RESEARCH PAPER

Cortactin And SIRT1 Increased Expression Levels Are Associated With Meningioma Patients.

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Abstract
Meningiomas are the most prevalent CNS neoplasms in adults and display various cytological and histological features associated with higher morbidity and mortality rates and diagnostic complexity. SIRT1 is the class III histone deacetylase (HDAC) that plays a role in the metabolism, aging, and carcinogenesis of organisms and regulates senescence and apoptosis in cells but its role in meningioma is unclear. This study aims to check the expression patterns and interactions of cortactin and SIRT1 proteins in different grades of meningiomas and control arachnoid tissue. In the experimental groups of meningioma, human tissue samples from the three grades of meningioma (n=28) and control arachnoid tissues (n=12) were employed for assessing the expression of cortactin and SIRT1 proteins via immunoblotting, immunohistochemical staining, and immunofluorescence staining. Results revealed a significant increase in cortactin and SIRT1 expression in high-grade meningiomas compared to arachnoid tissue. Co-expression analysis with ki67 revealed correlations between cortactin/SIRT1 and proliferative activity, particularly in grade II and grade III meningiomas. Co-immunoprecipitation assays confirmed a direct interaction between cortactin and SIRT1, which intensified with tumor severity. Here, we report the in vivo interaction between cortactin and SIRT1, revealed by fluorescence resonance energy transfer (FRET) microscopy. In conclusion, this study emphasizes the significance of cortactin and SIRT1 as biomarkers for diagnosis and prognosis in meningiomas, suggesting their potential as targets for therapy.

Keywords: Cortactin, SIRT1, invasion, proliferation, metastasis
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Introduction

Meningiomas are the most common intracranial tumors that originate in the meninges, with severity ranging from benign (WHO Grade I) to atypical (Grade II) and malignant (Grade III) tumors (Ogasawara C 2021). The development of meningiomas to higher grades substantially impacts patient prognosis, demanding a deeper insight into the underlying molecular pathways. Post-translational modifications (PTMs) are important in meningioma etiology and development because they influence the function, stability, and activity of essential proteins involved in cellular processes such as proliferation, apoptosis, migration, and DNA repair (Lee Y 2020). Acetylation, phosphorylation, ubiquitination, and methylation are among the modifications that affect the action of signaling molecules, transcription factors, and cytoskeletal proteins (Grimes M 2018). For instance, aberrant acetylation patterns controlled by enzymes such as histone deacetylases (HDACs) and sirtuins have been linked to dysregulated gene expression and cytoskeletal dynamics, which contribute to tumor development and invasion (Milazzo G 2020). Understanding the dysregulation of these modifications in meningiomas could provide valuable insights into tumor biology and aid in identifying new therapeutic targets for treating aggressive types of health conditions. Silent mating-type information regulation 2 homologue 1 (SIRT1), the mammalian counterpart of yeast Sir2, is a NAD+-dependent protein deacetylase that regulates lifespan, metabolism, and stress responses. Its activity is influenced by nutritional and environmental factors (Fangma et al 2023). Members of the Sirtuin family,

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which includes SIRT1, 2, 3, and 5, are principally deacetylases and mono-ADP-ribosyl transferases that play important roles in numerous signaling pathways (Nasiri 2020). This contributes to a variety of biological processes, including metabolism, rDNA transcription, aging, senescence, DNA repair, and cancer (Alves-Fernandes 2019; Majeed 2021; Yang 2022; Chen 2020). SIRT1 has been demonstrated to play a multifaceted function in cancer, contributing to tumorigenesis. It deacetylates key transcription factors including p53 and Hypermethylation in cancer 1 (HIC1), which are crucial for stress responses, cell cycle control, and apoptosis, thus inhibiting tumor-suppressive effects and accelerating oncogenesis (Chen et al 2005; Homayoun et al 2001). Through the regulation of these and additional variables, including Ku70, NF-kB, and FOXO, SIRT1 promotes cellular survival and proliferation, which aids in the advancement of cancer (Chen 2005, Yeung, 2004, Brunet 2004). SIRT1 promotes uncontrolled cell proliferation and resistance to apoptosis by deregulating essential regulatory systems, underlining its potential as a therapeutic target. Notably, the SIRT1 protein negatively regulates tumor suppressors such as p53 and HIC1 and is deleted in breast cancer 1 (DBC1), reducing its capacity to inhibit tumor development and maintain genomic integrity (Kim et al 2008). This regulatory function supports SIRT1's carcinogenic effect by promoting cell survival and proliferation while facilitating cancer development. Despite its ability to cause cancer, it can also able to reduce tumors by making cells more susceptible to apoptosis caused by tumor necrosis factor-alpha (TNF-α). SIRT1 enhances the cellular response to TNF-α by deacetylating critical proteins in apoptotic pathways, facilitating programmed cell death, and preventing uncontrolled proliferation (Yeung et al 2004). This tumor-suppressive activity demonstrates SIRT1's dual nature since its involvement in cancer varies depending on the cellular context and signaling pathways involved. Zhang et al (2009) demonstrated that Cortactin is a novel substrate for SIRT1 and a potential function for SIRT1 in cell motility via cortactin deacetylation.

Cortactin, an actin-binding protein encoded by the CTTN gene (previously known as EMS1), is a prominent substrate for Src kinase (Yin M 2017). It is important in cancer growth because it modulates processes necessary for cancer progression, such as cell motility and invasion (Jeannot P 2020). By regulating the dynamic rearrangement of the actin cytoskeleton, which leads to the formation of cellular protrusions such as lamellipodia and invadopodia, it promotes the migration and invasion of cancer cells (Bandela 2022). These structures help tumor cells to penetrate the extracellular matrix, facilitating metastasis; as a result, it is frequently overexpressed in several human cancers, including breast, head and neck, oesophageal, and colorectal cancer (Twafra et al 2022, Timpson et al 2007, Fsu et al 2009, Zhang 2020, Zhang et al 2006). Functional studies show that cortactin overexpression accelerates cancer development by regulating actin assembly, which leads to tumor cell mobility, invasion, and metastasis (12,14,17). However, its role in oncogenesis is highlighted by the fact that reduced cortactin expression stops lung cancer cells from growing in vitro (Bandela 2022). These findings highlight the importance of cortactin in the mechanisms that drive cancer aggression and its potential as a therapeutic target. Cortactin also enhances cancer cell adhesion to both the extracellular matrix and endothelial cells, allowing them to pass through blood vessel walls during metastasis. It also impacts cancer cell signaling pathways that are involved in cell proliferation, survival, and angiogenesis, all of which lead to tumor development and metastatic spread (Twafra 2022).

In this study, we examined, the expression levels of cortactin and SIRT1 proteins in human patient samples of grade I, II, and III meningiomas, as well as arachnoid tissue, using western blot, immunohistochemical, and immunofluorescence staining techniques. Furthermore, we investigated the colocalization and interaction between cortactin and SIRT1 proteins through immunofluorescence and immunoprecipitation analyses. Further, we investigate the in vivo interaction between cortactin and SIRT1 using fluorescence resonance energy transfer (FRET) technology. Targeting these proteins and their interaction could open up new possibilities for developing novel anticancer therapies that impede cancer cell motility and invasion, restricting metastatic dissemination and improving patient outcomes.

Materials & methods:

Clinical Specimens and Experimental Material

This study included meningioma biopsy tissue specimens from 28 patients who underwent surgery at the Department of Neurosurgery, Krishna Institute of Medical Sciences (KIMS), Secunderabad between January 2018 and December 2023. Standard control, arachnoid tissue (n=12) obtained from the National Institute of Mental Health & Neurosciences (NIMHANS) brain bank in Bangalore, India. To participate in this study, patients must have a primary meningioma diagnosis and comprehensive clinicopathological data for review. Table 1 summarizes the clinicopathological features acquired from the tumor registry's computerized database. The tumors comprised

Grade I, Grade II, and Grade III instances of meningioma, which are categorized using World Health Organisation (WHO) criteria. Patients with various grades of meningioma were histopathologically diagnosed and identified at the pathology department of KIMS hospital. Clinical data were obtained from KIMS hospital medical records, which included age, gender, resection grade, and tumor recurrence. This study group consisted of 18 females and 22 males with a mean age of 42 years (range, 22-65 years), and it contained twelve normal arachnoid tissue and twenty-eight (12-grade I, 12-grade II, 4-grade III) various meningioma-grade tissue specimens. Tissues were snap-frozen and preserved at -80 degrees Celsius. To use tumor specimens and clinicopathological details, written informed consent was obtained from the patients. The study was approved by the Institutional Ethical sUH/IEC/2019/159), University of Hyderabad, India.

Reagents and antibodies

Cell culture chemicals such as Dulbecco's modified eagle medium (DMEM) (AL007A), Trypsin-EDTA (TCL007), FBS (RM9955), antibiotic solution (A018), antimycotic antibiotics (A002), and various types of plates (T-25, 60mm, 100mm, 6-well) were obtained from HI media. Additionally, a protease inhibitor (SIGMAFAST TM S8820-2TAB) and phosphatase inhibitors (Sigma, Roche PhosSTOP Easy pack, 04906845001) were used. EBSS (E7510) and collagenase (C1-28) were also obtained from Sigma. Protein A/G beads were purchased from Santa Cruz (sc-2003). Cell signaling technology provided primary antibodies for GAPDH (D16H11), Cortactin (H222), SirT1 (1F3) Mouse mAb #8469, and Secondary antibodies (7076S and 7074) and fluorescent secondary antibodies (Anti-rabbit IgG (Alexa Fluor® 555 Conjugate) #4413, Anti-mouse IgG ((Alexa Fluor® 555 Conjugate) #4409).

Preparation of Human Tissue Samples for Western Blotting Analysis

Frozen human meningioma tissue and normal arachnoid tissue were procured from KIMS and NIMHANS, respectively. For each frozen sample, approximately 50 mg of frozen tissue was ground in a cold mortar with liquid nitrogen into fine powder and underwent lysis in NP-40 lysis buffer, comprising 50 mM Tris-HCl, 120 mM NaCl, and 0.5% NP-40, supplemented with 20µg/ml phenylmethylsulfonylfluoride (PMSF), 10µl/ml protease inhibitors, and 10µl/ml phosphatase inhibitors. Crude protein lysates were collected by centrifugation at 12,000 ×g and their concentration was measured using the Bradford assay (G-Biosciences, Page Avenue St. Louis, MO, USA). SDS-PAGE was performed on equal volumes of crude protein lysates from various grades. The separated proteins were electro-transferred onto nitrocellulose (NC) membranes overnight at 4 °C using a transfer buffer comprising 12mM Tris-HCl, pH 8.3, 96mM glycine, and 20% (v/v) methanol. Following the transfer, the membrane was blocked with TBST buffer containing 5% skimmed milk for two hours. It was then treated with primary antibodies GAPDH (D16H11) XP® Rabbit mAb #5174 CST, Cortactin (Cortactin H222) antibody# 3505 (1:1000), SirT1 (1F3) Mouse mAb #8469 (1:1000) CST overnight at 4 °C on a rocker. The membrane was washed four times (once in TBS, twice in TBST, and once in TBS), then incubated with secondary antibodies on a rocker plate for 120 minutes at room temperature. Another round of washing, this time for 7 minutes each (once in TBS, twice in TBST, and once in TBS), followed. Protein signals were identified using G-Biosciences' ECL™ detection reagents in the Bio-Rad Chemidoc imaging system.

Immunohistochemical staining

Formalin-fixed, paraffin-embedded (FFPE) tissue sections of 5 µm on positively charged glass slides from meningioma tumor tissue blocks were retrieved from the pathology lab of KIMS Hospital, Secunderabad, India. Arachnoid tissue sections were retrieved from NIMHANS brain bank, Bengaluru, Karnataka, India. The FFPE sections were preheated to 100°C for 10 minutes, deparaffinized with xylene, and rehydrated progressively with 90%, 80%, 70%, and 50% ethanol. The sections were immersed in an antigen retrieval solution [10 mM sodium citrate buffer (pH 6.0) with 0.05% Tween 20] for 15 minutes. To inactivate endogenous peroxidase, sections were treated with 5% BSA (Bovine Serum Albumin) for 60 minutes at room temperature, followed by a peroxidase blocker for 30 minutes. Sections have been washed with PBST twice. These sections were subsequently treated with Cortactin (Cortactin H222) antibody # 3505 (1:200) CST and SirT1 (1F3) Mouse mAb #8469 (1:200) CST antibody at 4 °C overnight. Tissue sections were rinsed twice with PBS and PBST for 5 minutes before incubating with a secondary HRP antibody. After incubation, the sections were washed again with PBS and PBST for 5 minutes. The antigen-antibody response was observed using 3',3'-diaminobenzidine (DAB) until a brown colour appeared. Following the chromogenic reaction, the sections were counterstained with haematoxylin and covered with coverslips.

Double Immunofluorescence Staining

Formalin-fixed, paraffin-embedded tissue sections were heated to 100°C for 10 minutes to induce antigen retrieval. Xylene was used to deparaffinize the specimens, which were then rehydrated using 100% and 95% ethanol solutions in sequence. The 5 µm tissue sections were washed with distilled water before being exposed to antigen retrieval in a 10 mM sodium citrate buffer (pH 6.0) containing 0.05% Tween 20, heated for 15 minutes. After antigen retrieval, the sections were washed twice with PBST (phosphate-buffered saline with Tween 20) for two minutes. The serum was used to block the section for 30 minutes and then incubated overnight at 4°C with primary antibodies such as Cortactin (Cortactin H222) antibody #3505 (1:200), CST, and SirT1 (1F3) Mouse mAb #8469 (1:100) CST. After incubation, the sections were rinsed twice with PBST and PBS for 5 minutes each. The sections were then treated for 60 minutes at room temperature with secondary antibodies Alexa Fluorochrome 488 and Alexa Fluorochrome 555. Following a final wash with PBST, the sections were DAPI-mounted for nuclear counterstaining. A confocal microscope was used to acquire images (Carl Zeiss Model: NLO710, software: ZEN 2010).

Immunoprecipitation analysis

50 mg of tissue was lysed with liquid nitrogen in a mortar and pestle. IP buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X 100, and 2 mM EDTA from a 0.5 M stock pH 8.0) was added along with 20 µl/ml phenylmethylsulfonylfluoride (PMSF), 10 µl/ml protease inhibitor (SIGMAFAST TM S8820-2TAB), and phosphatase inhibitors (Sigma, Roche PhosSTOP Easy pack, 04906845001). The sample was sonicated for 15 seconds at 25 amplitudes with 10-second intervals for four cycles. The lysate was then centrifuged at 16,000 rpm at 4 °C for 20 minutes using an Optima TM max-XP centrifuge with an MLA 150 fixed-angle rotor. The protein concentration in the supernatant was determined using the Bradford technique. Next, the required quantity of antibody was added to the 500 ul lysate, and the combination was incubated overnight at 4 °C with agitation. Protein A/G beads were equilibrated before being added to the protein lysate mixture, which was then incubated for 6 hours at 4 °C with agitation. The mixture was then microcentrifuged for 30 seconds at 4 °C to remove the supernatant. The pellet was rinsed with 1X lysis buffer and then resuspended in 3X SDS loading buffer before being vortexed and microcentrifuged for 30 seconds. The samples were heated to 100 °C for 6 minutes before being centrifuged for 2-3 minutes at 14,000 g. Finally, the samples were put into SDS-PAGE and analyse by western blotting.

Meningioma Primary culture establishment

Primary meningioma cultures were established from patient specimens using the procedure reported by Uhlmann et al. (2021), with minor variations (Uhlmann E. et al. 2021). Tissue specimens were carefully transported on ice in sterile containers containing antibiotic-treated DMEM immediately following meningioma surgery at KIMS hospital. Upon arrival, the specimens were placed in a sterile plate with fresh Dulbecco's Balances salt solution (DBSS) and properly washed. The tissue was then moved to another plate, where any undesired tissue was removed. The tissue was then carefully diced into 1-mm cubes using crossed scalpels and transferred to a 15-ml sterile centrifuge tube or universal container with a wide-tipped device. The tissue fragments were allowed to settle before being washed with DBSS to remove any remaining debris. The supernatant fluid was carefully discarded after each wash. This treatment was performed twice, followed by a single DMEM wash before transferring the tissue pieces to a 25-cm2 flask. The tissue fragments were mixed with growth media and collagenase in the flask to obtain a final concentration of 200 units/ml. The flask was then put in a humidified incubator set to 37°C with 5% CO2, and tissue disaggregation was examined daily. Disaggregated cells were centrifuged in 1.5 ml Eppendorf tubes and resuspended in a new 25-cm2 flask containing growth media, serum, and antibiotics. Adherent cultures were established by supplementing the medium with 4% fetal bovine serum for three days. The culture media were replaced every three days to maintain optimal conditions. A diagrammatic representation of the meningioma primary culture procedure is shown in S1 (Supplementary Figure 1). Once the cells attained sub confluency, they were removed using 0.25% trypsin-EDTA and resuspended for the Cortactin-SIRT1 FRET experiments.

Cortactin-SIRT1 FRET experiments

For fixed-cell FRET analysis, a meningioma primary culture was established from a meningioma patient's brain tissue, and the required number of cells was plated on a coverslip in 6 well plates. Cells were fixed and probed with a cocktail mixture of primary monoclonal antibody cortactin and SIRT1 overnight Cortactin antibody #3505 (1:200) CST, and SirT1 #8469 (1:100) CST. Immunofluorescence was performed using an anti-mouse human-SIRT1 antibody and anti-rabbit human-cortactin antibody labelled with Alexa fluor 488 and Alexa fluor 555 respectively (Anti-rabbit IgG (Alexa

Fluor® 555 Conjugate) #4413, Anti-mouse IgG ((Alexa Fluor® 555 Conjugate) #4409) CST. FRET measurements were based on spectral imaging of a sample containing both donor (Alexa fluor 488-cortactin-GFP) and acceptor (Alexa fluor 555-SIRT1-red) species, which were excited using kD488/kA543 laser lines. Alexa fluor 488 having (λ ex= 488 nm, λ em=520nm) and Alexa fluor 555(λ ex= 555nm, λ em=570nm). All the FRET experiments were performed as described by Chakraborty (2014). In brief, at the site where cortactin and SIRT1 colocalization were observed, the acceptor channel was bleached and changes in the fluorescent signal in the donor channel were measured and analysed. Images were acquired using a laser scanning microscope (Olympus FV3000 confocal microscope) and analysis was performed by using Fiji software. Change of fluorescence intensity (Δ IF) = I_{DA}-$I_{DB,}$ Where I_{DA} is the donor intensity after bleaching and I_{DB} is the donor intensity before bleaching.

Statistical analysis

Total data were subjected to statistical analysis using GraphPad Prism 8 and Sigma Plot 11.0 software. The results are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using One-way ANOVA for multiple groups, with p-values < 0.05 considered as statistically significant (ns: not-significant, *p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

Results

Analysis of cortactin and SIRT1 expression across varied grades of meningioma patient specimens.

In the present study, we investigated the expression levels of cortactin and SIRT1 proteins in biopsy specimens from human patients with meningiomas (n=12) of varying grades (I, II, and III) and arachnoid tissue as control (n=4) using western blotting and immunohistochemical staining. Western blot analysis revealed a significant elevation in cortactin protein levels in Grade II and Grade III meningiomas compared to control and Grade I tissues, which exhibited no notable changes in expression. The densitometric analysis further corroborated these findings, which quantified the differences and showed statistically significant variations in cortactin expression across the different grades of meningiomas and control tissue ($p < 0.007$) (Fig.1 a, b). This indicates that cortactin expression is associated with higher-grade meningiomas, suggesting a potential role in the progression or aggressiveness of these tumors. Western blot analysis of SIRT1 protein demonstrated a progressive increase in protein expression from Grade I to Grade III meningiomas. The densitometric analysis confirmed these results, showing a statistically significant ($p < 0.02$) in SIRT1 expression as the meningioma grade increased. This suggests that SIRT1 may also play a role in tumor grade progression, with higher expression levels correlating with more advanced tumor grades. The immunohistochemical (IHC) analysis revealed a significant increase in the expression of cortactin and SIRT1 proteins in Grade II and Grade III meningiomas compared to Grade I meningiomas and control arachnoid tissue. Quantitative IHC evaluation demonstrated a greater number of highly positive cells in Grade II and III meningiomas, while Grade I tumors and arachnoid tissues predominantly exhibited faint or negative staining. These results were statistically significant, (p CTTN <0.02, and p SIRT1<0.04) (Fig. 1c, d). This suggests a correlation between the progression of meningioma grades and the expression of these proteins. In line with this study, Immunofluorescence (IF) staining with fluorescent-labelled antibodies targeting cortactin and SIRT1 protein demonstrated higher protein expression levels in grade II and grade III meningioma compared with grade I tumor tissue and arachnoid tissue, as shown in (Fig. 1 e, f.). These findings indicate that cortactin and SIRT1 protein expression gradually increases with meningioma grade. Furthermore, immunofluorescence quantification revealed a statistically significant increase in cortactin (p CTTN< 0.001) and SIRT1 (p SIRT1 < 0.004) protein levels in grade II and III meningiomas as compared to grade I and control arachnoid tissue. The differential expression pattern suggests that cortactin and SIRT1 might play a role in meningioma development, potentially as a biomarker for tumor grading and prognosis.

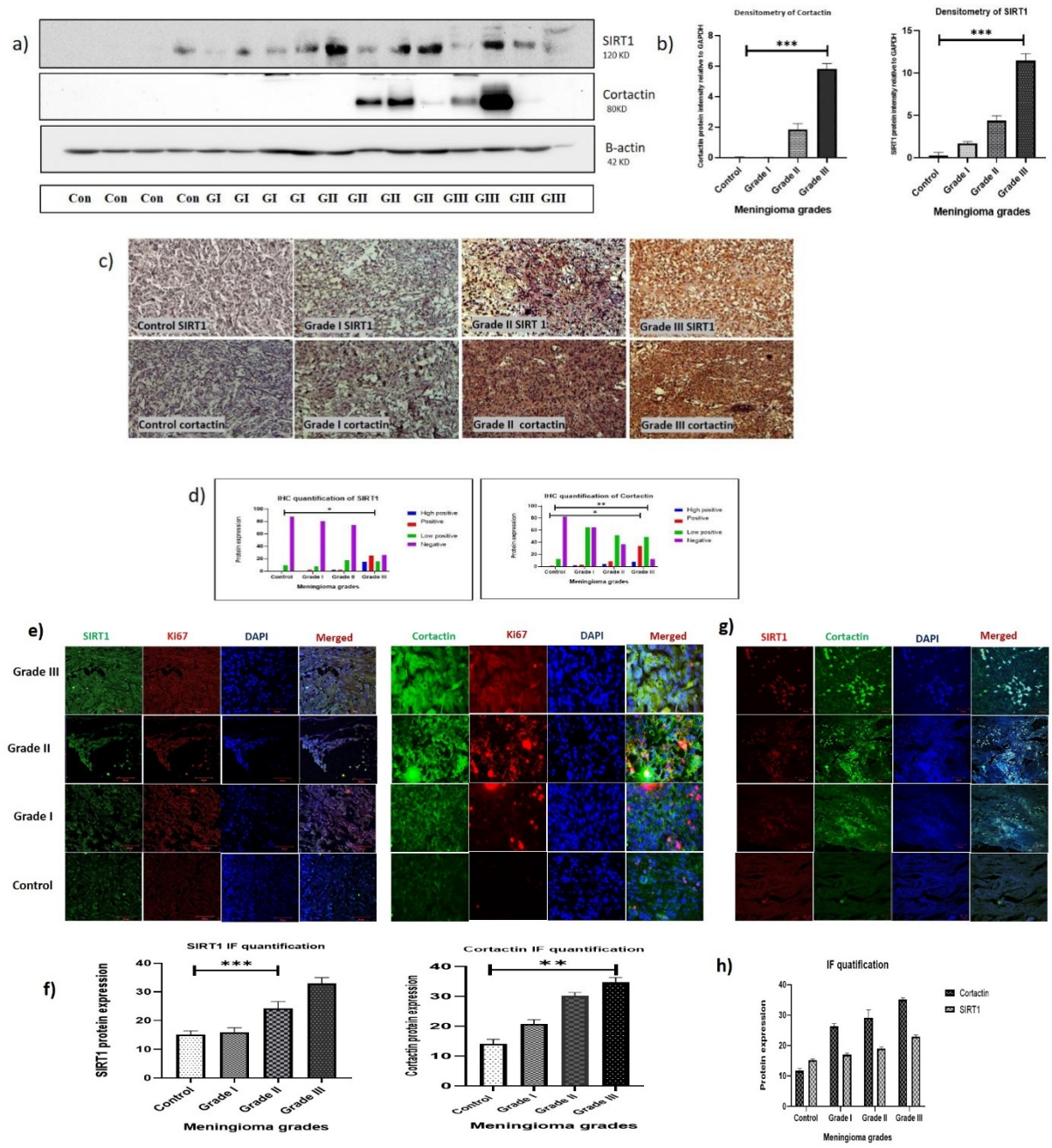

Fig 1 Cortactin and SIRT1 protein expression in meningioma tissue specimens. a) Western blot of cortactin and SIRT1 proteins in arachnoid tissues and grade I, II, and grade III of meningiomas. (Control n=4; grade I n= 4; grade II n=4; grade III n=4. b) Immuno-blot quantification of cortactin and SIRT1 proteins. (c) Immunohistochemical staining analysis of cortactin and SIRT1 proteins in arachnoid and WHO-grade meningioma tissue sections (Scale- 50 um). (d) Quantification of immunohistochemical staining analysis of these proteins in control and meningioma tissue. (e) Immunofluorescence staining analysis of SIRT1 (green) and Ki 67 (red) and cortactin (green) and Ki 67 (red) , and proteins in arachnoid tissue sections and biopsy tissue sections from three grades of meningioma. (f) Immunofluorescence quantification of these proteins arachnoid sections and three grades of meningioma tissue specimens (Scale-50 um). g) Immunofluorescence staining of cortactin (green) and SIRT1 (red) proteins. h) Immunofluorescence quantification of cortactin and SIRT1 proteins. (Scale-20um) (ns: not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

Co-expression and co-localization between cortactin, SIRT1 and Ki67 proteins

Further, we studied double immunofluorescence labelling of cortactin and SIRT1 with ki67 in meningioma tissue sections revealing distinct co-

expression patterns associated with tumor proliferation. The ki67 co-expression with cortactin and SIRT1 was absent in arachnoid tissue and very less in grade I meningioma, indicating low proliferative activity. This finding shows that cortactin may play a role in regulating cell proliferation, as its absence coincides with lower ki67 expression, a well-known marker of cellular proliferation. In contrast, grade II grade III meningiomas showed significantly higher levels of cortactin and ki67 co-expression, indicating increased tumor growth. This increase in cortactin expression is consistent with the aggressive proliferative phenotype found in grade II and grade III meningiomas. Additionally, SIRT1 and ki67 co-expression showed a parallel expression pattern, indicating its role in the progression of meningiomas (Fig. 1 e, f).

Further, we studied the colocalization of these proteins by employing double immunofluorescence in control arachnoid and grade I, II, and III meningiomas tissue sections. Results revealed a significant increase in cortactin and SIRT1 colocalization in grade II and grade III meningiomas compared with normal arachnoid tissue which suggests a stronger interaction between these proteins. Whereas the grading progressed from grade III to arachnoid tissue, the degree of colocalization declined indicating a gradual decrease in protein interaction. These findings point to a possible functional relationship between cortactin and SIRT1, which is exacerbated in higher grades of meningioma (Fig. 1 g, h).

Interaction of SIRT1 with cortactin in meningioma tissue.

To confirm the possible direct interaction between cortactin and SIRT1 proteins, we performed the co-immunoprecipitation assay. In this assay, specific antibodies targeting cortactin and SIRT1 were employed to isolate protein complexes from tissue lysates of meningioma grades and arachnoid tissue utilizing protein A/G beads. A subsequent western blot study shows higher SIRT1 immunoprecipitation in grade II and III meningiomas, demonstrating that the interaction between cortactin and SIRT1 becomes more prominent as meningioma progresses. This data suggests that cortactin and SIRT1 do interact, with the intensity of the interaction corresponding to the severity of the meningioma grades (Fig 2 a, b). The observed elevation in expression, co-localization, and interaction of cortactin and SIRT1 in grade II and III meningiomas suggests their potential involvement in tumor spread and progression. Further, we conducted FRET analysis to assess the direct interaction between cortactin and SIRT1 proteins. The data depicted a change in fluorescent intensity specifically within colocalized regions (Fig. 3a-d), indicating a potential direct interaction between these two proteins. This finding suggests a physical association between cortactin and SIRT1 in the examined cellular context.

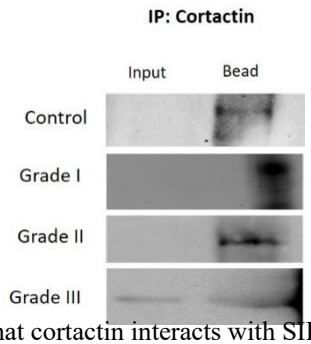

Fig 2 Co-immunoprecipitation results show that cortactin interacts with SIRT1 in grades II and III of meningioma but not in control and grade I.

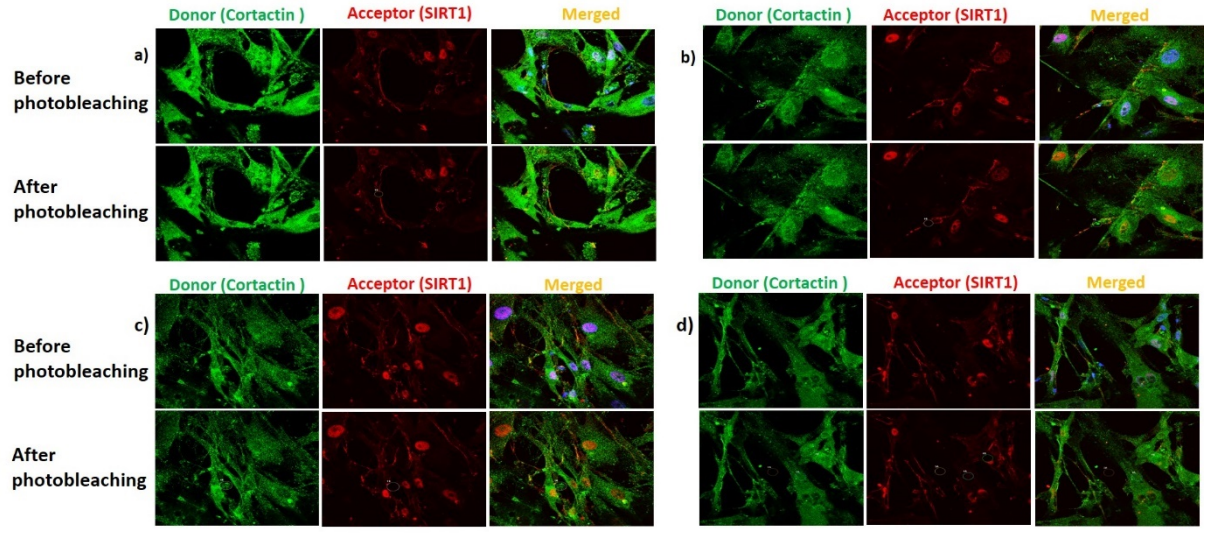

Fig 3 Photobleaching FRET analysis cortactin and SIRT1. Two frames before and after the bleaching interval were presented. The bleached region of SIRT1 was shown (arrows).

Cellular localization of cortactin and SIRT1 proteins in meningioma tissues.

This study used fluorescent-specific antibodies to explore the cellular localization patterns of Cortactin and SIRT1 proteins in arachnoid tissue and meningiomas of Grades I, II, and III. The findings revealed distinct localization patterns for these proteins in meningioma tissues. SIRT1 was predominantly localized in the nucleus across all grades of meningiomas, suggesting its involvement in nuclear processes such as gene regulation. In contrast, Cortactin exhibited primarily cytoplasmic localization in Grade I, II, and III meningiomas, consistent with its role in cytoskeletal regulation and cellular movement. Notably, in Grade III meningiomas, Cortactin was also observed within the nucleus, a distinct pattern not seen in lower-grade tumors or arachnoid tissue. This nuclear localization may indicate alterations in signaling pathways or protein interactions associated with the progression and aggressiveness of higher-grade meningiomas (Fig. 1g, h).

Discussion

Meningiomas are the most prevalent primary intracranial tumors, making up roughly one-third of all central nervous system neoplasms (Ogasawara 2021). Common symptoms include headaches, seizures, focal neurological abnormalities, and cognitive dysfunction (Ogasawara 2021, Gyawali S 2019). The diagnosis is mostly based on neuroimaging examinations such as MRI and CT scans, frequently followed by histological evaluation of biopsy specimens (Zhao L 2020). Treatment options include surgical resection, radiation, and, in some situations, adjuvant chemotherapy (Honore 2015). Despite advancements in treatment techniques, managing meningiomas remains difficult, particularly for high-grade and recurrent tumors, emphasizing the need for ongoing research into novel therapeutic approaches.

In the present investigation, we observed differences in cortactin and SIRT1 protein expression across different grades of meningiomas and normal arachnoid tissue by western blot, immunohistochemical staining, and immunofluorescence staining, indicating the possibility of their involvement in tumor growth. Western blot analysis indicates cortactin expression is significantly higher in grade II and III meningiomas, indicating that cortactin may have a role in tumor growth and aggressiveness. Katharina et al., (2019) studied cortactin and knock-out cortactin expression in pancreatic ductal adenocarcinoma (PDAC) tumor tissue and established pancreatic cell lines and they observed enhanced levels of cortactin in pancreatic tumor tissue is related to tumor metastasis (Stock 2019). Similarly, the gradual increase in SIRT1 expression from grade I to grade III meningiomas indicates that it may play a role in meningioma pathogenesis, probably via cell proliferation and survival mechanisms. These findings show the dynamic changes in protein expression patterns that occur as meningioma progresses, emphasizing the importance of cortactin and SIRT1 as potential indicators or therapeutic targets. Shuai et al., (2021) studied SIRT1 overexpression in pancreatic cancer cells and its interaction with Cullin 4B (CUL4B) protein that promotes autophagy, proliferation, and invasion of cancer cells (Leng 2021).

The study observed that cortactin and SIRT1 protein expression increased progressively from normal arachnoid tissues to grade III meningiomas, with considerably greater levels in grade II and III tumours compared to grade I tumours and controls. This pattern was constant across immunohistochemical staining and immunofluorescence labelling, implying a link between increased protein expression and higher meningioma grades. These results suggest that cortactin and SIRT1 may contribute to tumour growth and aggressiveness, thereby impacting clinical outcomes. The statistically substantial increase in these proteins in higher-grade meningiomas highlights their potential as tumour grading and prognostic indicators.

Supporting research further highlights the functional significance of these proteins. Sang et al. (2013) reported a strong link between cortactin and SIRT1 expression and advanced pathological stages in non-small cell lung cancer. Similarly, Motonishi et al. (2015) demonstrated that Sirt1 knockout animals had decreased podocyte-specific proteins, whereas cortactin knockdown created actin cytoskeleton dearrangements and cortactin-F-actin dissociation. These findings imply that cortactin and SIRT1 play critical roles in both cellular structural integrity and tumor growth.

Double immunofluorescence labelling of cortactin and SIRT1 with Ki67 in meningioma tissue revealed unique co-expression patterns associated with tumour growth. Cortactin and Ki67 co-expression was absent in lower-grade meningiomas and arachnoid tissues, showing minimal proliferative activity and suggesting that cortactin plays a regulatory function in cell proliferation. Higher-grade meningiomas, on the other hand, showed considerably increased cortactin and Ki67 co-expression, which corresponded to greater tumour proliferation and more aggressive character. Similarly, SIRT1 and Ki67 co-expression followed similar pattern, indicating their role in tumour growth. These findings identify cortactin and SIRT1 as possible proliferation drivers, as well as intriguing biomarkers or therapeutic targets in meningioma therapy. Yong et al. (2022) investigated Ki67-positive cells in SIRT1 knockout mice during corneal epithelial wound healing and discovered that SIRT1 deletion had no effect on proliferation or migration, highlighting SIRT1's tissue-specific functions in cell growth processes.

Cortactin and SIRT1 colocalization was much higher in grade III meningiomas than in normal arachnoid tissues, indicating greater protein interactions in high-grade malignancies. This interaction decreased gradually from grade III to control tissues, indicating a link with tumour aggressiveness. Cortactin-SIRT1 interactions may influence important signalling pathways, contributing to the proliferative capability and aggressive character of advanced meningiomas. These findings highlight their potential as therapeutic targets for high-grade meningioma treatment. Similarly, Keita et al. (2012) found that suppressing cortactin or SIRT1 inhibited migration and invasion in DU145 prostate cancer cells, confirming their function in tumor growth.

In our investigation, co-immunoprecipitation assays confirmed a direct interaction between cortactin and SIRT1 in meningioma tissue lysates, with stronger SIRT1 immunoprecipitation observed in grade II and III meningiomas. These findings, supported by FRET results, demonstrate that the cortactin-SIRT1 interaction intensifies with increasing tumor severity. Elevated expression, co-localization, and interaction of cortactin and SIRT1 in grade III meningiomas suggest their involvement in tumor progression and aggressiveness. This highlights their potential role in meningioma etiology and their significance in driving tumor spread and development. Similarly, Lin et al. (2022) confirmed cortactin-SIRT1 interaction during corneal epithelial wound healing through co-immunoprecipitation. Therapeutically targeting this interaction could offer a promising strategy for inhibiting meningioma growth and dissemination.

The cellular localisation patterns of cortactin and SIRT1 in meningioma tissues provide critical insight into their functions in tumour biology. SIRT1's constant nuclear localisation across all tumour grades demonstrates its participation in nuclear processes such as gene regulation, which corresponds to its recognised functional activities. Cortactin is predominantly cytoplasmic, which reflects its role in cytoskeletal control and cell movement. However, the presence of cortactin in grade III meningiomas indicates a change in its subcellular location, which might be connected to altered signaling pathways or protein interactions that drive tumor growth. This dynamic localization emphasizes the complicated interaction of cortactin and SIRT1 in meningioma pathogenesis. Understanding these patterns is critical for determining how they contribute to tumor development and aggressiveness. Akihiro Ito et al. (2015) demonstrated SIRT1's nuclear expression and cortactin's capacity to shuttle between the cytoplasm and nucleus, emphasizing their varied roles in cancer development.

In conclusion, this work examines the expression, localization, and interactions of cortactin and SIRT1 in the development of meningioma. Their changing expression patterns across tumor grades highlight their role in aggressiveness and patient outcomes. The established interaction and dysregulation of these proteins emphasize their potential as therapeutic targets

in meningioma therapy. Understanding the molecular mechanisms that control cortactin and SIRT1 is critical for understanding their roles in tumor growth. More investigation into the mechanisms behind their overexpression in advanced meningiomas is needed to evaluate their significance as prognostic markers and therapeutic targets.

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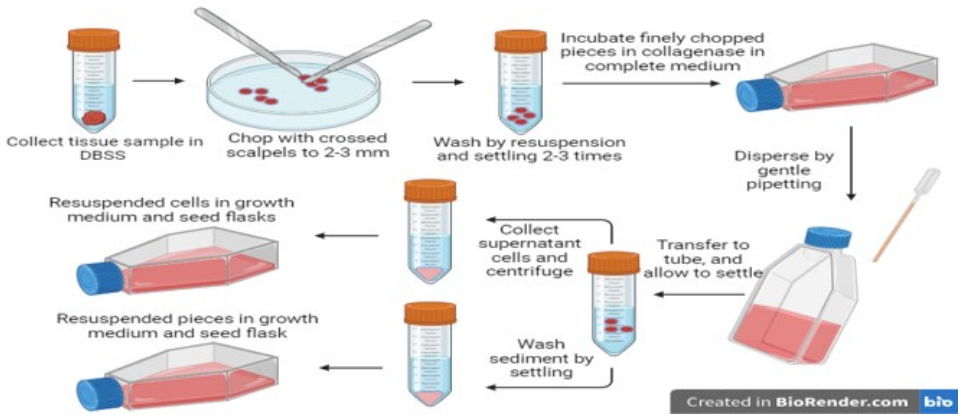

Fig S1: Diagrammatic representation of the tissue processing protocol for establishing a primary meningioma culture. Tissue is initially collected in Dulbecco's Balanced Salt Solution (DBSS) and finely chopped to approximately 2–3 mm pieces using crossed scalpels. The tissue fragments are incubated in a collagenase solution in a complete medium, followed by washing through repeated cycles of resuspension and settling (2–3 times). Afterward, the pieces are dispersed through gentle pipetting. The supernatant cells are collected and centrifuged, while the sediment is further washed and allowed to settle. Finally, the cells and tissue pieces are resuspended in a growth medium, seeded in culture flasks, and incubated to establish the primary meningioma cell culture.

Sr no	Age	Gender	Grade	Meningioma Subtype
1	25	Female	Grade I	Meningothelial
2	56	Female	Grade I	Fibroblastic
3	48	Male	Grade I	Transitional
4	37	Female	Grade I	fibroblastic
5	28	Male	Grade I	Angiomatous
6	22	Male	Grade I	Meningothelial
7	35	Male	Grade I	Meningothelial
8	42	Male	Grade I	Meningothelial
9	46	Female	Grade I	Transitional
10	29	Female	Grade I	Fibroblastic
11	38	Male	Grade I	Fibroblastic
12	57	Female	Grade I	Meningothelial
13	51	Female	Grade II	Clear cell
14	36	Male	Grade II	Chordoid
15	24	Male	Grade II	Atypical
16	50	Female	Grade II	Clear cell
17	32	Male	Grade II	Chordoid
18	39	Female	Grade II	Atypical
19	29	Female	Grade II	Atypical
20	48	Male	Grade II	Chordoid
21	47	Female	Grade II	Clear cell
22	33	Male	Grade II	Chordoid
23	42	Female	Grade II	Clear cell
24	35	Male	Grade II	Chordoid
25	34	Female	Grade III	Rhabdoid
26	46	Female	Grade III	Anaplastic
27	51	Female	Grade III	Rhabdoid
28	49	Female	Grade III	Anaplastic

29	24	Male	Arachnoid	Control
30	25	Male	Arachnoid	Control
31	23	Male	Arachnoid	Control
32	65	Male	Arachnoid	Control
33	43	Male	Arachnoid	Control
34	25	Male	Arachnoid	Control
35	32	Male	Arachnoid	Control
36	20	Male	Arachnoid	Control
37	33	Male	Arachnoid	Control
38	42	Male	Arachnoid	Control
39	56	Male	Arachnoid	Control
40	28	Male	Arachnoid	Control

Table 1: Clinicopathological Characteristics of Meningioma and Control Patients in This Study.