Eribulin prolongs survival in an orthotopic xenograft mouse model of malignant meningioma

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Abstract

Meningioma is the most common intracranial tumor, with generally favorable patient prognosis. However, patients with malignant meningioma typically experience recurrence, undergo multiple surgical resections, and ultimately have a poor prognosis. Thus far, effective chemotherapy for malignant meningiomas has not been established. We recently reported the efficacy of eribulin (Halaven) for glioblastoma with a telomerase reverse transcriptase (TERT) promoter mutation. This study investigated the anti-tumor effect of eribulin against TERT promoter mutation-harboring human malignant meningioma cell lines in vitro and in vivo. Two meningioma cell lines, IOMM-Lee and HKBMM, were used in this study. The strong inhibition of cell proliferation by eribulin via cell cycle arrest was demonstrated through viability assay and flow cytometry. Apoptotic cell death in malignant meningioma cell lines was determined through vital dye assay and immunoblotting. Moreover, a wound healing assay revealed the suppression of tumor cell migration after eribulin exposure. Intraperitoneal administration of eribulin significantly prolonged the survival of orthotopic xenograft models of both malignant meningioma cell lines implanted in the subdural space.
1 | INTRODUCTION

Meningiomas are the most common intracranial tumors, with an incidence of 23.8%-36.8% among all primary brain tumors.¹ ² Up to 98% of meningiomas are World Health Organization (WHO) grade I or II, and their prognosis is generally favorable. Surgery alone or in combination with radiation therapy could mostly cure or control low-malignancy meningiomas. Malignant meningiomas (WHO grade III: anaplastic meningioma, papillary meningioma, and rhabdoid meningioma) are rare, accounting for only 1.6%-1.7% of all meningiomas.¹ ² Previous studies have revealed a high recurrence rate (5-year progression-free survival: 13.0%-34.5%),¹ ³ despite repetitive surgical treatments. Due to the limited efficacy of radiation therapy and lack of effective chemotherapy, patients typically have a dismal prognosis (5-year and 10-year overall survival: 40.0%-54.2% and 27.9%-57.4%).¹ ³ ⁴

Many studies have analyzed the molecular profiles of meningiomas to elucidate their pathogenesis and seek therapeutic targets. Earlier studies have examined copy number alterations in meningiomas, including 1p, 4p, 6q, 7p, 9p, 10q, 11p, 14q, 18q, and 22q loss and 1p, 9q, 12q, 15q, 17q, and 20q gain.⁵ ⁶ Subsequently, mutational profiling has become the focus of biological analysis. The first genetic alteration identified was in the NF2 gene, which was found mutated in NF2-associated and in a subset of sporadic meningioma.⁷ Recent developments in next-generation sequencing technologies have led to the discovery of several potentially targetable gene mutations in meningioma (eg, SMO, KLF4, AKT1, TRAF7, POLR2A, PIK3CA, SUFU, SMARCB1, SMARCE1, and BAP1).⁷ ¹⁰ A recent study addressed the efficacy of the mTOR inhibitors sirolimus and everolimus using malignant meningioma cell lines in vivo, leading to the initiation of the ongoing clinical trials.¹¹ Irinotecan also exhibited anti–tumor activity in vivo, which contributed to upcoming clinical trials.¹² Oncolytic herpes virus, gemcitabine, and protein phosphatase 2A inhibitor LB-100 generated positive data in vivo, and may serve as a candidate for future clinical trials.¹³ ¹⁵ However, no therapeutic agents have demonstrated efficacy in malignant meningiomas.¹⁶ ¹⁸

Point mutations in the promoter region of the telomerase reverse transcriptase (TERT) gene are frequently observed in various malignant tumors.¹⁹ ²⁰ TERT is a reverse transcriptase subunit of telomerase, together with the telomerase RNA component. Aberrant telomerase activation induces telomere elongation, enabling the incessant proliferation of tumor cells.²¹ The TERT promoter mutations most commonly occur in two hot spots: -124C>T (C228T) and -146C>T(C250T). These mutations generate new Ets-binding motifs for GA-binding proteins, leading to transcriptional upregulation and telomerase activation.²² ²³

Previous studies have revealed that TERT promoter mutations frequently occur in central nervous system tumors, particularly in glioblastoma, oligodendroglioma, and IDH-wildtype astrocytomas.²⁴ ²⁵ This has led to TERT promoter mutations playing an important role in molecular diagnosis in adult gliomas. The cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) Update 3 recommended that adult WHO grade II or III diffuse astrocytic, IDH–wild-type gliomas should be diagnosed as diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV.²⁶ Consequently, TERT promoter mutation in meningioma has recently received considerable attention. In previous reports, the mutation was found in 5.5% to 11% of all analyzed meningiomas, at a higher frequency among tumors with high WHO grades (WHO grade I: 0%-5.3%; WHO grade II: 4.1%-11.4%; WHO grade III: 13.3%-20.0%).²⁷ ²⁹ ³¹ Meningioma patients harboring TERT promoter mutations tend to undergo an aggressive clinical course and have a high recurrence rate with shorter overall survival.²⁹ ³¹

Telomerase reverse transcriptase has been demonstrated to possess RNA-dependent RNA polymerase (RdRP) activity, which is associated with double-strand RNA synthesis, as one of the noncanonical functions of TERT.²² Moreover, TERT-RdRP may maintain stem cell phenotype and control cancer cell proliferation independent of its function as telomerase reverse transcriptase.²³ ³⁴ Recent findings suggest that RdRP activity is directly involved in tumor formation and is, thus, considered a prognostic marker in the liver and pancreatic cancers by clinicopathological analysis of clinical specimens.³⁵ Thus, RdRP activity as a telomere-independent mechanism may serve as an actionable target in cancers.³⁶ Eribulin methyrate (Halaven, Eisai), a nontaxane microtubule inhibitor, has been identified as a specific RdRP inhibitor through pharmacological screening.³⁷ Our recent study demonstrated that eribulin efficiently suppresses the growth of glioblastoma cell lines harboring TERT promoter mutations in vitro and in vivo.³⁸ This work has led to an ongoing multi-center phase II clinical trial investigating the efficacy and safety of eribulin in recurrent glioblastoma (UMIN000030359).

This study investigated the anti–tumor efficacy of eribulin in cell lines derived from malignant meningiomas with TERT promoter mutations in vitro and in vivo. Our study demonstrated that eribulin-induced apoptosis via cell cycle arrest in vitro and prolonged the space (P < .0001). Immunohistochemistry confirmed apoptosis in brain tumor tissue treated with eribulin. Overall, these results suggest that eribulin is a potential therapeutic agent for malignant meningiomas.

KEYWORDS
apoptosis, eribulin, malignant meningioma, microtubule inhibitor, TERT promoter mutation
survival of orthotopic xenograft mouse models. Thus, our findings identified eribulin as a new therapeutic agent for malignant meningioma to prevent calamitous patient outcomes.

2 | MATERIALS AND METHODS

The malignant meningioma cell lines IOMM-Lee and HKBMM (Figure S1) were obtained from the American Type Culture Collection (ATCC; #CRL-3370) and RIKEN cell bank (RIKEN BioResource Research Center; #RCB0680), respectively. The details of the cell culture conditions and gene mutation status are presented in the supporting document (Doc S1). The RdRP activity status of IOMM-Lee and HKBMM cells are illustrated in Figure S2.

The details of the cell viability assay, flow cytometric cell cycle analysis, immunoblotting, tubulin polymerization assay, siRNA-mediated TERT knockdown, real-time PCR, dye exclusion assay, migration assay, animal experiments, immunohistochemistry, immunoprecipitation-RdRP assay, and statistical analysis are provided as supporting information (Doc S1).

3 | RESULTS

3.1 | Eribulin strongly inhibited cell proliferation of malignant meningioma cell lines via cell cycle arrest in vitro

To evaluate the drug sensitivity of malignant meningioma cell lines for eribulin, a cell viability assay was performed on IOMM-Lee and HKBMM cells. The WST assay demonstrated that an extremely low concentration of eribulin suppressed the proliferation of both cell lines in a dose-dependent manner (Figure 1A). The IC50 values were at the nanomolar scale (IOMM-Lee cells: 0.42 nmol/L, HKBMM cells: 3.02 nmol/L).

Based on the previous findings demonstrating that eribulin inhibits mitosis through microtubule inhibition, the cell cycle state under eribulin treatment was evaluated via flow cytometry. A dose-dependent accumulation of G2/M phase cells was observed in both cell lines (Figure 1B). In addition, the changes in cell cycle phase distribution were detected shortly after eribulin exposure (Figure 1C). Thus, eribulin was considered to induce cell cycle arrest promptly with a small amount of eribulin, which prevented cell proliferation in malignant meningioma cell lines.

3.2 | Eribulin suppressed the growth of IOMM-Lee cells in a tubulin inhibition-independent and telomerase inhibition-independent manner

To further investigate the functional consequences of eribulin for malignant meningioma cells, the tubulin expression under eribulin exposure was assayed via immunoblotting, and the role of eribulin as an inhibitor of tubulin polymerization was evaluated. The tubulin expression could be reduced by treating IOMM-Lee cells with 100 nmol/L eribulin but not with 10 nmol/L eribulin (Figure 2A,B). The tubulin polymerization was reduced to a level comparable to that of vincristine when treated with 100 nmol/L eribulin but not with 10 nmol/L eribulin (Figure 2C,D). Subsequently, the effects of TERT inhibition, telomerase inhibition, and eribulin exposure on the suppression of IOMM-Lee cell viability were assayed. Treatment with TERT siRNA decreased the levels of TERT mRNA and the rates of cell proliferation in IOMM-Lee cells (Figure 2E,F). The cell viability of IOMM-Lee cells treated with 6-Thio-2′-deoxyguanosine (6-Thio-dG), which triggers telomere dysfunction, in the presence or absence of eribulin, was then assayed. Consequently, 6-Thio-dG alone exhibited significant suppression of cell growth in IOMM-Lee cells (Figure 2G). Eribulin alone also exhibited strong suppression of cell viability at 1 or 10 nmol/L, the concentration at which no inhibition of tubulin polymerization was observed (see above). Although 6-Thio-dG did not exhibit any additional effects on eribulin-induced growth suppression, adding eribulin to 6-Thio-dG significantly enhanced 6-Thio-dG-induced suppression of cell viability in IOMM-Lee cells (Figure 2G).

3.3 | Eribulin induced apoptotic cell death in malignant meningioma cell lines

A vital dye exclusion assay was used to quantitatively assess eribulin-induced cell death. The results showed the dose- and time-dependent cytotoxic effects of eribulin in IOMM-Lee and HKBMM cells (Figure 3A,B). Although the concentration of eribulin that was cytotoxic to the tumor cells in both cell lines was higher than the IC50 value, it was still considerably low.

To determine whether cell death was caused by apoptosis, the effect of Z-VAD-FMK (pan-caspase inhibitor) pretreatment was assayed. IOMM-Lee and HKBMM cells demonstrated a significant reduction in mortality after exposure to Z-VAD-FMK (Figure 4A). Immunoblotting was performed to observe the behavior of several hallmark proteins involved in apoptosis (Figure 4B). The cells exposed to eribulin exhibited increased levels of cleaved PARP and caspase-3. Pretreatment with Z-VAD-FMK blocked the cleavage of both proteins in malignant meningioma cell lines.

To further understand the molecular mechanism of eribulin-induced apoptosis in malignant meningioma cells, the cell lysates of eribulin-treated IOMM-Lee and HKBMM cells were analyzed through immunoblotting, focusing on the DNA damage response, endoplasmic reticulum stress response (ERSR), and plasma membrane-localizing death receptor-mediated signaling, known as the representative cell death-inducing machinery triggered by anticancer agents (Figure 4C). The enhanced expression of p53, a marker of DNA damage response signaling activation, and upregulation of DR5, a death receptor in the plasma membrane, were confirmed in both IOMM-Lee and HKBMM cells following eribulin treatment. However, the degree of upregulation of these molecules
FIGURE 1  Legend on next page
did not correlate with the eribulin dose. Conversely, the expression of Bip, a marker of ERSR activation, was not detected. Collectively, these results suggest the presence of complex molecular mechanisms of eribulin-induced malignant meningioma cell apoptosis regulated by DNA damage response and death receptors in the plasma membrane.

### 3.4 Suppression of cellular migration following eribulin exposure

As malignant meningiomas typically infiltrate surrounding tissues, our study focused on tumor cell migration behavior. The effect of eribulin on cell migration in malignant meningioma cell lines was evaluated using the IncuCyte ZOOM 96-well scratch wound cell migration assay system. The cells were incubated in the system, and the real-time wound healing process in all wells was monitored and quantified. Eribulin caused a dose-dependent decrease in the rate of wound closure in both IOMM-Lee and HKBMM cell lines (Figure 5A,B). Thus, the results suggest that eribulin suppresses cell migration in malignant meningioma cell lines.

### 3.5 Eribulin administration prolongs the survival of orthotopic malignant meningioma xenograft mouse models

Finally, in vivo studies were conducted to substantiate the anti-tumor efficacy of eribulin in malignant meningioma mouse models. First, IOMM-Lee and HKBMM cells were injected subcutaneously into the left flank of athymic nude mice (5 × 10⁶ cells). Successful engraftment was accomplished with the aggressive proliferation of the tumor xenograft (IOMM-Lee: Figure S3A; HKBMM: not shown). To test the efficacy of eribulin, subcutaneous IOMM-Lee xenografted mice were intraperitoneally injected with saline or three different doses of eribulin (0.125, 0.25, and 0.5 mg/kg) three times a week. Eribulin significantly suppressed subcutaneous tumor growth in a dose-dependent manner (Figure S3A). There was no significant weight loss in mice during eribulin administration; therefore, it can be concluded that eribulin did not exhibit any drug-induced toxicity (Figure S3B). The mice were euthanized 1 h after the final injection of eribulin on day 16, and the subcutaneous tumors were collected and subjected to the RdRP activity assay. A dose-dependent reduction in RdRP activity caused by eribulin was observed in subcutaneous meningioma xenografts (Figure S3C).

Afterward, malignant meningioma cell lines were injected stereotactically 1 mm beneath the dura mater of athymic nude mice (2.5 × 10⁵ cells). Stable orthotopic malignant meningioma xenograft mouse models exhibited phenotypes similar to those of human malignant meningioma (IOMM-Lee: Figure 6A; HKBMM: not shown). The tumor under mouse convexity destructively grew into the frontal bone and protruded outside the skull. In addition, the infiltration of tumor cells into the mouse brain parenchyma was observed in the mouse model.

Overall survival was analyzed among the three groups: the control group (saline injection; n = 8), a two-cycle eribulin administration group (n = 8), and a group continuously administered eribulin (n = 8). Mice received saline or eribulin (0.5 mg/kg) intraperitoneally three times per week, which was defined as one cycle. Eribulin administration significantly improved survival in IOMM-Lee cell-implanted mice, and continuous administration further prolonged survival compared to the short-term administration group (Figure 6B). Although HKBMM-xenografted mice that received eribulin for two cycles survived slightly longer than the control group without statistical significance, long-term eribulin administration significantly prolonged overall survival (Figure 6B).

To further investigate whether the induction of apoptosis was recapitulated in an in vivo model, a histological study was performed using the IOMM-Lee tumor model. In the H&E-stained tissue sections, apoptotic body formation was observed in the tumors from mice exposed to eribulin (Figure 6C). Cleaved PARP1 and cleaved caspase-3, reliable apoptosis markers, were immunohistochemically stained on serial sections adjacent to the H&E-stained sections. The expression of cleaved PARP1 and cleaved caspase-3 was detected in the same region (Figure 6D,E).

### 4 DISCUSSION

Our current study suggests that eribulin may function as an effective anti-cancer drug against malignant meningiomas. Little information is available on the efficacy of several other microtubule-targeted agents, such as paclitaxel, vinblastine, and vincristine, against meningiomas. Tichomirowa et al. described apoptosis induction by paclitaxel in meningioma cells (WHO grade I and II) in vitro. Chamberlain described the outcome of malignant meningioma patients who received combination chemoradiotherapy, including vincristine, cyclophosphamide, and Adriamycin. However, further studies have not been conducted.

Previous studies have demonstrated that the binding site and inhibition mechanism of eribulin in microtubules are distinct from
FIGURE 2  Legend on next page
Eribulin binds only to the extended ends of microtubules and inhibits microtubule polymerization without depolymerization. As eribulin selectively binds to the polymerization site of microtubules with high affinity, it potently affects microtubules by an extremely small amount compared to other agents. Moreover, unlike other anti-microtubule agents, eribulin induces irreversible complete mitotic block at the G2/M phase. Following prolonged mitotic obstruction, the development of mitotic spindles is disrupted, initiating apoptosis. Our in vitro study in malignant meningioma is consistent with the findings of previous studies (Figures 1-5). Generally, most conventional microtubule-targeted agents reversibly bind to tubulin; however, there is no long-term retention in cells. This may partially explain the lack of positive results in microtubule-targeting agents against malignant meningioma. Our findings suggest that eribulin may be effective in treating malignant meningiomas. Notably, our results showed that eribulin suppressed malignant meningioma cell growth at a concentration far below that induced by the inhibition of tubulin polymerization (Figure 2A-D). This suggests that eribulin may have a tubulin inhibition-independent mechanism to suppress
NAKANO et al. demonstrated that the knockdown of TERT by siRNA or the inhibition of telomerase by 6-Thio-dG suppressed the growth of malignant meningioma cells, suggesting that TERT may control cell growth. However, 6-Thio-dG did not enhance eribulin-mediated growth inhibition, suggesting that eribulin may also target telomerase. Eribulin enhanced the 6-Thio-dG-mediated telomerase inhibition and cell growth suppression, suggesting that eribulin exerts a telomerase inhibition-independent growth suppressive mechanism. Although further experimental evidence is required to support this hypothesis, the anti-tumor mechanism of eribulin appears to be highly complex.

Considering that meningioma is generally an extra-axial brain tumor, there is a question of whether malignant meningioma cells were inoculated into the brain parenchyma of model mice in the
current study. However, our model mice demonstrated 100% tumor induction by successfully locating the tumor in the subdural space and recapitulating the histological characteristics in human settings (Figure 6A). Meningioma typically arises from a dura mater and has rich vascularity supplied by conspicuous feeding arteries via dural arteries, which normally provide a dural supply.\(^49\) Moreover, meningiomas lack a blood-brain barrier, suggesting that meningioma is more exposed to eribulin than gliomas.\(^50\) Nonetheless, aggressive malignant meningiomas typically infiltrate surrounding tissues, including the brain parenchyma (Figure 6A). Our previous study revealed that eribulin penetrated and accumulated in the tumor tissue of glioblastoma.\(^38\) Thus, eribulin may reach malignant meningioma tissues even when the tumor cells infiltrate the brain tissue.

Not all underlying mechanisms contributing to the anti-tumor effects were addressed in this study. In fact, IOMM-Lee cells were consistently more susceptible to eribulin than HKBMM cells in current results in vitro and in vivo. These results might be explained by the fact that IOMM-Lee cells proliferate more aggressively than HKBMM cells in vitro (Figure S4). In contrast, the Ki-67 proliferation index was not significantly different between IOMM-Lee and HKBMM cells in vivo (Figure S5). Eribulin induces aberrant mitotic spindle collection in highly proliferative malignant meningioma cells, leading to eribulin-induced apoptosis.\(^51,52\) However, the anti-tumor mechanism has not been fully elucidated in this study, and it remains unclear why IOMM-Lee is more sensitive to eribulin in our study. Another limitation is that we could not elucidate the impact of \(TERT\) promoter mutation among malignant meningioma cells because there were no \(TERT\) promoter wild-type malignant meningioma cell lines available. Previously established malignant meningioma cell lines, IOMM-Lee and HKBMM, and CH157-MN, which we could not obtain, all harbored \(TERT\) promoter mutation (\(-124C > T/C228T\)).\(^53,54\) Establishing an immortal meningioma cell line is challenging, particularly from a \(TERT\) promoter wild-type tumor. Spiegl-Kreinecker et al. reported the results of meningioma primary cell culture from their cohort. Only two cases out of 121 developed into stable cell lines with both harboring \(TERT\) promoter mutations.\(^31\) They also suggested that the aggressive growth in malignant meningioma cells could be attributable to \(TERT\) promoter mutation. This is also reflected in the clinical observation that \(TERT\) promoter-mutated malignant meningioma patients exhibit significantly shorter time to recurrence and shorter overall survival.\(^29-31\) We demonstrated the efficacy of eribulin against exceedingly aggressive malignant meningioma cell lines harboring \(TERT\) promoter mutations. Thus, in conjunction with previous knowledge that eribulin is a possible \(TERT\) inhibitor, eribulin appears to be a promising agent against highly proliferative malignant meningiomas.
FIGURE 6: Legend on next page.
In conclusion, this study revealed the promising anti–tumor effect of eribulin against malignant meningioma cells harboring TERT promoter mutations in vitro and in vivo. Eribulin significantly inhibited cell proliferation and migration. The induction of apoptosis by eribulin was consistent with cell-based assays and animal models, demonstrating a potent survival advantage in orthotopic malignant meningioma xenograft mice. Therefore, eribulin may serve as a potential agent for improving clinical outcomes in patients with notoriously aggressive malignant meningiomas.

ACKNOWLEDGMENTS

We are grateful to Yuko Matsushita and Yoko Hibiya for their valuable technical advice and Yuki Yomoda for her generous secretarial assistance.

DISCLOSURE

The authors have no conflicts of interest to declare for this study.

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43. How to cite this article: Nakano T, Fujimoto K, Tomiyama A, et al. Eribulin prolongs survival in an orthotopic xenograft mouse model of malignant meningioma. *Cancer Sci.* 2022;113:697-708. doi:10.1111/cas.15221