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Original Research

Molecular mechanisms and therapeutic targeting implications of ER/mTOR signaling axis-driven tumor progression in aggressive meningiomas

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ABSTRACT

Purpose: Aggressive meningiomas pose substantial clinical challenges because of their high rates of recurrence. The aim of this study is to investigate the mechanistic role of estrogen receptor (ER)-mediated mTOR hyperactivation in promoting meningioma progression and to evaluate the therapeutic potential of targeting this signaling axis with tamoxifen.

Methods: Analyses involving data from clinical registries have established sex as a predictor of adverse outcomes, while multiomics investigations have revealed the overexpression of ER in advanced-grade and recurrent tumors. Functional validation was conducted using ER knockdown models. Mechanistic insights were obtained through RNA sequencing, with orthogonal validation performed via qPCR and Western blotting, with a focus on regulators of the mTOR pathway (RICTOR, PIK3CA, and DEPTOR). Therapeutic efficacy was evaluated in meningioma cell models through pharmacological inhibition using tamoxifen.

Results: Clinical analysis was used to identify sex as a predictor of adverse outcomes, revealing that ER overexpression is significantly correlated with advanced tumor grades. Silencing of the ER markedly reduced malignant phenotypes, leading to decreased cell proliferation and invasion, while also inducing apoptosis. Mechanistically, ER activation resulted in the upregulation of RICTOR and PIK3CA expression, alongside suppression of DEPTOR, which directly activated mTOR signaling. Tamoxifen exhibited potent antitumor effects by reversing this oncogenic signaling cascade.

Conclusion: This study revealed that the ER/mTOR axis is associated with sex-linked therapeutic vulnerability in aggressive meningiomas. This finding provides mechanistic evidence for ER-driven mTOR activation via the dysregulation of RICTOR/PIK3CA-DEPTOR. The demonstrated efficacy of tamoxifen supports its clinical repurposing as a targeted therapy for ER-positive meningiomas, offering a biologically rational strategy to address therapeutic resistance in this challenging malignancy.

Introduction

Meningiomas, which originate from arachnoid cap cells, represent the most prevalent primary intracranial tumors in adults and constitute approximately 13–26 % of all primary intracranial neoplasms [1,2]. According to the 2019 Central Brain Tumor Registry of the United States report, the annual age-adjusted incidence rate in the US reached 8.58 per 100,000 population during the period from 2012 to 2016, demonstrating a strong age-dependent progression with a significantly increased incidence after the age of 65 [3]. The WHO classification system categorizes meningiomas into three grades: benign (Grade I), atypical (Grade II), and anaplastic (Grade III). The latest WHO Classification of Central Nervous System Tumors (5th edition, CNS5) further delineates 15 histological subtypes [4]. Approximately 20 % of patients

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present malignant progression corresponding to Grade II/III tumors, whereas 80 % maintain benign Grade I histopathology [5]. Tumor recurrence remains the primary prognostic determinant, particularly for aggressive subtypes; however, even benign cases demonstrate substantial recurrence rates [6,7]. Studies have revealed postoperative recurrence rates of up to 20 % following Simpson Grade I resection for benign meningiomas, which increase to 40 % and 80 % for atypical and anaplastic variants, respectively [8]. Although stereotactic radiotherapy shows partial efficacy in preventing recurrence, current immunotherapeutic approaches remain suboptimal [9–12]. This clinical landscape highlights the critical need to elucidate the molecular mechanisms underlying meningioma aggressiveness, identify novel prognostic biomarkers, and develop targeted therapeutic strategies.

Meningiomas exhibit significant sex-based epidemiological disparities, with females having approximately twice the incidence rate of males [5]. This disparity is particularly pronounced among women during pregnancy or those receiving estrogen-based therapies [13], which strongly correlates with the expression profiles of sex hormone receptors in meningiomas. Molecular analyses have indicated that 76 % of meningiomas express progesterone receptors (PRs), whereas ER expression is detected in 6 % of cases [14]. Although ER and PR are well-established therapeutic targets in the management of breast cancer [15–19], their clinical utility in the treatment of meningiomas remains unvalidated by conclusive evidence. In previously reported cases of aggressive meningiomas, ERα is rarely expressed, whereas ERβ has been consistently detected and is considered to play a role in the pathobiological processes of these tumors.Therefore, systematic investigations into the mechanistic roles of these hormone receptors in the progression of meningiomas may reveal novel therapeutic avenues for targeted interventions.

Materials and methods

SEER data inclusion exclusion criteria and statistics

Data were procured from the SEER Research Plus Database, which includes 17 registries, for the November 2021 submission covering the years 2000 to 2019. See the supplementary materials for full methodological details.

GEO database

We conducted a search in the GEO database, a public repository for functional genomics data, utilizing high-throughput gene expression datasets with the following keywords: “meningioma” or “gender” and “WHO classification” . Following a systematic review, we downloaded three gene expression datasets (GSE16181, GSE74385, and GSE149923) for further analysis. For detailed information, please refer to the supplementary materials.

Patients and specimens and immunohistochemistry

Data from the pathological repository of Sun Yat-sen Memorial Hospital at Sun Yat-sen University were procured for this investigation (sanctioned by the Medical Ethics Committee of Sun Yat-sen Memorial Hospital, Opinion No SYSKY-2023–477–01). Additional details are available in the supplementary materials.

Immunohistochemical analysis methods and statistical processing

Positive cells were quantified under 40x magnification. Cells were classified as positive if the proportion of positive cells was $\geq$1 %. A 9-point scoring system was employed: Samples with $\leq$10 % positive cells received 1 point, those with 10 %−50 % received 2 points, and those with >50 % received 3 points. Staining intensity was also scored as follows: samples with negative staining received 0 points, those with light blue staining received 1 point, those with moderate yellow staining received 2 points, and those with brown–yellow staining received 3 points. The total score was computed as the product of the positive cell score and the staining intensity score. A total score of 6 was classified as strongly positive. Statistical analysis was performed using R version 4.0.3, with the Hmisc package utilized for correlation analysis. Spearman correlation was employed for analysis, with a two-sided p value <0.05 indicating a significant difference. Within standard clinical investigations, a correlation coefficient (r) of 0.7 is considered strong.

Cell lines

The IOMM-Lee meningioma cell line was obtained from the ATCC. The cells were kept at 37 °C in a 5 % carbon dioxide atmosphere with a minimum humidity of 95 %. They were maintained in DMEM (Gibco, USA) supplemented with 10 % fetal bovine serum (HyClone, USA). Routine testing confirmed that the cell line was free from mycoplasma contamination.

Cell transfection

The designed small interfering RNAs (siRNAs) were produced by IGEBIO (Guangzhou, China) and subsequently transfected into the cells for a duration of 24 h using Lipofectamine 2000 (Invitrogen, Shanghai, China) in accordance with the supplier’s protocols. The RNA oligoribonucleotides are detailed in Supplementary Table 2.

Western blot (WB) analysis

Western blot (WB) analysis was conducted following established protocols [20]. The primary antibodies used included Estrogen Receptor (ERβ, ab3576, Abcam, USA), Rapamycin-Insensitive Companion of mTOR (RICTOR, ab219950, Abcam, USA), Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA, ab40776, Abcam, USA), DEP-Domain Containing mTOR Interacting Protein (DEPTOR, ab309531, Abcam, USA), Phosphorylated Akt (Serine 473) [p-AKT (Ser473), ab81283, Abcam, USA], and Phosphorylated Akt (Threonine 308) [p-AKT (Thr308), D25E6, Cell Signaling, USA]. The secondary antibody used was Goat Anti-Rabbit H&L (HRP) (ab6721; Abcam, USA).

Normal PCR and qPCR assays

Normal PCR and qPCR were conducted according to established protocols [20]. The primer sequences are listed in Supplementary Table 2.

Apoptosis assay

In this study, an Annexin V-FITC apoptosis detection kit (Beyotime, China) was used. A total of 6×10^4 cells were harvested and resuspended in 200 microliters of Annexin V-FITC binding buffer. Subsequently, 5 microliters of Annexin V-FITC reaction solution combined with 5 microliters of propidium iodide (PI) were introduced at ambient temperature under dark conditions. The mixture was incubated for 20 min at 37 °C in the dark, after which the number of apoptotic cells was evaluated via flow cytometry analysis (Beckman Coulter, USA).

Colony formation assay

In 6-well plates, 500 cells were plated per well and cultured for 10 days. Colonies were counted after they were stained with 0.2 % crystal violet for 10 min.

CCK8 assay

Proliferation assays were conducted on IOMM-Lee cells in 96-well plates using the Cell Counting Kit 8 (CCK8) reagent (Beyotime, China) daily for five days. The cells were incubated at 37 °C for 2 h, after which the plate was examined with a microplate reader at 450 nm to determine the absorbance.

Migration and invasion assays

Established protocols [20] were used to evaluate the invasive and migratory potential of IOMM-Lee cells. For quantification, the average cell counts were calculated across all five fields of view observed under the microscope.

5-Ethynyl-2,-deoxyuridine (EdU) assay

Cells were plated on glass slides, and 5-ethynyl-2′-deoxyuridine (EdU) was added at a concentration of 10 micromoles per liter. The cells were assessed with the E-Click EdU Cell Proliferation Imaging Assay Kit (Elabscience, China). Following the supplier's protocol, the specimens were labeled with Alexa Fluor 488 and DAPI and subsequently visualized under a fluorescence microscope (Olympus IX83, Japan). The percentage of proliferating cells was determined by computing the ratio of the number of EdU-positive nuclei to the total number of nuclei.

RNA-seq and gene set enrichment analysis

The cells were subjected to transcriptome sequencing and enrichment analysis using methods outlined in previously published articles [20].

Scratch assay

In the scratch wound-healing assay, cells were placed in 6-well plates and maintained until they reached 90 % confluency. A scratch wound was then generated utilizing a 20 μL pipette tip, followed by washing with PBS to eliminate any detached cells. The distance of cell migration was assessed at both 0 h and 24 h post-scratching, and images were captured for analysis.

Statistical analysis

Statistical analyses were conducted with SPSS version 20.0. An independent sample t-test was employed to calculate the P value for the in vitro experiments. To assess the correlation between variables, Spearman's rank correlation analysis was performed. In all the statistical analyses, two-sided P values were used. The experiments were independently repeated three times, yielding consistent results. The relevant representative data are presented in the figures and tables. Survival analysis was executed utilizing Kaplan–Meier (KM) curves and log-rank statistical tests to assess overall survival (OS) and survival outcomes of individuals with meningioma.

Results

Sex as a key risk factor for aggressive meningioma

To investigate the risk factors associated with the development of aggressive meningiomas, we analyzed 497 patients from the SEER database diagnosed between 2000 and 2019 (Supplementary Table 1) using Cox proportional hazards regression. Our analysis revealed six significant predictors: age at diagnosis, tumor laterality, disease stage, sex, chemotherapy status, and tumor size ($P < 0.05$). Survival analysis revealed strong associations between these factors and poor prognosis, as demonstrated by Kaplan–Meier curves and forest plots (Fig. 1a–b). Given the notable sexual dimorphism in meningioma incidence (female-to-male ratio = 2:1), we subsequently focused our analyses on sex-specific pathogenic mechanisms.

To investigate the molecular mechanisms involved in aggressive meningioma development, we analyzed RNA sequencing data from 252 meningioma specimens obtained from the GEO database and stratified them by WHO grade (164 Grade I, 52 Grade II, and 36 Grade III). Principal component analysis (PCA) revealed distinct transcriptional profiles across the histological grades (Fig. 1c). A comparative analysis between the aggressive (combined Grades II/III) and nonaggressive (Grade I) subgroups revealed 148 differentially expressed genes (DEGs), which are presented as a volcano plot (Fig. 1d). Functional enrichment analyses, including KEGG and GO analyses, revealed significant associations between the DEGs and tumor progression pathways (Fig. 1e–f). Additionally,protein−protein interaction network analysis further identified EGFR (epidermal growth factor receptor) and ERBB3 (erb-b2 receptor tyrosine kinase 3) as central regulatory nodes (Fig. 1g–h).

ER predicts advanced WHO grade and poor prognosis in meningioma

Building upon bioinformatics evidence linking aggressive meningioma phenotypes with sexual dimorphism and potential involvement of the EGFR/ERBB3 pathway, we prospectively collected 38 surgical specimens from Sun Yat-sen Memorial Hospital for immunohistochemical validation (Fig. 2a–b). Quantitative analysis revealed significant positive correlations between WHO tumor grade and the expression levels of EGFR ($p < 0.05$), ErbB3 ($p < 0.01$), ER ($p < 0.05$), and nuclear progesterone receptor (nPR) ($p < 0.001$) (Fig. 2c). Although the expression patterns of EGFR, ErbB3, and nPR in meningiomas are well documented, the controversial importance of ER in clinical practice prompted our mechanistic investigation.

To identify the specific ER subtype and its clinical significance, we performed a systematic analysis of ERβ. Our results showed that ER expression was significantly higher in tumors from female patients compared to males (Fig. 3a), and markedly elevated in WHO grade II–III tumors (Fig. 3b). Consistent with these findings, analysis of the GEO dataset confirmed that ESR mRNA levels increased with tumor grade (Fig. 3c). Moreover, ESR2 expression was significantly higher in recurrent meningiomas than in non-recurrent cases (Fig. 3d), suggesting that ERβ may contribute to tumor progression and recurrence. Immunohistochemical staining further validated strong ERβ positivity in tumors from female patients, whereas those from male patients were predominantly negative or weakly positive (Fig. 3e). Western blot analysis revealed that protein levels of RICTOR, PIK3CA, DEPTOR, and ERβ were generally higher in tumor tissues from female patients, while PTEN showed no consistent trend (Fig. 3f), indicating that ERβ may promote aggressive meningioma behavior through modulation of key mTOR pathway components.

ER knockdown suppresses meningioma malignancy and induces apoptosis

To investigate the functional role of ER in aggressive meningiomas, we designed three small interfering RNA (siRNA) constructs targeting ER in the IOMM cell line. The knockdown efficiency was systematically validated through quantitative polymerase chain reaction (qPCR) and Western blot analyses (Fig. 4a–b), with siER-2 demonstrating the greatest silencing efficiency. Subsequent functional analyses utilized siER-2 for mechanistic exploration.

To assess the functional consequences of ER knockdown on the stemness of meningioma cells, we conducted comprehensive phenotypic analyses in IOMM cells. ER silencing significantly impaired cellular migration capacity, as demonstrated by the results of the wound healing assays (Fig. 4c). Proliferation was markedly suppressed, as evidenced by the results of the EdU incorporation assays (Fig. 4d) and colony formation tests (Fig. 4e–f). Transwell invasion assays revealed a decreased capacity for matrix penetration in the knockdown groups (Fig. 4g–h).

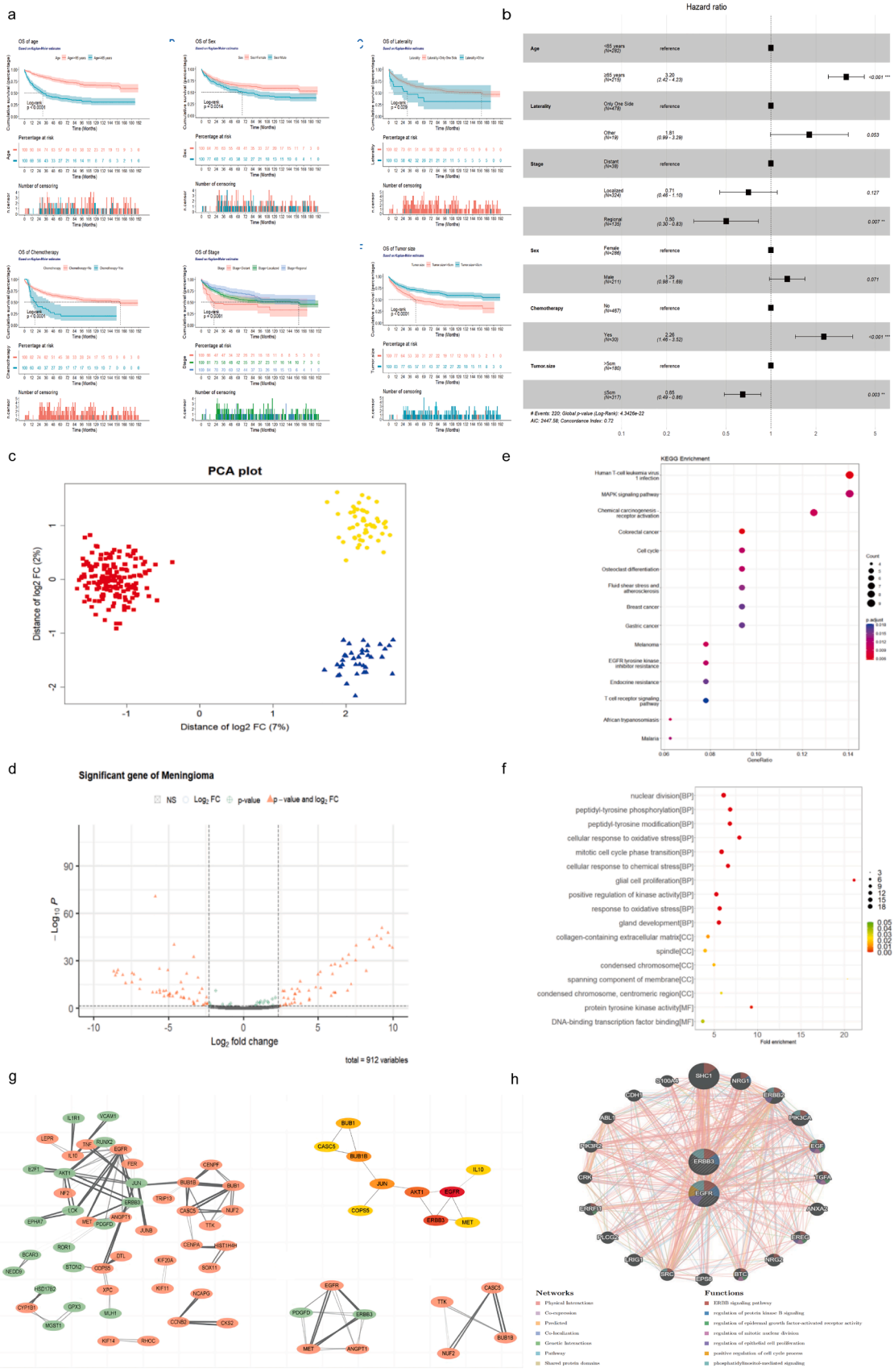

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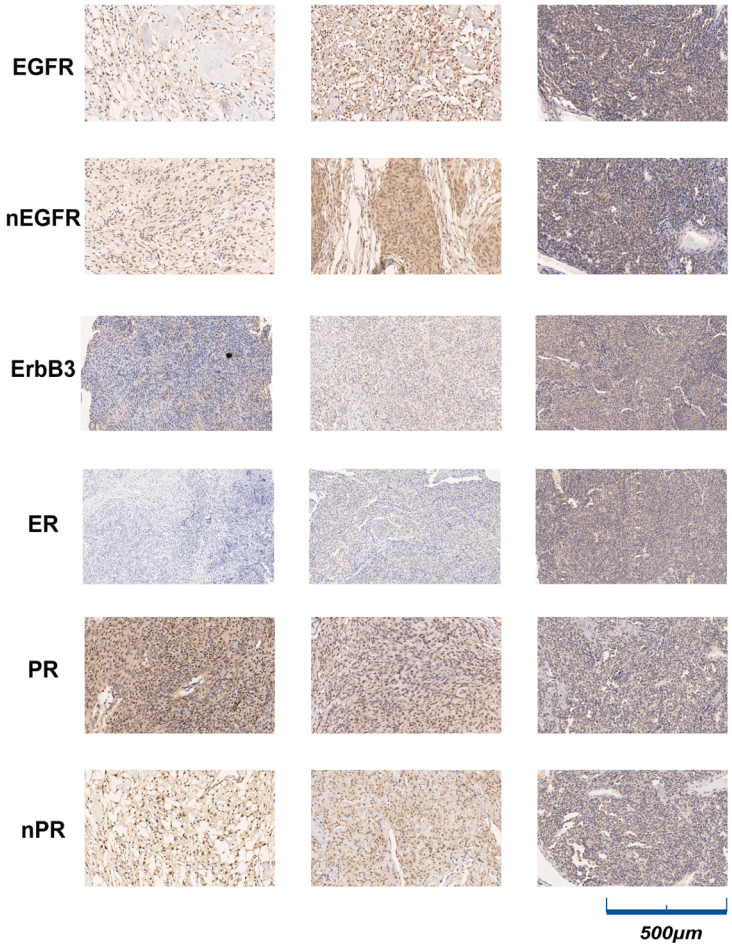
Fig. 1. Sex is a key risk factor for aggressive meningioma. a) KM survival analysis of factors such as age, sex, tumor laterality, chemotherapy status, tumor progression and tumor size. The KM curve, censored data and risk table, P value and median survival point were included in the analysis of each factor, and the different curve color areas represent the confidence intervals of the corresponding factors. **b)** Forest plot of six significant factors influencing MM prognosis after multivariate analysis. **For a-b)**, data were obtained from Incidence-SEER Research Plus Data, 17 Registries, Nov 2021 Sub (2000–2019). **c)** PCA plot of samples; red, yellow and blue correspond to WHO Grade I, II and III meningiomas, respectively. **d)** Volcano plots illustrating the differentially expressed miRNAs between WHO II/III and WHO I meningiomas. **e-f)** Bubble plots depicting KEGG and Gene Ontology (GO) enrichment analyses of different miRNAs between WHO II/III and WHO I meningiomas. **g)** Protein-protein interaction network highlighting EGFR and ErbB3 as hub genes (red/green: up/downregulated proteins). **h)** Key genes and their coexpressed genes were analyzed using GeneMANIA. **c-h)** Data from the GSE16181 dataset (GEO).

a



b

Baseline table of clinical characteristics and immunohistochemical results of patients in single-center database

Characteristics		Sample size
WHO classification	Grade III	1 (2.632%)
	Grade II	19 (50%)
	Grade I	18 (47.368%)
Gender	female	25 (65.789 %)
	male	13 (34.211%)
Age	≥50 years old	21 (55.263%)
	< 50 years old	17 (44.737%)
Postoperative treatment	Loss to follow-up	5 (13.158%)
	Radiation therapy	5 (13.158%)
	No radiotherapy	28 (73.684%)
Is it recurrent?	Relapse	8 (21.053%)
	Loss to follow-up	3 (7.895%)
	No recurrence	27 (71.053%)
Survival status	Loss to follow-up	5 (13.158%)
	death	6 (15.789%)
	Survival	27 (71.053%)
Pathological diagnosis	Papillary meningioma	1 (2.632%)
	Metaplastic meningioma	1 (2.632%)
	Epithelial-cell meningioma	1 (2.632%)
	Fibrous meningioma	2 (5.263%)
	Chordoid meningioma	1 (2.632%)
	Meningioma (unclassified)	1 (2.632%)
	Meningothelial meningioma	7 (18.421%)
	Angiomatous meningioma	3 (7.895%)
	Transitional meningioma	3 (7.895%)
	Clear cell meningioma	1 (2.632%)
	Atypical meningioma	17 (44.737%)
EGFR	Strong positive	4 (10.526%)
	Positive	30 (78.947%)
	Negative	4 (10.526%)
ErbB3	Strong positive	23 (60.526%)
	Positive	15 (39.474%)
ER	Positive	36 (94.737%)
	Negative	2 (5.263%)
PR	Strong positive	8 (21.053%)
	Positive	30 (78.947%)
nEGFR	Strong positive	2 (5.263%)
	Positive	8 (21.053%)
	Negative	28 (73.684%)
nPR	Strong positive	25 (65.789%)
	Positive	10 (26.316%)
	Negative	3 (7.895%)

c

Results of Spearman correlation analysis between immunohistochemical score and WHO grade

Genes	Correlation coefficient (r)	Significance (p)	95% confidence interval (95%CI)	Sample size (n)
EGFR	0.396	0.014	[0.077, 0.641]	38
nEGFR	0.061	0.717	[0.382, 0.273]	
ErbB3	0.487	0.002	[0.703, 0.189]	
ER	0.396	0.014	[0.641, 0.077]	
PR	0.247	0.189	[0.532, 0.089]	
nPR	0.519	< 0.001	[0.724, 0.229]	

Fig. 2. ER predicts advanced WHO grade and poor prognosis in meningioma. a) Immunohistochemical sections of different proteins for each grade of meningioma under a 40x objective. **b)** Baseline clinical characteristics and immunohistochemical results of patients in the single-center database. **c)** Results of the Spearman correlation analysis between the immunohistochemical score and WHO grade.

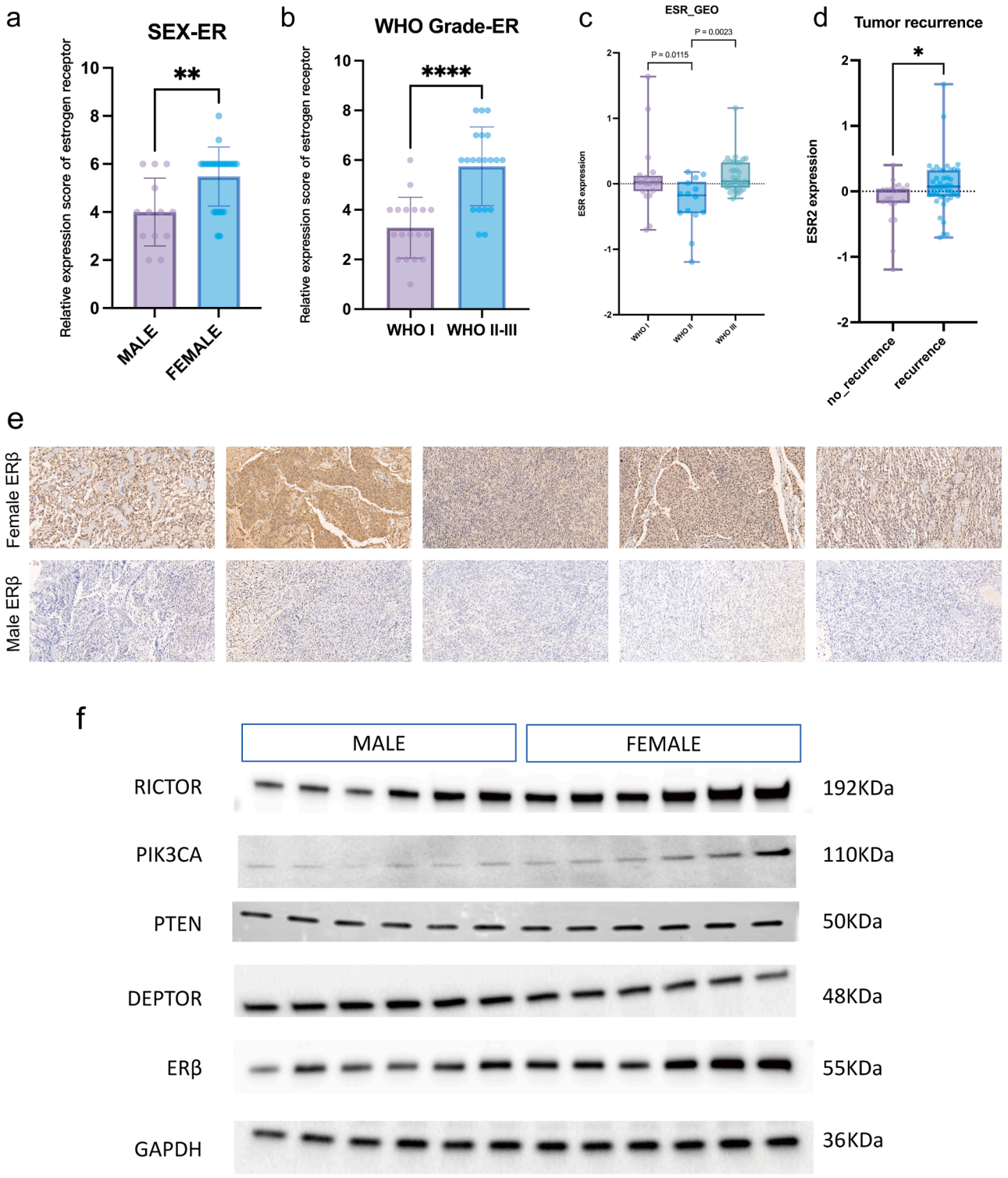


Fig. 3. ER expression is elevated in female patients and high-grade/recurrent meningiomas, and correlates with key mTOR pathway components. a) Relative expression score of estrogen receptor (ER) in meningiomas stratified by patient sex (male vs. female). Data are presented as box plots showing median, interquartile range, and outliers. Statistical significance was assessed using the Mann-Whitney U test. $P < 0.01$. b) Relative ER expression score in meningiomas grouped by WHO grade (WHO I vs. WHO II–III). Significance was determined by Mann-Whitney U test. $P < 0.0001$. c) ESR2 mRNA expression levels in meningioma samples from the GEO dataset, categorized by WHO grade. Box plots represent median and interquartile ranges; P-values were calculated using Kruskal-Wallis test with Dunn's post hoc correction. $P = 0.0023$ for overall comparison. d) ESR mRNA expression in recurrent versus non-recurrent meningiomas from the same GEO dataset. Wilcoxon signed-rank test was used; $P < 0.05$. e) Representative immunohistochemical staining of ERβin meningioma tissues from female (top row) and male (bottom row) patients. Brown staining indicates positive ERβ expression. Scale bars: 100 μm. f) Western blot analysis of ERβ and mTOR-related proteins (RICTOR, PIK3CA, PTEN, DEPTOR) and loading control (GAPDH) in meningioma tissue lysates from male and female patients. Molecular weight markers are indicated on the right.

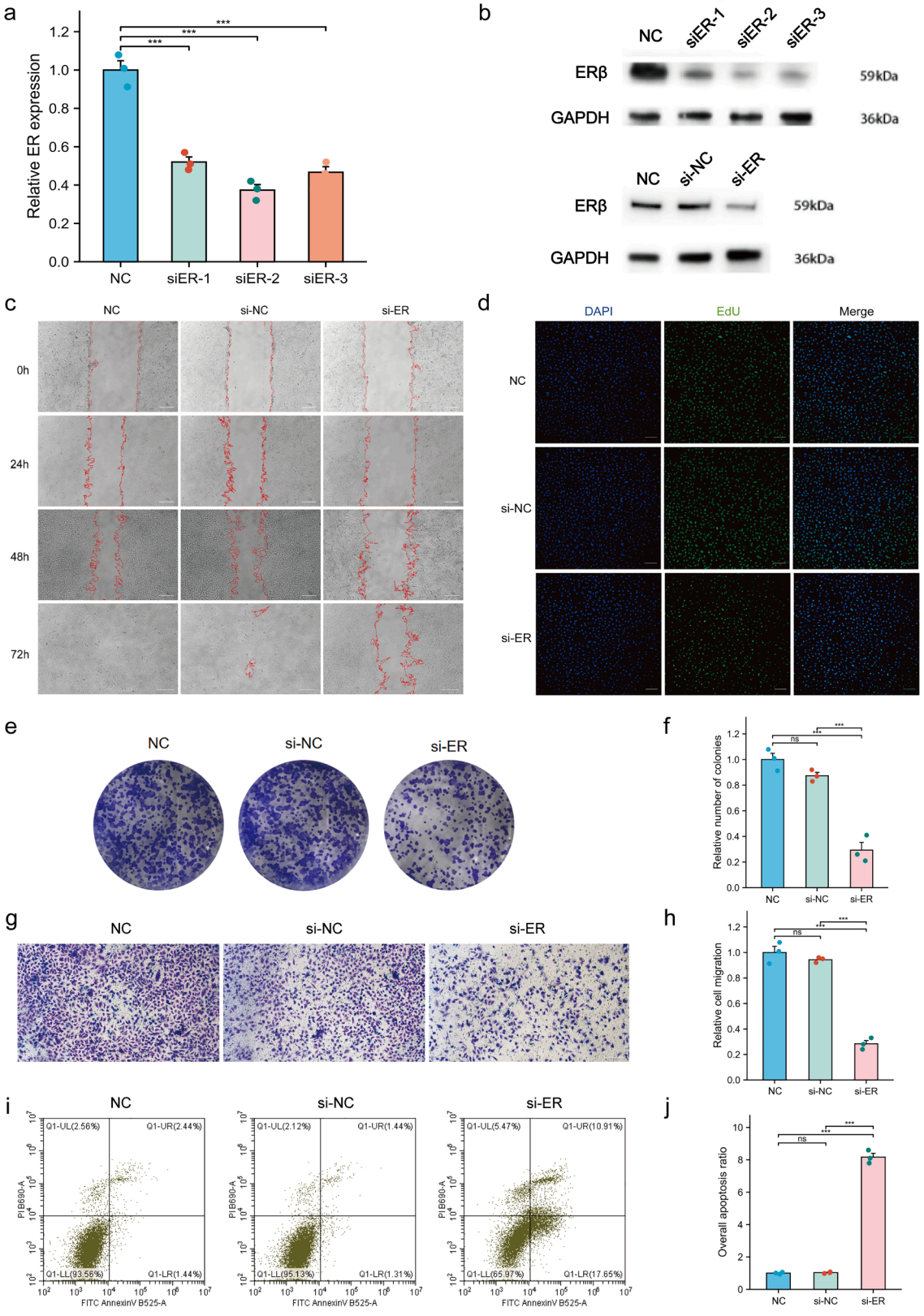


Fig. 4. ER knockdown suppresses meningioma malignancy and induces apoptosis. a-b) Validation of ER knockdown efficiency via qRT−PCR and Western blotting. **c)** Scratch assay results demonstrating impaired migration capacity after ER silencing. Scale bar, 100 μm. **d)** Results from the EdU assay of cell proliferation following ER knockdown. Scale bar, 200 μm. **e-f)** Colony formation assay for assessing cell proliferation after ER knockdown. Scale bar, 200 μm. **g-h)** Transwell invasion assay showing reduced invasive potential following ER knockdown. Scale bar, 100 μm. **i-j)** Flow cytometric analysis of ER knockdown-induced apoptosis in IOMM cells. ***, $P < 0.001$; nc, negative control; ns, not significant.

Most notably, flow cytometric analysis revealed an eightfold increase in total apoptosis rates (Fig. 4i–j).

ER silencing attenuates pathological mTOR signaling in meningioma cells

To elucidate the mechanism underlying ER-mediated meningioma aggressiveness, we performed transcriptome sequencing on ER-knockdown and control meningioma cells ($n = 3$ per group). Principal component analysis (PCA) and hierarchical clustering revealed distinct transcriptomic profiles between the groups (Fig. 5a–b). KEGG and GO enrichment analyses of the differentially expressed genes revealed significant associations with the mTOR signaling pathway and organic

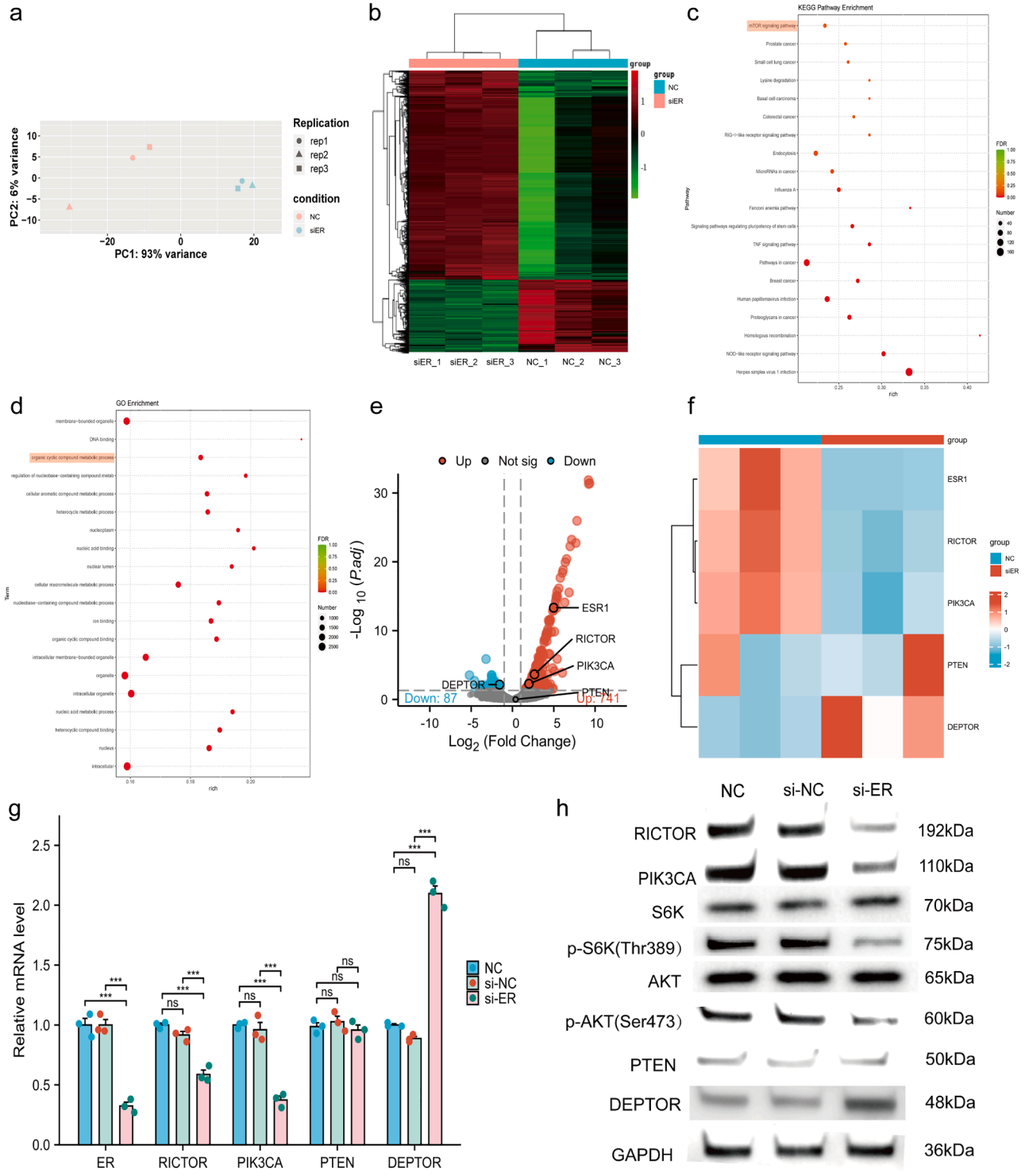


Fig. 5. ER silencing attenuates pathological mTOR signaling in meningioma cells. a) Principal component analysis (PCA) of global transcriptomic profiles in ER-silenced versus control IOMM cells, demonstrating distinct transcriptional clustering. **b)** Heatmap of RNA-seq-derived differentially expressed mRNAs. **c–d)** Bubble plots illustrating the results of the KEGG and Gene Ontology (GO) enrichment analyses of different miRNAs. **e)** Differentially expressed genes are illustrated using volcano plots. **f)** Heatmap of RNA sequencing analyses focusing on mTOR signaling pathway regulatory proteins. **g-h)** Validation of mTOR pathway protein suppression via qPCR and Western blotting. ***, $P < 0.001$; nc, negative control; ns, not significant.

cyclic compound binding processes, which encompass ER−ligand interactions (Fig. 5c–d).

Dysregulated activation of the mTOR signaling cascade is strongly linked to tumor progression. To validate its role in aggressive meningiomas, we conducted an in-depth analysis of transcriptomic sequencing data, which revealed the differential expression of mTOR pathway components. Volcano plot and heatmap analyses revealed the upregulation of mTOR-activating proteins (RICTOR and PIK3CA) and the downregulation of mTOR inhibitors (DEPTOR) in the control groups compared with the knockdown groups (Fig. 5e–f). Notably, the expression of PTEN, another negative regulator of mTOR, remained comparable between the groups.

Complementary qPCR and Western blot analyses in IOMM cells corroborated these findings. The control groups exhibited increased phosphorylation of mTORC1/2 downstream effectors, specifically p-AKT and p-S6K (Fig. 5g–h).Importantly, total AKT and total S6K protein levels showed no significant differences between groups, indicating that ER silencing modulates mTOR pathway activity through post-translational regulation rather than altering the abundance of these core signaling molecules.

Tamoxifen suppresses ER+ meningioma via mTOR pathway inhibition

Tamoxifen, a selective ER modulator (SERM) recognized for its proven efficacy in ER+ breast cancer, was evaluated for its therapeutic potential in ER+ meningiomas. Dose−response assays revealed a meningioma cell IC50 of 8.02 μM (Fig. 6a). At this concentration, tamoxifen had no significant modulatory effect on ER expression levels (Fig. 6b–c); therefore, this dose was established as the optimal dose for subsequent functional analyses.

Tamoxifen treatment recapitulated the phenotypic effects observed in ER knockdown models. Wound healing assays demonstrated a reduction in migration capacity (Fig. 6d). Proliferation was significantly attenuated, as evidenced by the results of the EdU incorporation assays (Fig. 6e) and colony formation analysis (Fig. 6f–g). Transwell invasion assays revealed decreased invasion (Fig. 6h–i), whereas apoptosis rates increased by 4.2-fold (Fig. 6j–k). Mechanistically, tamoxifen treatment suppressed mTOR signaling activation, as confirmed by decreased phosphorylation of key effectors, p-AKT (Ser473) and p-S6K (Thr389) (Fig. 6l–m). Notably, total AKT and total S6K protein levels remained unchanged across all treatment conditions, indicating that tamoxifen specifically modulates the activation state—rather than the abundance—of these core mTOR pathway components.

Western blot analysis further revealed that tamoxifen treatment led to a marked decrease in the protein levels of RICTOR and PIK3CA, along with an increase in the mTOR inhibitor DEPTOR, while PTEN expression was unaffected (Fig. 6m). Importantly, these regulatory effects were abolished upon ERβ knockdown, confirming that tamoxifen-mediated modulation of mTOR signaling is strictly dependent on ERβ expression (Fig. 6n). Together, these findings demonstrate that tamoxifen exerts its anti-tumor effects in ER+ meningiomas through ERβ-dependent suppression of mTOR pathway activity.

Discussion

Our study revealed that the upregulation of ERβ expression is significantly correlated with high tumor grade, high recurrence risk, and poor prognosis in patients with meningioma. These findings are consistent with epidemiological evidence that implicates estrogen signaling as a critical driver of tumor aggressiveness. Despite the low prevalence of ER expression in meningiomas, its functional relevance has remained contentious [13,21,22]. Through an integrated analysis of GEO datasets, immunohistochemical (IHC),Western blot,validation in clinical specimens, and cellular transcriptomic profiling, we demonstrated that ER overexpression mechanistically promotes meningiomagenesis via the constitutive activation of the PI3K/AKT/mTOR signaling axis. However, due to the limited follow-up duration in our current clinical cohort, meaningful survival analyses could not be performed. We are actively continuing prospective follow-up of these patients to further evaluate the association between ER expression and long-term clinical outcomes.

The PI3K/AKT/mTOR signaling axis is a well-established driver of tumor progression, and its mechanistic role has been extensively validated in ER-positive (ER+) breast cancer [23–28]. Studies have demonstrated that the ER interacts with multiple key components of this pathway to increase oncogenic signaling in ER+ breast malignancies [25]. Furthermore, the constitutive activation of this pathway is a critical factor underlying therapeutic resistance to endocrine therapies in ER+ breast cancer [29,30]. Our findings revealed conserved pathway dynamics in meningiomas: ER knockdown significantly suppressed mTOR pathway activity and induced parallel phenotypic consequences, including inhibited proliferation, impaired migration, and enhanced apoptosis. Notably, these ER-mediated regulatory mechanisms reflect functional patterns observed in breast cancer models.

Tamoxifen, a selective ER modulator (SERM), has been shown to reverse ER-driven phenotypes in vitro by suppressing tumor cell viability and restoring apoptotic sensitivity [31]. In ER+ breast cancer, tamoxifen serves as a standard adjuvant therapy, effectively inhibiting tumor progression and improving survival outcomes [32,33]. However, its therapeutic potential in meningiomas remains controversial; epidemiological studies have reported a reduced incidence of meningiomas among tamoxifen users, yet no conclusive evidence supports its efficacy in ER+ meningiomas [34–36]. Our cellular studies demonstrate that tamoxifen suppresses aberrant activation of the PI3K/AKT/mTOR pathway in meningioma cells, thus suppressing stemness features, including clonogenicity and invasive capacity. These mechanistic insights provide preclinical evidence supporting the repurposing of tamoxifen for the management of ER+ meningiomas. Critically, rescue experiments in the context of ERβ knockdown revealed that the inhibitory effects of tamoxifen on mTOR signaling and its anti-tumor activity were markedly attenuated or abolished, demonstrating that its pharmacological action is strictly dependent on ERβ expression.

This study revealed a significant association between ERβ activation and aberrant PI3K/AKT/mTOR signaling in meningiomas. Tamoxifen exerts antitumor effects by suppressing this hyperactivated pathway. Paradoxically, in ER+ breast cancer, overactivation of the PI3K/AKT/mTOR pathway is a recognized driver of tamoxifen resistance [37–40]. Given these complexities, investigating the underlying mechanisms of tamoxifen in ER+ meningiomas particularly its dual role in pathway modulation and therapeutic efficacy represents an imperative and promising research frontier.

Conclusion

In summary, our findings demonstrate that meningioma cells contribute to tumor progression through the upregulation of ER and the subsequent aberrant activation of the PI3K/AKT/mTOR signaling axis. Conversely, both genetic silencing of ER and pharmacological intervention with tamoxifen effectively reversed this oncogenic signaling cascade. These results suggest that the ER is a promising therapeutic target for ER-positive meningiomas, offering a mechanistic rationale for the exploration of endocrine-based therapies in this molecular subset.

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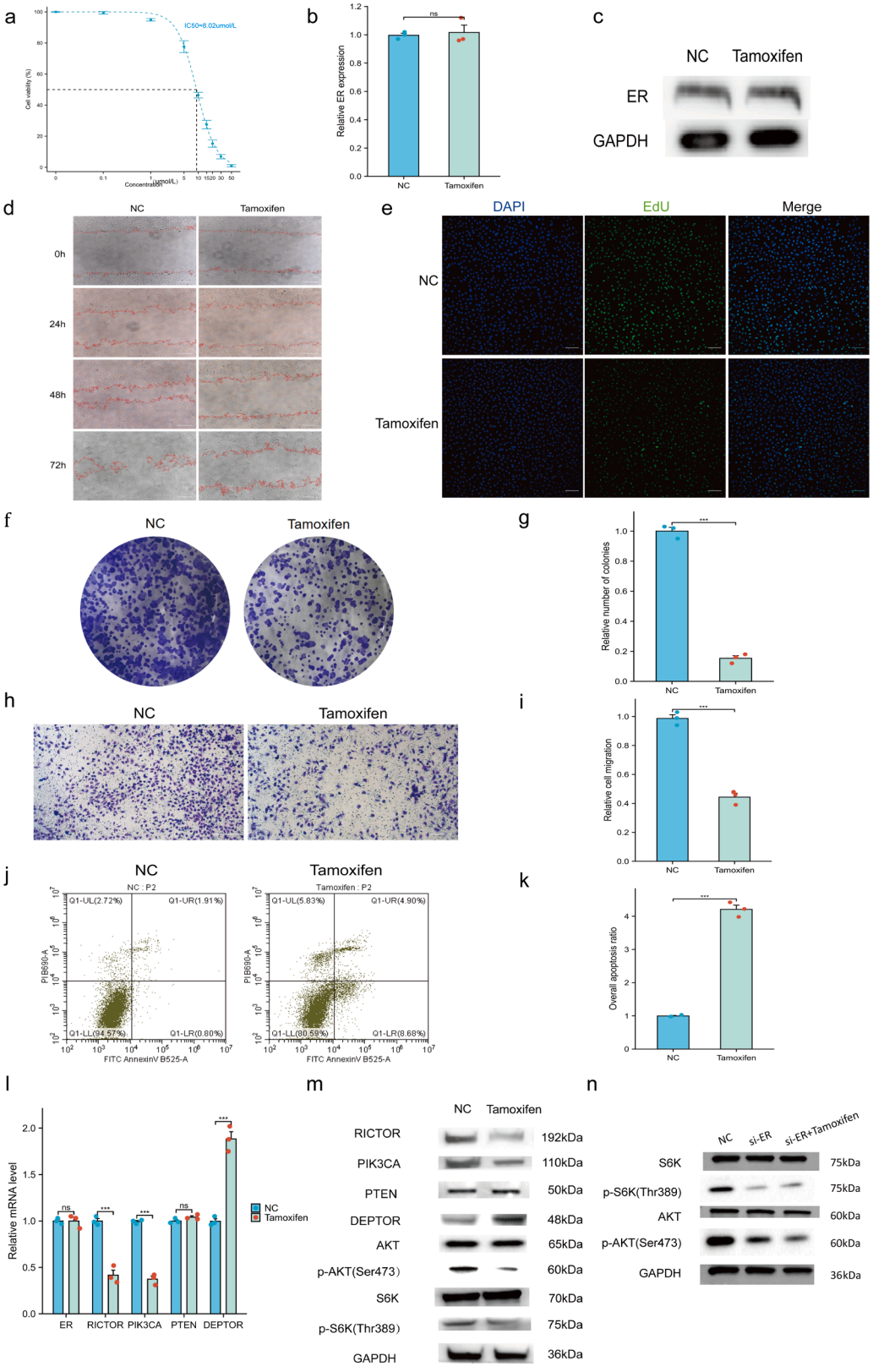

Fig. 6. Tamoxifen suppresses ER+ meningioma via mTOR pathway inhibition. a) Dose–response curve showing tamoxifen cytotoxicity in IOMM cells (IC50≈8.02 μmol/L). **b-c)** Tamoxifen treatment minimally affected.ER expression, as assessed by qRT–PCR and Western blot analysis. **d)** Scratch assay demonstrating impaired migration. Scale bar, 100 μm. **e)** The results of the EdU assay revealed that proliferation was suppressed. Scale bar, 200 μm. **f-g)** Colony formation assay showing reduced clonogenicity. Scale bar, 200 μm. **h-j)** Transwell invasion assay indicating reduced invasive potential. Scale bar, 100 μm. **j-k)** Flow cytometric analysis of apoptosis induced by tamoxifen treatment in IOMM cells. **l-m)** Validation of mTOR pathway protein suppression via qRT–PCR and Western blotting following tamoxifen treatment. ***, $P < 0.001$; nc, negative control; ns, not significant. **n)**Western blot analysis of mTOR pathway proteins in meningioma cells following ERβ knockdown and tamoxifen treatment.

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Institutional review board statement

Data from the pathological repository of Sun Yat-sen Memorial Hospital at Sun Yat-sen University were procured for this investigation (sanctioned by the Medical Ethics Committee of Sun Yat-sen Memorial Hospital, Opinion No SYSKY-2023–477–01, 2023–05–25)

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

The following abbreviations are used in this manuscript:

ER	Estrogen receptor
RICTOR	Rapamycin-Insensitive Companion of mTOR
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
DEPTOR	DEP-Domain Containing mTOR Interacting Protein
p-AKT	Phosphorylated Akt
CCK8	Cell Counting Kit 8
EdU	5-ethynyl-2′-deoxyuridine
PCA	Principal component analysis
DEGs	Differentially expressed genes
siRNA	Small interfering RNA
qPCR	Quantitative polymerase chain reaction
SERM	Selective ER modulator

CRediT authorship contribution statement

Hongru Yao: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Kaitao Zhu:** Validation, Supervision. **Shiwei Li:** Formal analysis, Data curation. **Jilong Hei:** Data curation. **Shuwen Wang:** Investigation. **Wenpeng Li:** Validation, Software, Conceptualization. **Tongxin Ye:** Methodology. **WenG Jiang:** Writing – review & editing, Supervision, Funding acquisition. **Tracey Martin:** Writing – review & editing, Supervision, Funding acquisition. **Shanyi Zhang:** Supervision, Formal analysis.

Declaration of competing interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2026.102723.

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