$) (**Figure 1**).

P16 protein expression detected via IHC

IHC results showed that P16 was expressed in the cytoplasm (**Figure 2**). There were 15 positive cases of P16 protein in meningioma tissues with a positive rate of 23.81%. In comparison, there were 58 positive cases of P16 protein in para-carcinoma tissues with a positive rate of 92.06%. Similar to the mRNA level, the positive rate of P16 protein in meningioma tis-

sues was significantly lower than that in para-carcinoma tissues ($P < 0.05$) (**Table 1**).

CT signs of tumor in patients

CT scan results from 63 cases of malignant meningioma were shown in **Figure 3**. Of note, the tumor diameter was 3.1-8.3 cm with an average of 5.3 cm. According to the CT scan, there were 28 cases of irregular tumor morphology, 42 cases of peritumoral edema, 39 cases of significant bone destruction and 24 cases of intratumoral calcification.

Correlation between CT signs and P16 expression in patients

The focal shape, profile, tumor necrosis, calcification, perifocal edema and skull changes were compared between P16 positive group and P16 negative group (**Table 2**). Among 63 patients with malignant meningioma, there were no significant differences in the shape, profile and calcification in CT scan between the P16 positive group and P16 negative group ($P > 0.05$), but there were evident differences in the focal necrosis, edema and bone changes, suggesting that P16 deletion may be closely related to necrosis, edema and bone changes ($P < 0.05$).

Correlation between P16 expression and CT enhancement

The focal edge, density and its changes were compared after enhancement scan. After enhancement scan, the SCT enhancement value in patients with negative P16 expression was noticeably higher than that in patients with positive P16 expression ($P < 0.05$) (**Table 3**). The expression of P16 in carcinoma tissues of patients was significantly negatively correlated with CT enhancement.

Discussion

Previous study has mostly focused on the analysis of CT signs of general pathological changes in meningioma, but less attention is paid to the correlation between CT scan results and tumor suppressor genes in meningioma. It has been revealed that there were no significant differences in CT results between vascular and nonvascular meningioma (i.e., epithelial type, fibrous type and transitional type) [9].
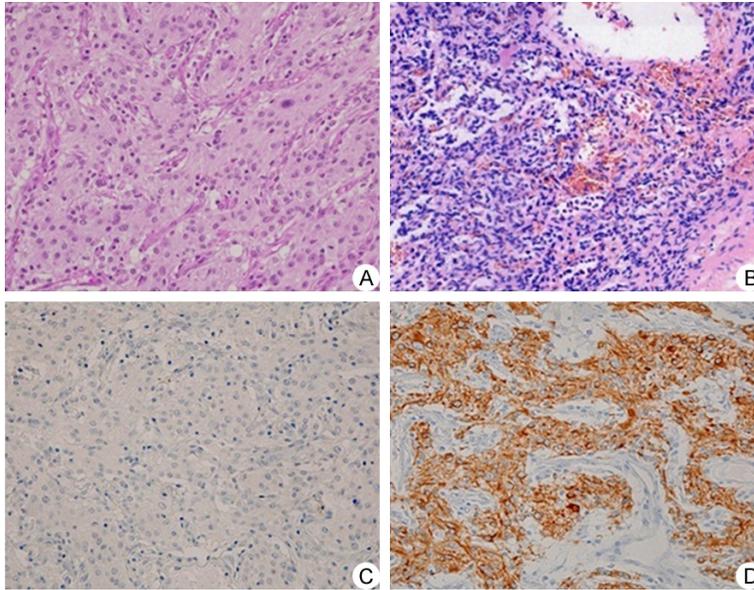
Figure 2. P16 protein expression detected via IHC. A: Morphology of meningioma tissues detected via hematoxylin and eosin (H&E) staining. B: Morphological data of para-carcinoma tissues. C: IHC staining of meningioma tissues shows that the P16 protein expression in meningioma tissues is negative. D: P16 protein in para-carcinoma tissues is expressed in the cytoplasm, and positive cells are brown yellow or dark brown. Magnification 100×.

Table 1. Difference in P16 protein expression between meningioma tissues and para-carcinoma tissues

Group	n	P16	
		Negative	Positive
Carcinoma tissue	63	48 (76.19%)	15 (23.81%)
Para-carcinoma tissue	63	5 (7.94%)	58 (92.06%)
χ^2		15.61	
P		0.000	

The occurrence and development of meningioma is a gradually evolving process, which also involves the activation of protooncogenes and inactivation of tumor suppressor genes [9]. P16, as a tumor suppressor gene, contains 4 ankyrin repetitive sequences required for protein-protein interaction. These ankyrin repetitive sequences form a concave structural domain, which binds to the non-catalytic domain of human CDK4/CDK6 to inhibit the catalytic activity of CDK4-6/cyclinD enzyme complex and arrest the cell cycle in deoxyribonucleic acid (DNA) presynthetic phase (G1 phase) [10-12]. In the case of P16 inactivation, its competitive binding capacity declines, the binding between Cyclin D1 and CDK1 increases, the cell division accelerates, and the cycle regulation gradually becomes out of control, resulting in cancer. Numerous studies have found that there are P16 point mutations and gene deletion-induced inactivation in the occurrence and development of most human tumors [13-15]. Notably, the P16 gene deletion rate is as high as 87.5% in gliomas. In addition to gene deletion and mutations in the mechanism of P16 inactivation, the aberrant methylation of CpG Island in the 5'-end promoter region is also commonly found in the tumor, and its mutation inhibits the transcription, namely gene expression deletion. Moreover, studies have also revealed that the methylation of CpG Island serve as promoters of tumor suppressor genes and appear in the early stages of a tumor, which is closely related to the occurrence and development of tumors [16, 17]. In this study, the P16 protein expression in 63 pairs of carcinoma tissues and para-carcinoma tissues was detected via IHC. It was found that P16 was mainly expressed in the cytoplasm in carcinoma tissues and para-carcinoma tissues. The positive rate of P16 protein was 85.71% (72/84) in para-carcinoma tissues and 30.95% (26/84) in carcinoma tissues.

The basic imaging characteristics of malignant meningioma are as follows: it frequently occurs in the hemispherical convexity, cerebral falx, tentorium of cerebellum, sagittal sinus, etc., where the broad base contacts the cranial plate, the tumor is enhanced significantly [18]. The correlation between positive P16 expression and CT parameters was analyzed in this study. The focal shape, profile, tumor necrosis, calcification, perifocal edema and skull changes were compared between P16 positive and P16 negative groups. Among 63 patients with malignant meningioma, despite the fact there were no significant differences in the shape,
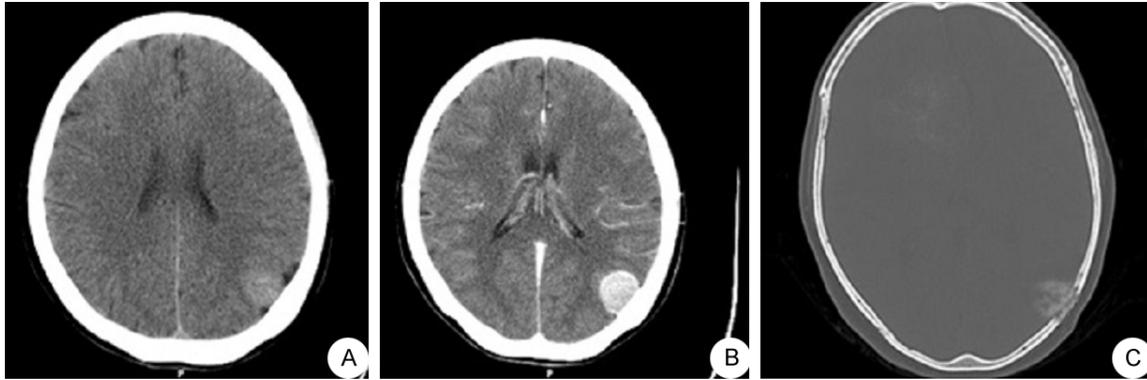
Figure 3. CT scans of tumors in patients. A male patient aged 63 years old with left occipital meningioma. A: There is slightly higher-density shadow in a quasi-circular shape in the left occiput, and the broad base attaches the inner plate of cranial bone. B: The tumor is obviously enhanced after enhancement, and dural tail signs can be seen. C: The bone window indicates the significant destruction of parietal bone.

Table 2. Correlation between CT scans and P16 expression in patients

CT characteristic		n=63	P16 Negative (48)	P16 Positive (15)	P
Shape	Quasi-circular	35	26	9	0.651
	Irregular	28	22	6	
Profile	Flat	39	29	10	0.812
	Lobular	9	6	3	
	Uneven	15	13	2	
Necrosis	Yes	26	23	3	0.019
	No	37	25	12	
Calcification	Yes	24	16	8	0.686
	No	39	32	7	
Edema	Yes	42	35	7	0.037
	No	21	13	8	
Skull changes	Yes	39	36	3	0.015
	No	24	11	12	

Table 3. Correlation between P16 expression and CT enhancement

Detection index		n	SCT enhancement value > 50 Hu	SCT enhancement value ≤ 50 Hu	P
P16	Negative	48	39	9	0.004
	Positive	15	4	11	

profile and calcification in CT scan between P16 positive and P16 negative groups, there were marked differences that were observed in the focal necrosis, edema and bone changes: suggesting that P16 deletion may be closely related to necrosis, edema and bone changes (P<0.05).

Therefore, patients were qualitatively diagnosed after surgery in this study. According to previous data and related literature [19], malignant meningioma can be diagnosed with the references of the following features: (1) Tumor necrosis: the deletion of tumor suppressor P16 leads to the rapid proliferation of tumor. Necrosis often occurs due to malnutrition in some tumor regions. (2) Peritumoral edema: studies have demonstrated that peritumoral edema is associated with benign and malignant type of meningioma [20], which is consistent with the findings in this study. There are many reasons for edema in meningioma, such as the increased capillary permeability, venous sinus occlusion, tumor angiogenesis, endocrine changes in tumor and production of vascular endothelial growth factor. This study indicates that the deletion of tumor suppressor P16 may be a regulatory factor for edema. (3) Bone changes: the tumor breaks through the capsule and invades the adjacent brain parenchyma, there is even destruction in the inner and outer plates of the cranial bone or extracranial infiltrative growth of the tumor, and soft tissue masses are formed. In this study, we detected bone changes in 39 patients, and P16 positivity was found in tumor tissues in 36 patients. (4) Due to uneven tumor density and signal as well as rapid tumor growth, ischemia and necrosis

are often found in the center of the tumor, leading to uneven density in the malignant meningioma, which also reflects the malignant and invasive features of the tumor and is considered as one of the important signs in the diagnosis of malignant meningioma.

Conclusion

In conclusion, this study indicates that P16 is deleted in malignant meningioma tissues, which is associated with CT scan diagnosis. The conventional CT scan, along with the detection of tumor suppressor P16 levels, facilitates the diagnosis of meningioma in a noninvasive manner, which provides new leads for the evaluation of severity, prognosis, as well as clinical treatment of meningioma.

Disclosure of conflict of interest

None.

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References

[1] Aizer AA, Bi WL, Kandola MS, Lee EQ, Nayak L, Rinne ML, Norden AD, Beroukhim R, Reardon DA, Wen PY, Al-Mefty O, Arvold ND, Dunn IF and Alexander BM. Extent of resection and overall survival for patients with atypical and malignant meningioma. Cancer 2015; 121: 4376-4381.

[2] Pereira BJA, de Almeida AN, de Aguiar PHP, Paiva WS, Cabrera HN, da Silva CC, Teixeira MJ and Marie SKN. Atypical and malignant meningiomas: neurooncologic management in a brazilian cohort. World Neurosurg 2018; 110: e20-e23.

[3] Cheng G, Zhang L, Lv W, Dong C, Wang Y and Zhang J. Overexpression of cyclin D1 in meningioma is associated with malignancy grade and causes abnormalities in apoptosis, invasion and cell cycle progression. Med Oncol 2015; 32: 439.

[4] Weber DC, Ares C, Villa S, Peerdeman SM, Renard L, Baumert BG, Lucas A, Veninga T, Pica A, Jefferies S, Ricardi U, Miralbell R, Stelmes JJ, Liu Y, Collette L and Collette S. Adjuvant postoperative high-dose radiotherapy for atypical and malignant meningioma: a phase-II parallel non-randomized and observation study (EORTC 22042-26042). Radiother Oncol 2018; 128: 260-265.

[5] Nowak A, Dziedzic T, Krych P, Czernicki T, Kunert P and Marchel A. Benign versus atypical meningiomas: risk factors predicting recurrence. Neurol Neurochir Pol 2015; 49: 1-10.

[6] Sahm F, Schrimpf D, Stichel D, Jones DTW, Hielscher T, Schefzyk S, Okonechnikov K, Koelsche C, Reuss DE, Capper D, Sturm D, Wirsching HG, Berghoff AS, Baumgarten P, Kratz A, Huang K, Wefers AK, Hovestadt V, Sill M, Ellis HP, Kurian KM, Okuducu AF, Jungk C, Drueschler K, Schick M, Bewerunge-Hudler M, Mawrin C, Seiz-Rosenhagen M, Ketter R, Simon M, Westphal M, Lamszus K, Becker A, Koch A, Schittenhelm J, Rushing EJ, Collins VP, Brehmer S, Chavez L, Platten M, Hanggi D, Unterberg A, Paulus W, Wick W, Pfister SM, Mittelbronn M, Preusser M, Herold-Mende C, Weller M and von Deimling A. DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. Lancet Oncol 2017; 18: 682-694.

[7] Domingues P, Gonzalez-Tablas M, Otero A, Pascual D, Ruiz L, Miranda D, Sousa P, Goncalves JM, Lopes MC, Orfao A and Tabernero MD. Genetic/molecular alterations of meningiomas and the signaling pathways targeted. Oncotarget 2015; 6: 10671-10688.

[8] Yu DH, Waterland RA, Zhang P, Schady D, Chen MH, Guan Y, Gadkari M and Shen L. Targeted p16 (Ink4a) epimutation causes tumorigenesis and reduces survival in mice. J Clin Invest 2014; 124: 3708-3712.

[9] Zhu M, Yu BB, Zhai JL and Sun G. Case of Langerhans cell histiocytosis that mimics meningioma in CT and MRI. J Korean Neurosurg Soc 2016; 59: 165-167.

[10] Gu K, Mes-Masson AM, Gauthier J and Saad F. Analysis of the p16 tumor suppressor gene in early-stage prostate cancer. Mol Carcinog 1998; 21: 164-170.

[11] Feng W, Han Z, Zhu R, Liu P and Liu S. Association of p16 gene methylation with prostate cancer risk: a meta-analysis. J BUON 2015; 20: 1074-1080.

[12] Pan FP, Zhou HK, Bu HQ, Chen ZQ, Zhang H, Xu LP, Tang J, Yu QJ, Chu YQ, Pan J, Fei Y, Lin SZ, Liu DL and Chen L. Emodin enhances the demethylation by 5-Aza-CdR of pancreatic cancer cell tumor-suppressor genes P16, RASSF1A and ppENK. Oncol Rep 2016; 35: 1941-1949.

[13] Geraldes C, Goncalves AC, Cortesao E, Pereira MI, Roque A, Paiva A, Ribeiro L, Nascimento-Costa JM and Sarmento-Ribeiro AB. Aberrant p15, p16, p53, and DAPK gene methylation in myelomagenesis: clinical and prognostic implications. Clin Lymphoma Myeloma Leuk 2016; 16: 713-720, e2.

[14] Mohd Ridah LJ, A Talib N, Muhammad N, Hussain FA and Zainuddin N. p16 tumor suppressor gene methylation in diffuse large B cell lymphoma: a study of 88 cases at two hospitals in the East Coast of Malaysia. Asian Pac J Cancer Prev 2017; 18: 2781-2785.

[15] Supsavhad W, Dirksen WP, Hildreth BE and Rosol TJ. p16, pRb, and p53 in feline oral squamous cell carcinoma. Vet Sci 2016; 3.

[16] Zhang H, Li X, Ge L, Yang J, Sun J and Niu Q. Methylation of CpG island of p14(ARK), p15(INK4b) and p16(INK4a) genes in coke oven workers. Hum Exp Toxicol 2015; 34: 191-197.

[17] Cui C, Gan Y, Gu L, Wilson J, Liu Z, Zhang B and Deng D. P16-specific DNA methylation by engineered zinc finger methyltransferase inactivates gene transcription and promotes cancer metastasis. Genome Biol 2015; 16: 252.

[18] Karki P, Yonezawa H, Bohara M, Oyoshi T, Hirano H, Moinuddin FM, Hiraki T, Yoshioka T and Arita K. Third ventricular atypical meningioma which recurred with further malignant progression. Brain Tumor Pathol 2015; 32: 56-60.

[19] Zhao P, Hu M, Zhao M, Ren X and Jiang Z. Prognostic factors for patients with atypical or malignant meningiomas treated at a single center. Neurosurg Rev 2015; 38: 101-107; discussion 107.

[20] Loewenstern J, Aggarwal A, Pain M, Barthelemy E, Costa A, Bederson J and Shrivastava RK. Peritumoral edema relative to meningioma size predicts functional outcomes after resection in older patients. Oper Neurosurg (Hagerstown) 2019; 16: 281-291.