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Role and mechanism of autophagy in the occurrence and development of meningothelial meningioma

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Abstract: This paper aims to investigate the role and mechanism of autophagy in meningioma. A total of 182 meningiomas which diagnosed in the ultrastructural pathology department of Beijing Neurosurgical Institute, Beijing Tiantan Hospital from 1993 to 2021. Light microscope, electron microscope, western blotting and immunohistochemical staining were used. 182 meningiomas, 104 males and 78 females. There were 174 WHO grade 1 benign meningiomas, including 62 meningothelial types, 39 fibrous type, 58 transitional types, 15 angiomatous types, 6 WHO grade 2 cases, including 4 clear cell types and 2 atypical types, 2 WHO grade 3 anaplastic types. Transmission electron microscopy (TEM) showed that the incidence of autophagosomes as a high expression in 27 cases, most of which were meningothelial meningiomas. Immunohistochemical markers EMA, PR, Vimentin, SSTR-2, Ki67, and CD34 were positively expressed in most of the tumors, GFAP, S-100, desmin, C-erbB-2 expression were negative. Western blotting was used to detect the up-regulated proteins of LC3, Beclin1, and down-regulated proteins of Bax, Caspase3, mTOR, PI3K, Akt, S6 and 4EBP1in meningothelial meningiomas compared with the none –meningothelial control group.Autophagy is induced by down-regulating PI3K/Akt/mTOR signaling pathway molecules in meningothelial meningiomas, which can inhibit tumor apoptosis and promote tumorigenesis.

Keywords: Meningothelial meningioma; Autophagy; Occurrence and development.

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INTRODUCTION

Meningioma is a common clinical intracranial tumor of non-neuroepithelial origin, and its incidence is second only to glioma [Vernooij *et al.*, 2007]. It is more common among women, and the peak incidence is 50-70 years old. As the most common primary intracranial benign tumor in adults [Goldbrunner *et al.*, 2021; Huang *et al.*, 2019], meningioma cells originate from arachnoid granule cells, and the predilection sites are also related to the distribution of arachnoid granules. The most common sites are the convexity of the cerebral hemisphere (parietal, temporal, frontal, and occipital lobes), parasagittal sinus, and falx cerebral wait. Meningiomas grow slowly, often compressing peripheral nerve tissue and causing related symptoms and signs [Fountain *et al.*, 2017; Nakasu *et al.*, 2020]. The first diagnosis of patients is mainly headache and seizures. There are many subtypes of meningiomas, most of which are benign (WHO grade 1), and some subtypes have a poor prognosis, equivalent to borderline tumors (WHO grade 2)

or even malignant tumors (WHO grade 3) [Siempis *et al.,* 2020]. There are many histological types of meningiomas, various clinical manifestations, and variable incidence sites, which are easily misdiagnosed clinically [Poillet *et al.,* 2019]. Among them, meningothelial meningiomas are the most common. More and more studies have shown that autophagy is ubiquitous in tumor tissues [Shintani *et al.,* 2004; Ichimiya *et al.,* 2020]. It can not only inhibit cell apoptosis by removing its own waste and providing nutrients, but also participate in the process of cell death together with apoptosis [Mawrin *et al.,* 2010]. However, it has not been reported in sporadic meningiomas. Therefore, this paper focuses on the role of autophagy in the occurrence and development of meningothelial meningiomas and their related mechanisms.

MATERIALS AND METHODS

1. Case data: A total of 182 patients diagnosed with meningioma from 1993 to 2021 in the ultrastructural pathology department of Beijing Institute of Neurosurgery, Beijing Tiantan Hospital affiliated with Capital Medical University were collected, including 104 males (57%) and 78 females (43%). Ages occurred between 12-75 years, with an average age of 48. The 182 specimens included three grades and seven types of meningiomas, mainly WHO grade 1, including 62 meningothelial types, 39 fibrous types, 15 angiomatous types, and 58 transition types. Meningothelial type is more common. WHO grades 2-3 were prone to recurrence after surgery, including 4 clear cell types, 2 atypical meningioma of WHO grade 2 and 2 cases of WHO grade 3 anaplastic meningioma. The main clinical manifestations of the patient at the first visit were headache, dizziness. Other symptoms included vomiting, memory loss, blurred vision, unsteady walking, hearing impairment, epilepsy, and upper limb tremors.

2. Specimen preparation and instruments:

2.1. Preparation of Transmission electron microscope specimens

2.1.1. Immediately place the sporadic meningioma specimens excised during surgery into 2% paraformaldehyde-2.5% glutaraldehyde fixative for fixation, and rinse with 0.1 mol/L sodium cacodylate buffer (pH=7.4); After fixation with 1% osmium tetroxide, rinsed with double-distilled water, dehydrated to propylene oxide with graded ethanol, embedded in SPI812 resin; 1 μm semi-thin sections were prepared using a Leica EM UC7 microtome, and azure-methylene blue staining was used to observe the positioning under a light microscope. Then, 60nm ultrathin sections were prepared by Leica EM UC7 microtome, stained with uranyl acetate and lead citrate, and observed and filmed by HITACHI H-7650 transmission electron microscope.
2.1.2 Determination of results: The morphology and number of autophagosomes in different pathological types of meningioma were observed. Statistical methods: 3 or more autophagosome cells were observed as positive cells, 100 cells were randomly observed, and the incidence of autophagosomes was counted.

2.2. Western blot detection of autophagy-related proteins and the expression of PI3K/AKT/mTOR signaling pathway molecules

2.2.1. Take specimens from patients with meningothelial meningioma, incubate them with RIPA cell lysate on ice, and detect the protein concentration. Routine gel preparation, sample loading, electrophoresis, transfer and blocking. Primary antibodies: Beclin1 (1:1000), LC3II(1:1000), Akt(1:1000), Phospho-AKT (1:1000), m-TOR(1:1000), Phosphor-mTOR(1:1000), S6(1:1000), Phosphor-S6(1:1 000), 4E-BP1(1:1000), Phospho-4E-BP1 (1:1 000). Secondary antibody: goat anti-rabbit IgG antibody (1:2000), incubate at room temperature for 2 hours, and wash the membrane. Each band's integrated absorbance value (Integrated Absorbance, IA) was developed, exposed, developed and scanned, with GAPDH as the internal reference. The relative expression level of the protein to be tested was expressed as $IA_{\text{target protein}}/IA_{\text{GAPDH}}$.

2.2.2. Statistical analysis

SPSS 19.0 statistical software was used for data processing of experimental results, expressed as $x \pm s$, and a t-test was used for differences between groups. $p<0.05$ was set as statistically significant.

2.3. Auxiliary diagnosis by light microscopy and immunohistochemical detection

2.3.1. Meningioma specimens were fixed in 4% neutral formaldehyde for 12 to 24 hours, embedded

in paraffin, and HE sections were made. Some cases were subjected to immunohistochemical detection to assist in the diagnosis. Immunohistochemical detection of vimentin (Vimentin), cytokeratin (CK), S-100 protein (S-100), epithelial membrane antigen (EMA), glial fibrillary acidic protein (GFAP), blood progenitor cell antigen (CD34), Somatostatin receptor 2 (SSTR-2).

2.3.2. The pathological diagnosis and histological typing criteria of WHO (2016 edition) in "Pathology and Genetics of Nervous System Tumors" were used to carry out pathological diagnosis and classification. The pathological diagnosis was completed by 3 pathologists.

RESULTS

1. The clinical data of human meningioma showed that among the 182 cases of meningioma, there were 104 males and 78 females. Tumor clinical prognosis staging and classification: 174 cases of WHO grade 1, including 62 cases of meningothelial type, 39 cases of fibrous type, 58 cases of transition type, 15 cases of angiomatous type, 6 WHO grade 2 cases including 4 clear cell type and 2 atypical type, and 2 WHO grade 3 interclass variant. See Table 1 for details and Table 2 for immunohistochemical staining results in different meningioma variants.

Grading	Variants	NO. [Case(%)]
WHO I	Meningothelial	62 (34.1)
	Fibrous	39 (21.4)
	Transitional	58 (31.9)
	Angiomatous	15 (8.2)
WHO II	Clear cell	4 (2.2)
	Atypical	2 (1.1)
WHO III	Anaplastic	2 (1.1)

Table 1. Analysis of prognosis and variants of 182 meningiomas.

Variants	EMA	Vimentin	SSTR-2	CD34	PR	GFAP	S-100
Meningothelial	51	58	61	1	30	0	6
Fibrous	33	37	38	1	9	0	4
Transitional	45	47	55	1	8	0	5
Angiomatous	10	15	11	9	5	0	6
Clear cell	3	4	4	2	0	2	0
Atypical	2	2	2	1	0	0	0
Anaplastic	2	2	2	2	0	0	0

Table 2. Analysis of immunohistochemical results in different variants of meningioma.

2. The incidence of autophagosomes in different tissue types of meningioma was observed under TEM (transmission electron microscope) (Table 3, Figure 1). Statistical methods: 3 or more autophagosome cells were observed as positive cells, 100 cells were randomly observed, and the incidence of autophagosomes was counted under TEM (Figure 1). Combined with the results in Table 1 and Table 3, it is shown that autophagy was found in 27 of 182 meningiomas, including 11 cases of meningothelial meningioma (11 cases/62cases, 17.7%), 5 cases of fibrous meningioma (5cases/39cases, 12.8%), 9 cases of transitional meningioma (9cases/58cases, 15.5%) and 2 cases of angiomatous meningioma (2cases/15cases, 13.3%). Among them, transitional type (mainly meningothelial type) and meningothelial meningiomas have a higher incidence of autophagosomes, accounting for 17.7% and 15.5%, respectively. In addition, fibrous type accounts for 12.8%, angiomatous accounts for 13.3%, and the ratio is low. Figure 1 showed that the incidence of autophagosomes in the meningothelial variant was high, but there were no apoptotic body cells.

Grading	Typing	NO. [Case(%)]
WHO I	Meningothelial	11 (17.7)
	Fibrous	5 (12.8)
	Transitional	9 (15.5)
	Angiomatous	2 (13.3)
WHO II	Clear cell	0 (0)
	Atypical	0 (0)
WHO III	Anaplastic	0 (0)

Table 3. The incidence of autophagosomes in different histological types of meningiomas

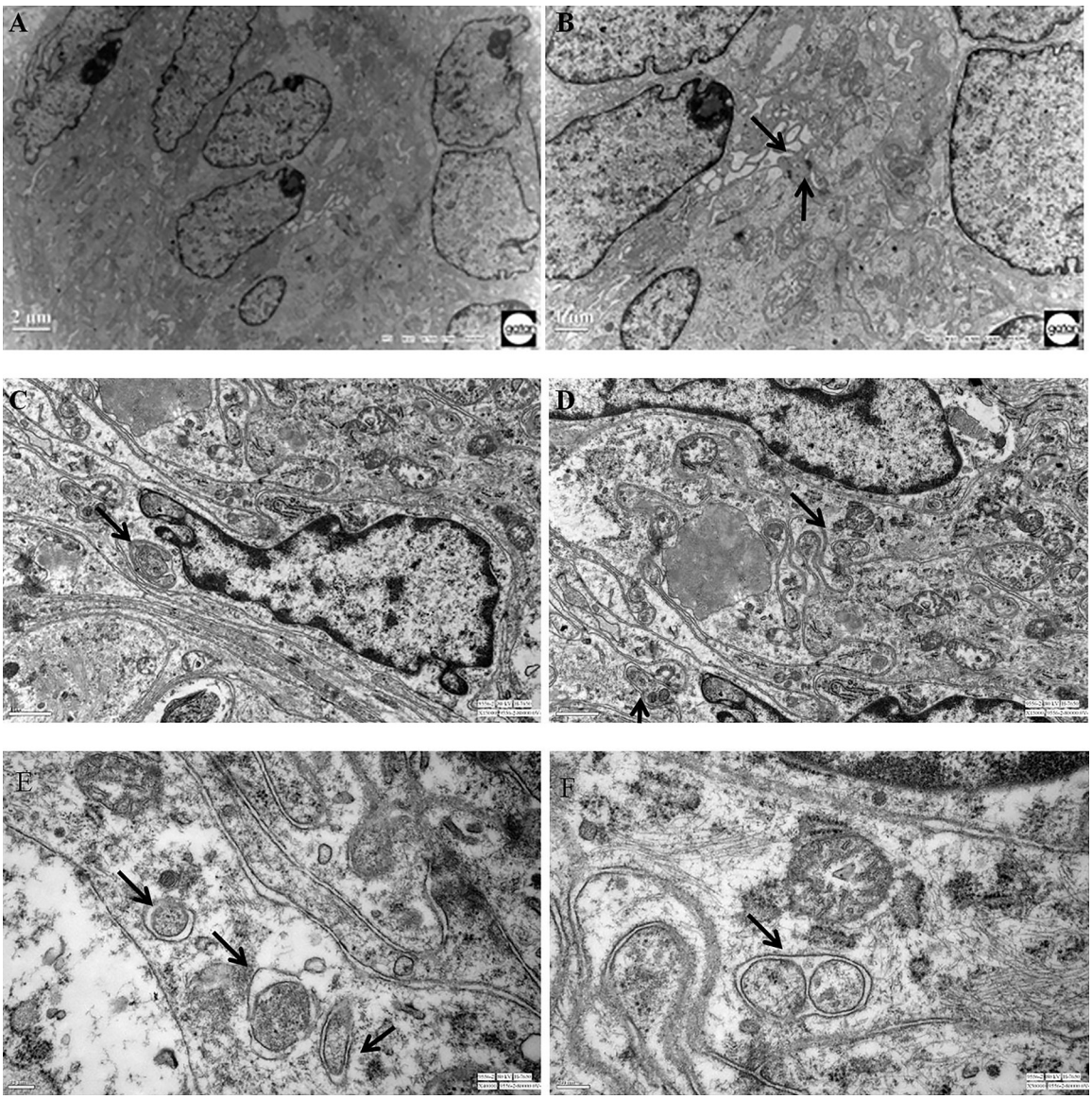


Figure 1. Morphology of autophagosomes in meningothelial meningiomas observed by TEM.

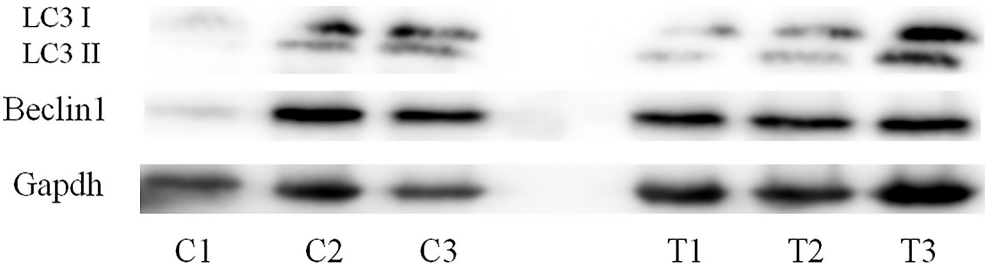
A-B tumor cells are densely distributed in clusters (A, Bar=2μm), with a large number of finger-like protrusions on the cell membrane surface, and desmosome connections between cells are common (B, Bar=1μm)

C-D The nuclei of tumor cells were obviously atypia, and a large number of autophagosomes were found in the cytoplasm (Bar=1μm indicated by the arrow)

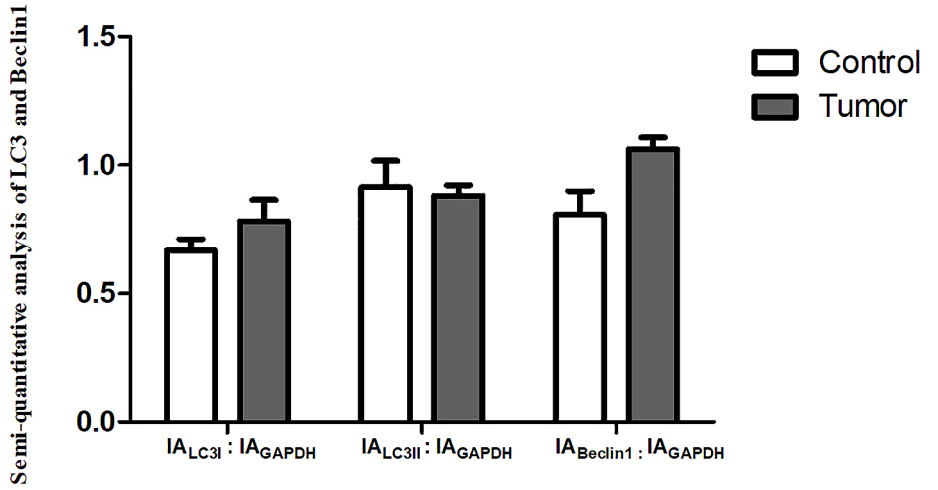
E-F Autophagosomes (Bar=200nm indicated by arrows)

3. Western Blot detection of Beclin1, LC3, Bax, Caspage3 expression levels in 3 meningothelial meningiomas (T1, T2, T3). 3 fibrous meningiomas as a control group (C1, C2, C3). Scan each band's integrated absorbance value (Integrated Absorbance, IA), with GAPDH as the internal reference. The relative expression levels of the proteins to be tested were expressed by the $IA_{\text{target protein}}$ and IA_{GAPDH}. In 3 meningothelial meningiomas, LC3 and Beclin1 were highly expressed, but Bax and Caspage3 were low expressed compared with the non-meningothelial control group. (Figure 2A-2B).

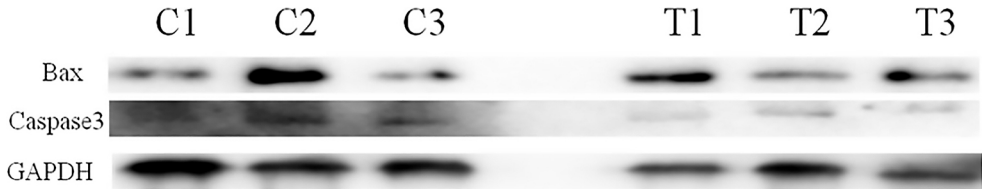
A



IA	IA_{LC3I}	IA_{LC3II}	$IA_{Beclin1}$	IA_{GAPDH}	IA_{LC3I}:IA_{GAPDH}	IA_{LC3II}:IA_{GAPDH}	$IA_{Beclin1}$:IA_{GAPDH}
C1	1864	1573	1952	2684	0.694	0.586	0.727
C2	2536	2479	3461	3089	0.821	0.803	1.120
C3	2149	2325	3093	3126	0.687	0.744	0.989
T1	2675	2740	3335	2889	0.926	0.948	1.065
T2	2442	2784	3570	3132	0.780	0.889	1.140
T3	2080	2625	3192	3240	0.641	0.810	0.985



B



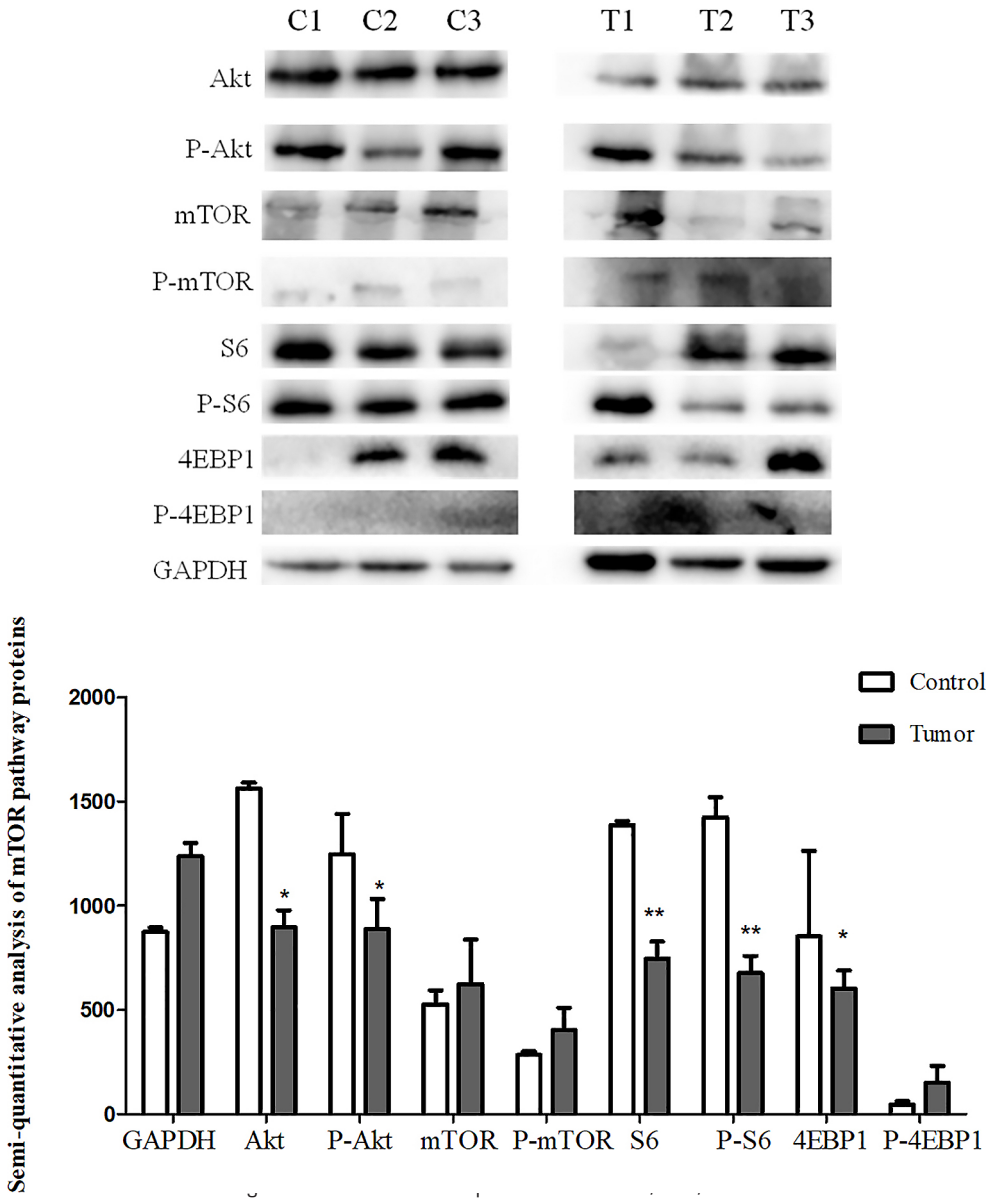
IA	IA_{Bax}	$IA_{Caspase3}$	IA_{GAPDH}	I_{ABax}:IA_{GAPDH}	$IA_{Caspase3}$:IA_{GAPDH}
C1	2190	01980	1952	1.122	1.014
C2	2890	2340	3461	0.835	0.676
C3	2149	1990	3093	0.687	0.744
T1	1980	850	3335	0.695	0.255
T2	2098	900	3570	0.587	0.252
T3	1920	650	3192	0.602	0.204

Figure 2. High expression of LC3 and Beclin1 in meningothelial meningiomas compared with non-meningothelial meningiomas

A: The expression of LC3I, LC3II and Beclin1 protein was detected by western blots in different meningothelial meningiomas and normalized with an internal control (GAPDH). Quantification levels of LC3I, LC3II and Beclin1 use Image J software. (C1-C3: Fibrous meningiomas; T1-T3: Meningothelial meningiomas)

B: The expression of Bax, Caspage3 protein was detected by western blots in different meningothelial meningiomas and normalized with an internal control (GAPDH). Quantification levels of Bax and Caspage3 use Image J software. (C1-C3: Fibrous meningiomas; T1-T3: Meningothelial meningiomas)

4. The regulation of upstream and downstream signaling molecules of PI3K/AKT/mTOR pathway in meningothelial meningioma (T1, T2, T3). 3 fibrous meningiomas as a control group (C1, C2, C3). By semi-quantitative analysis of the upstream and downstream molecules of the mTOR pathway, it was found that AKT, mTOR, S6, and 4EBP1 protein expressions decreased in meningothelial meningioma but up-regulated in the control group (Figure 3). Scan each band's integrated absorbance value (Integrated Absorbance, IA), with GAPDH as the internal reference.



signaling pathway molecules in meningothelial meningiomas.

The protein levels of AKT, P-AKT, mTOR, p-mTOR, S6, P-S6, 4EBP1, and P-4EBP1 were confirmed by western blot. (*p<0.05, **p<0.005, ***p<0.001)

C1, C2, C3 represent 3 fibrous meningioma; T1, T2, T3 represent 3meningothelial meningioma.

DISCUSSION

Meningioma is a common clinical intracranial tumor of non-neuroepithelial origin, and its incidence is second only to glioma, accounting for about 30% of primary intracranial tumors [Vernooij *et al.*, 2007]. It is generally believed to originate from the arachnoid cap cells on the arachnoid membrane and arachnoid granules [Barthélemy *et al.*, 2016]. The arachnoid cap cells generally appear as fibroblast-like monolayers or nests of multicellular epithelial cells. With age, the arachnoid cap cells aggregate to form spirals or sand bodies, so from the point of view of histopathology, meningiomas are considered to originate from arachnoid cap cells [Barthélemy *et al.*, 2016; Qu *et al.*, 2019].

Clinical studies have shown that more than 90% of meningiomas are benign (WHO 1), and the rest are atypical meningiomas (about 6.26%, WHO 2) and malignant meningiomas (about 1.7%, WHO 3). This is a retrospective study. The study has some shortcomings and limitations in the imbalance of the samples we collected. The sample size of high-grade (WHO 2-3) meningiomas was small in the research. This study found that among 182 patients with meningioma, 178 (98%) were benign meningiomas of WHO grade 1, 2 cases (1%) of WHO grade 2 atypia, and 2 cases (1%) of WHO grade 3 anaplastic. Among the benign meningiomas, 62 cases (35%) of meningothelial type, 39 cases (22%) of the fibrous type, 58 cases (33%) of the transition type, 15 cases (8%) of angiomatous, and 4 cases (2%) of clear cell type, of which meningothelial meningiomas accounted for the highest proportion.

Currently, clinicians use EMA, PR, and SSTR2 as the first-line markers for the diagnosis of meningiomas. Epithelial membrane antigen(EMA) widely exists in many kinds of epithelial cells and epithelial tumors. Both arachnoid cap cells and meningioma cells have EMA expression. Progesterone receptor (PR) is generally used to diagnose breast, endometrial, and ovarian cancel cancer, and PR is also found in meningioma, somatostatin receptor (SSTR) expresses in neuroendocrine tumors. SSTR2 has developed into an important SSTR2 and is anessential marker of central nervous system tumors and is present in most meningiomas.

Immunohistochemical results showed that 146 cases were positive for epithelial marker EMA, 165 cases were positive for mesenchymal tissue marker Vimentin, 173 cases were positive for SSTR-2, with positive rates of 80.2%, 90.7% and 95%respectively. The above results showed that although the positive rates of EMA Vimentin and SSTR-2 were high, there were still a small number of cases with negative expression. CD34 expressed in vascular meningothelial cells, 9 angiomatous meningiomas showed positive CD34 expression. The PR progesterone receptor protein is expressed in epithelial cells, most often in meningothelial meningiomas, but only 54 cases were positive. Both S-100 and GFAP are acidic proteins distributed in the nervous system, which are mainly used for the diagnosis and differential diagnosis of glial cell tumors. Immunohistochemical staining methods can detect tumor types by molecular markers. However, the sensitivity and specificity of existing biological markers are uneven, and many molecular markers do not exist exclusively in a particular tumor [Langford *et al.*, 1996], which limits the application of immunohistochemical staining technology in tumor classification to certain extent applications. Therefore, the observation of ultrastructural pathology changes by TEM can help us to determine the differentiation direction and malignant degree of meningiomas, but it can still play an irreplaceable role in tumor diagnosis [Song *et al.*, 2017].

Autophagy is a highly conserved process ubiquitous in eukaryotes. It wraps damaged or dysfunctional proteins and organelles in the cytoplasm through a double membrane structure and transports them to lysosomes for digestion and degradation, thereby maintaining cell survival [Kimmelman *et al.*, 2017; Saha *et al.*, 2018] differentiation, development, and homeostasis. As an essential stress response mechanism, autophagy is involved in the occurrence and development of various diseases and regulates related signaling pathways [White *et al.*, 2019]. It is like a double-edged sword. It is an essential pathway for growth and development, and in the early stage of tumorigenesis, autophagy inhibits tumorigenesis and evolution; on the other hand, cancer cells can activate autophagy under nutrient deficiency or stress state and can resist chemotherapeutic drug treatment, thereby promoting the occurrence of cancer development [Shaul *et al.*, 2021; Baird *et al.*, 1989].

The proportion of meningothelial variant was higher in the recurrent meningiomas, which accounted for more than half of the recurrent cases. It has been reported that meningothelial meningiomas have a thin envelope, often accompanied by cystic changes, which are more adhesive to the surrounding brain tissue, cranial nerves, or blood vessels, so this subtype is more likely to recur [Jiang *et al.,* 2023; Sun *et al.,* 2022]. In our study, we observed differences in the incidence of autophagosomes in different histological types of meningiomas using transmission electron microscopy, respectively. Table 3 showed that transitional type (mainly meningothelial type) and meningothelial type meningioma have a higher incidence of autophagy, accounting for 15.5% (9 positive cases in 58 transitional variants) and 17.7% (11 positive cases in 62 meningothelial variants), respectively, fibrous type accounts for 5% (5 positive cases in 39 fibrous variants), and angiomatous type accounts for 13.3% (2 positive cases in 15 angiomatous variants). Protein level detection found that LC3, and Beclin1 expression was increased in meningothelial meningiomas. The above results also indicate that autophagy is more prominent in meningothelial meningiomas. Combined with the high occurrence and recurrence of the meningothelial variant, we speculated that a high autophagy rate but low apoptosis rate in meningothelial meningioma might promote the occurrence of cancer development compared with other non-meningothelial meningiomas. Next, we further investigated the mechanism of autophagy in the occurrence and development of meningothelial meningiomas.

The literature has reported that the mTOR signaling pathway is a classical inhibitory pathway of autophagy [Wu *et al.,* 2015; Laplante *et al.,* 2012]. mTOR is the main target of the mammalian autophagy signaling pathway, regulating cell transcription, translation, autophagy and apoptosis, participating in protein synthesis, tumor cell invasion and metastasis. This pathway can inhibit apoptosis and promote tumor growth [Zheng *et al.,* 2022]. Under nutrient-enriched conditions, growth factors, glucose, and amino acids can directly interact with autophagy-related factor serine/threonine kinase 1 (ULK1) through mTOR complex 1 (mTORC1) of the mTOR signaling pathway to phosphorylate. Under starvation conditions, mTORC1 is inhibited, ULK1 dephosphorylation is activated, ULK1 complex is formed, and autophagy is promoted [Yang *et al.,* 2022]. Therefore, our team is committed to studying whether the PI3K/AKT/mTOR signaling pathway is involved in the occurrence and development of meningothelial meningioma patients by regulating autophagy. It is speculated that there may be two roles: to inhibit tumor growth and invasion, and to promote tumor growth and invasion. Through semi-quantitative analysis of the upstream and downstream proteins of the mTOR pathway, we found that the protein expressions of upstream molecules AKT, mTOR, and downstream molecules S6 and 4EBP1 were significantly decreased in meningothelial meningioma. But in a control group, the protein expression of AKT, S6 and 4EBP1 was increased considerably, and the mTOR expression was slightly increased. The above results indicate that in meningothelial meningiomas, autophagy is induced by down-regulating PI3K/AKT/mTOR signaling pathway molecules, thereby promoting tumorigenesis and development.

Because the growth of meningioma has particular invasiveness, it often invades the adjacent soft tissue or skull. Some meningiomas cannot be removed entirely due to the growth site or adjacent vital structures [Rebchuk *et al.,* 2022; Brokinkel *et al.,* 2017]. Clinical radiotherapy is often supplemented for patients who cannot be completely resected or recur, including γ-knife, stereotactic radiotherapy, etc.. Still, the long-term effect remains to be seen. Currently, there is still no particularly effective drug for the chemotherapy of meningioma, and it is only recommended for malignant or inoperable meningioma [Phonwijit *et al.,* 2017; Duntze *et al.,*2012]. Therefore, how to effectively treat meningiomas that are difficult to remove completely altogether or recur has always been a complex problem for neurosurgeons to overcome.

Although most meningiomas are WHO grade 1 benign tumors, due to their aggressive growth, they often invade adjacent soft tissues or skullsherefore, patients are often unable to achieve T during surgical treatment due to the particular tumor growth site or invasion of adjacent important tissues [Marciscano *et al.,* 2016; Karabagli *et al.,* 2020]. Even benign meningiomas still have a recurrence rate of 7% to 25%, and no effective non-surgical treatment option exists. Therefore, how to effectively treat meningiomas that are difficult to remove or recur by surgery completely has always been a difficult problem for neurosurgeons to overcome [Streckert *et al.,* 2019]. Studying the pathological mechanism of meningioma development is the topic aim. All the above results showed that in meningothelial

meningioma, PI3K/AKT/mTOR signaling pathway inhibited could induce autophagy but inhibit apoptosis. We speculate that autophagy and apoptosis in the meningothelial variant are antagonistic relationships, in which autophagy does not promote apoptosis, instead, it can inhibit apoptosis and then promote cell survival. In this relationship, inhibition of autophagy may improve the sensitivity of tumor cells to apoptotic signals, and increasing autophagy may prevent apoptosis from occurring. Further, we speculate that autophagy may be an early event in meningioma rather than tumor progression. Autophagy may play an essential role in the development of meningothelial meningioma by apoptosis inhibition and provide new ideas and therapeutic directions for the clinical treatment of meningioma patients.

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