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Loss of *Tsc1* accelerates malignant gliomagenesis when combined with oncogenic signals

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Glioblastomas frequently harbour genetic lesions that stimulate the activity of mammalian target of rapamycin complex 1 (mTORC1). Loss of heterozygosity of *tuberous sclerosis complex 1* (*TSC1*) or *TSC2*, which together form a critical negative regulator of mTORC1, is also seen in glioblastoma; however, it is not known how loss of the TSC complex affects the development of malignant gliomas. Here we investigated the role of Tsc1 in gliomagenesis in mice. *Tsc1* deficiency up-regulated mTORC1 activity and suppressed the proliferation of neural stem/progenitor cells (NSPCs) in a serial neurosphere-forming assay, suggesting that *Tsc1*-deficient NSPCs have defective self-renewal activity. The neurosphere-forming capacity of *Tsc1*-deficient NSPCs was restored by $p16^{Ink4a}p19^{Arf}$ deficiency. Combined *Tsc1* and $p16^{Ink4a}p19^{Arf}$ deficiency in NSPCs did not cause gliomagenesis *in vivo*. However, in a glioma model driven by an active mutant of epidermal growth factor receptor (EGFR), EGFRvIII, loss of *Tsc1* resulted in an earlier onset of glioma development. The mTORC1 hyperactivation by *Tsc1* deletion accelerated malignant phenotypes, including increased tumour mass and enhanced microvascular formation, leading to intracranial haemorrhage. These data demonstrate that, although mTORC1 hyperactivation itself may not be sufficient for gliomagenesis, it is a potent modifier of glioma development when combined with oncogenic signals.

Keywords: gliomagenesis/mTORC1/neural stem/progenitor cells/TSC1.

Abbreviations: AKT, v-Akt murine thymoma viral oncogene; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGF2, fibroblast growth factor 2; GAP, GTPase activating protein; GBM, glioblastoma; LST8, lethal with SEC13 protein 8; mTOR, mammalian target of rapamycin; NF1, neurofibromatosis type 1; NSPCs, neural stem/progenitor cells; PI3K, phosphatidylinositol 3-phosphate; PRAS40, proline-rich Akt substrate of 40 kDa; RHEB, Ras homolog enriched in brain; RTK, receptor tyrosine kinase; TAM, tamoxifen; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.

Glioblastoma (GBM) is the most common high-grade malignant glioma in humans. GBM is categorized as a WHO grade IV astrocytoma, a very aggressive, invasive and destructive brain tumour (*1*). Primary GBM arises *de novo* in the absence of pre-existing low-grade lesions, whereas secondary GBM develops progressively from low-grade astrocytomas, generally over a period of 5–10 years. Alterations in several signalling pathways are associated with gliomagenesis, including the RTK/RAS/PI3K pathway and the p53 and retinoblastoma tumour suppressor pathways (*2*).

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that belongs to the PI3K-related kinase family and participates in two complexes, designated mTOR complex 1 (mTORC1) and 2 (mTORC2). mTOR regulates tumorigenesis by controlling multiple cellular processes, including protein synthesis, glucose metabolism, fatty acid and sterol synthesis, and autophagy (*3*). mTORC1 is a multi-protein complex composed of Raptor, mTOR, LST8 and PRAS40, and it is activated by growth factors and cytokines. A heterodimer of tuberous sclerosis complex (TSC) 2 (also known as tuberin) and TSC1 (also known as hamartin) exhibits GTPase activating protein (GAP) activity towards RHEB, an activator of mTORC1, and therefore, negatively regulates mTORC1. Activation of PI3K via RTKs leads to activation of AKT (also known as protein kinase B). AKT phosphorylates TSC2 and blocks the GAP activity of the TSC complex. The TSC complex can also be inactivated by the RAS-ERK pathway. In GBM, RTK/RAS/PI3K signalling is frequently altered by genetic mutations, including amplification and alteration of the *epidermal growth factor receptor* (*EGFR*) gene or *NF1* deletion (*2*). Thus, malignant gliomas frequently harbour genetic lesions that stimulate the activity of mTORC1.

Mutations in either *TSC1* or *TSC2* cause tuberous sclerosis, a multi-system genetic disorder that is characterized by tumour or hamartoma formation and neurological manifestations mediated by hyperactivation of the mTOR pathway (*4*, *5*). Brain lesions, including cortical tubers, subependymal nodules (SENs) and subependymal giant cell astrocytomas

(SEGAs) have been observed in TSC patients. *Tsc1* deletion in embryonic telencephalic neural stem/progenitor cells (NSPCs) has been reported to cause TSC neuropathological lesions, such as SENs (*6*). Postnatal deletion of *Tsc1* in NSPCs of tamoxifen (TAM)-inducible *Nestin-CreER^{T2}* transgenic mice leads to development of SEGAs, as well as SEN-like structures (*7*). In addition, loss of heterozygosity of *TSC1* or *TSC2* has been found in GBM, although the frequency is relatively rare (*8*). However, whether loss of the TSC complex affects malignant gliomagenesis is not known.

In this study, we investigated the effects of *Tsc1* deletion on NSPCs and gliomagenesis. *Tsc1* deficiency inhibited the self-renewal of NSPCs via activation of tumour suppressors. Although *Tsc1* deficiency was not sufficient for gliomagenesis, it accelerated glioma development and enhanced malignant properties in a glioma model. Thus, increased mTORC1 activity enhances GBM initiation and malignant progression in gliomas.

Materials and Methods

Mice

Rosa26-CreER^{T2} mice were kindly provided by Dr Tyler Jacks (Massachusetts Institute of Technology) (*9*). *p16$^{Ink4a+/-}$p19$^{Arf+/-}$* mice were obtained from the Mouse Models of Human Cancers Consortium of NCI-Frederick (*10*). *Tsc1$^{f/f}$* mice were purchased from Jackson Laboratory. BALB/c nu/nu mice used as recipients were purchased from Sankyo Labo Service. All animal experiments were approved by the Committee on Animal Experimentation of Kanazawa University and performed in compliance with the University's Guidelines for the Care and Use of Laboratory Animals.

Primary NSPC culture

To culture primary NSPCs, subventricular zone (SVZ) regions were harvested from brains of 4-week-old male mice. After mechanical dissociation, cells were cultured in Coaster Ultra-low attachment plates (Corning) in DMEM/F12 (Life Technologies) containing B27 (Life Technologies), 20 ng/ml human FGF2 (Wako), 20 ng/ml human EGF (Sigma) and penicillin/streptomycin (Life Technologies) for 7 days, then treated with 0.1 μM 4-hydroxy TAM (4-OHT, Sigma) for 3 days to activate Cre recombinase, followed by removal of 4-OHT by washing.

Sphere-forming assay

The cultured primary NSPCs were dissociated with Accutase (Life technologies) and filtered through a 40-μm cell strainer (BD Biosciences). The dissociated cells in single-cell suspension (300 cells/200 μl) were cultured in a 96-well Coaster Ultra-low attachment plate in the same medium containing 1% methylcellulose (Wako) for 7 days. These primary spheres that larger than 50 μm in diameter were counted. For the serial sphere-forming assays, the formed spheres were dissociated with Accutase and again cultured for 7 days in the same conditions.

Retrovirus preparation and infection

pLERNL-human *EGFRvIII* was a kind gift from Dr Frank Furnari (University of North Carolina). The digested human *EGFRvIII* segment was subcloned into pGCDNsamIRES*huKO*, which was provided by Dr Masafumi Onodera (National Research Institute for Child Health and Development) (*11*). Cells of the Plat-E packaging line, a gift from Dr Toshio Kitamura (The Institute of Medical Science University of Tokyo), were transfected with this construct, and retrovirus-containing supernatants were concentrated by centrifugation at 6,000 × *g* for 16 h. The cultured primary NSPCs were infected with the retroviruses for 24 h and then cultured until transplantation.

Intracranial inoculation

EGFRvIII-transduced NSPCs were dissociated and re-suspended in 5% FBS/PBS at a concentration of 10^4 cells/μl. The cells were transplanted into anesthetized 4-week-old female Balb/c nu/nu mice as described previously (*12*).

Immunohistological analysis

Mice were deeply anesthetized with pentobarbital and then perfused with 4% paraformaldehyde (PFA). Dissected brain samples were soaked in 4% PFA overnight and embedded in paraffin. The samples were sectioned into 5-μm slices, and antigens were reactivated with target retrieval solution (Dako). The following primary antibodies were used: anti-human EGFR (Thermo Fisher Scientific), pS6(S235/236) (Cell Signaling Technologies), p4E-BP1(T34/46) (Cell Signaling Technologies), Ki-67 (BD Biosciences), VEGF-A (Santa Cruz Biotechnologies) and CD34 (Abcam). Signals were visualized with horseradish peroxidase (HRP)-conjugated secondary antibodies (GE Healthcare). ImPACT 3,3′-diaminobenzidine (DAB) (Vector laboratories) was used for DAB staining of tumour sections, which were counterstained with Mayer's haematoxylin (Wako). For haematoxylin and eosin (H&E) staining, sections were stained with Mayer's H&E solution (Wako). Histological analysis of tumour specimens was performed using BZ-9000 (Keyence) and Axio Imager A1 (Carl Zeiss) microscopes.

Western blotting

Proteins were extracted with lysis buffer (0.1 M Tris [pH 6.7], 4% SDS, phosphatase inhibitor (Thermo Fisher Scientific), complete mini (Roche)) and quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo). Protein (5 μg) was loaded, and the separated proteins were transferred onto 0.45-μm polyvinylidene difluoride (PVDF) membranes (Millipore), which were blocked in 5% (w/v) BSA/0.02% (v/v) Tween 20/PBS and incubated with primary antibodies overnight at 4°C, followed by incubation with HRP-conjugated antibodies (GE Healthcare) and detection with ECL Prime (GE Healthcare).

Administration of rapamycin

Rapamycin (LC Laboratories) was resuspended in 5% (v/v) Tween 80/5% (v/v) PEG400 in PBS, and 20 mg/kg was administered by intraperitoneal injection.

Statistical analysis

Student's *t*-test and log-rank test were performed with Prism6. All *P*-values were two-tailed, and $P < 0.05$ was considered significant. Data are shown as mean ± SD.

Results

Loss of Tsc1 inhibits the sphere-forming capacity of NSPCs

To investigate the role of mTORC1 in the self-renewal of NSPCs, we performed a neurosphere-forming assay using *Tsc1*-deficient NSPCs. We generated *Tsc1$^{f/f}$; Rosa26-CreER^{T2}* (*Tsc1$^{f/f}$CreER*) mice, in which *Tsc1* could be depleted in all tissues by a TAM-inducible Cre-loxP system. We isolated NSPCs derived from the SVZ in the brains of these or control *Tsc1$^{+/+}$ CreER* mice and cultured them in standard media for neurosphere formation, which included EGF and FGF2 without serum. The deletion of *Tsc1* by treatment of NSPCs with 4-OHT did not affect the number or size of primary neurospheres (Fig. 1A, Supplementary Fig. S1A). However, in secondary and tertiary neurosphere assays, *Tsc1*-deficient NSPCs formed fewer spheres than control NSPCs. In the quaternary assay, control cells still had sphere-forming capacity, whereas *Tsc1*-deficient cells did not, indicating that the self-renewal of NSPCs is inhibited by *Tsc1* deletion *in vitro* (Fig. 1A). Because mTORC1

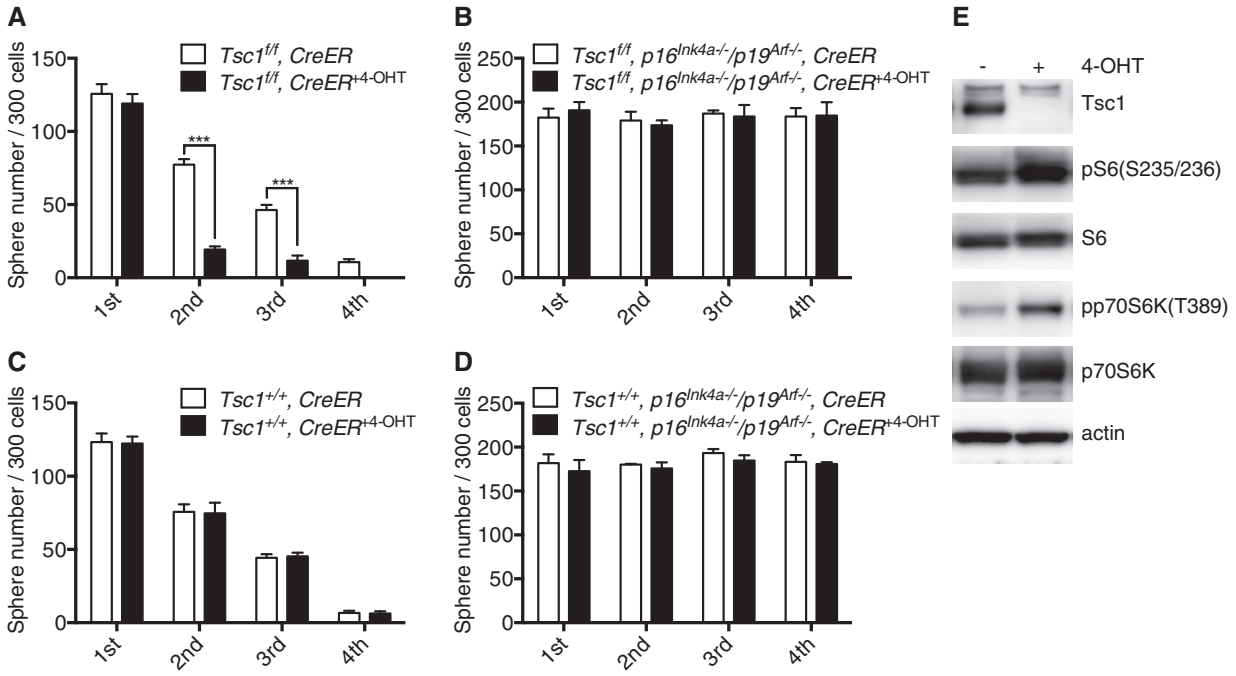

Fig. 1 mTORC1 hyperactivation by *Tsc1* deletion suppresses NSPC sphere formation through p16^{Ink4a}/p19^{Arf}. (A–D) Serial sphere formation assay. Dissociated *Tsc1$^{f/f}$CreER* NSPCs (A) or *Tsc1$^{f/f}$p16$^{Ink4a-/-}$p19$^{Arf-/-}$CreER* NSPCs (B) treated with or without 4-OHT were cultured at low density to evaluate their sphere-forming abilities. *Tsc1$^{+/+}$CreER* NSPCs (C) and *Tsc1$^{+/+}$p16$^{Ink4a-/-}$p19$^{Arf-/-}$CreER* NSPCs (D) were cultured as controls to evaluate the effect of 4-OHT or Cre. Data shown are the mean sphere number ± SD ($n = 3$). ***$P < 0.001$ (Student's *t*-test). *Tsc1*-deficient cells did not form any spheres in the quaternary assay in (A). (E) Phosphorylation of mTORC1 downstream targets in NSPCs. Lysates were prepared from primary culture of *Tsc1$^{f/f}$ p16$^{Ink4a-/-}$p19$^{Arf-/-}$CreER* NSPCs with or without 4-OHT and subjected to western blotting.

hyperactivation is reported to induce senescence by activating tumour suppressors, including p53, p16^{Ink4a} and p19^{Arf}, we further generated *Tsc1$^{f/f}$CreER* mice in a *p16^{Ink4a}p19^{Arf}*-deficient background. As previously reported (*13*), loss of *p16^{Ink4a}p19^{Arf}* increased sphere formation compared with wild type and reversed the inhibitory effect of *Tsc1* deficiency on NSPCs (Fig. 1B, Supplementary Fig. S1B). The 4-OHT did not affect sphere formation of the corresponding control *Tsc1$^{+/+}$CreER* cells (Fig. 1C and D, Supplementary Fig. S1C and D). Depletion of Tsc1 protein increased phosphorylation of S6 and p70S6K, indicating mTORC1 activation in *Tsc1*-deficient NSPCs (Fig. 1E). These data suggest that mTORC1 hyperactivation inhibits the self-renewal capacity of NSPCs by activating p16^{Ink4a}p19^{Arf}.

Loss of Tsc1 is not sufficient for gliomagenesis

Because *p16^{Ink4a}p19^{Arf}* deficiency reversed the inhibitory effect of mTORC1 activation on NSPC proliferation, we hypothesized that *Tsc1* deletion in combination with *p16^{Ink4a}p19^{Arf}* deficiency induces gliomagenesis. Therefore, we inoculated *Tsc1$^{f/f}$CreER* neurosphere cells (*p16^{Ink4a}p19^{Arf}*-deficient background) into the basal ganglia of immunocompromised mice, followed by administration of TAM to induce *Tsc1* deletion. However, no mice inoculated with control or *Tsc1*-deficient neurospheres showed symptoms of glioma development within 5 months (Fig. 2A). Like *Tsc1* deficiency, the constitutively active EGFR mutant (EGFRvIII) increased mTORC1 activity in neurospheres (Fig. 2B). Most recipients inoculated with *p16^{Ink4a}p19^{Arf}*-deficient neurosphere cells transduced with *EGFRvIII* gene developed gliomas, as reported previously (*14*). Therefore, the loss of *Tsc1* by itself may not be sufficient for gliomagenesis even in a *p16^{Ink4a}p19^{Arf}*-deficient background in this experimental setting.

Loss of Tsc1 accelerates glioma development

Next we examined the effects of *Tsc1* deletion on tumour development using the EGFRvIII-driven glioma model. *Tsc1$^{f/f}$CreER* (*p16$^{Ink4a-/-}$p19$^{Arf-/-}$* background) neurosphere cells were infected with a retrovirus carrying *EGFRvIII* and used to inoculate mouse brains. We then administered TAM to the mice to delete *Tsc1* only in glioma cells *in vivo*. Mice injected with vehicle (corn oil) developed gliomas as early as 5 weeks after transplantation (Fig. 3A). However, mice injected with *Tsc1*-deficient glioma cells began to develop tumours from 4 weeks after transplantation, and all died within 5 weeks (Fig. 3A). Genomic analysis revealed that the *Tsc1* allele was completely deleted in glioma tissues *in vivo* (Fig. 3B). Because TAM administration did not affect the survival of control *Tsc1$^{+/+}$CreER* gliomas (Supplementary Fig. S2), the earlier onset was caused by *Tsc1* deletion, not by Cre recombinase or TAM itself. Anti-human EGFR immunostaining showed that tumour mass increased remarkably in the absence of *Tsc1* (Fig. 3C). *Tsc1*-deficient gliomas showed higher expression of pS6, p4E-BP1 and Ki-67 than controls (Fig. 3D), suggesting that mTORC1 hyperactivation caused the earlier death of recipient mice.

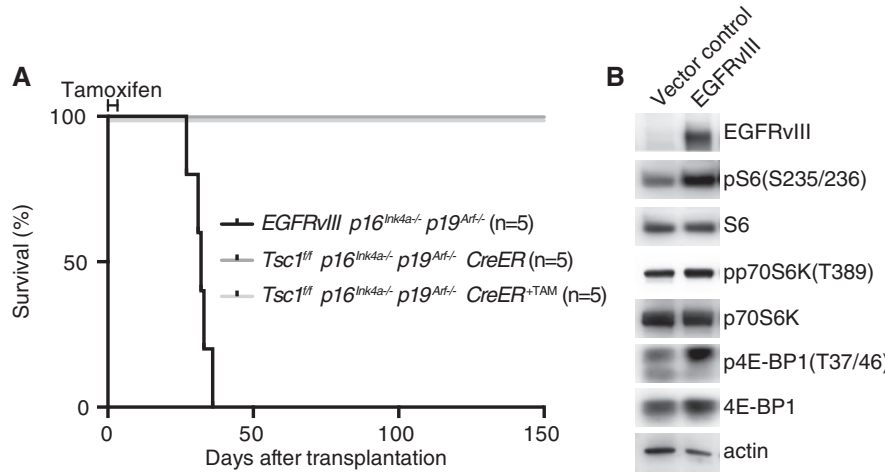

Fig. 2 mTORC1 hyperacvation is not sufficient for gliomagenesis. (A) Effects of *Tsc1* deficiency or EGFRvIII overexpression on survival of mice inoculated with $p16^{Ink4a}/p19^{Arf}$-deficient NSPCs. Mice were intracranially inoculated with *Tsc1*-deficient NSPCs isolated from $Tsc1^{f/f}$ $p16^{Ink4a-/-}p19^{Arf-/-}CreER$ mice and then given TAM. For EGFRvIII overexpression, $p16^{Ink4a-/-}p19^{Arf-/-}$ NSPCs were infected with retrovirus carrying *EGFRvIII* intracranially inoculated into immunocompromised mice. (B) Lysates were prepared from primary cultures of EGFRvIII-transduced $p16^{Ink4a-/-}p19^{Arf-/-}$ NSPCs and subjected to western blotting with antibodies against the indicated proteins.

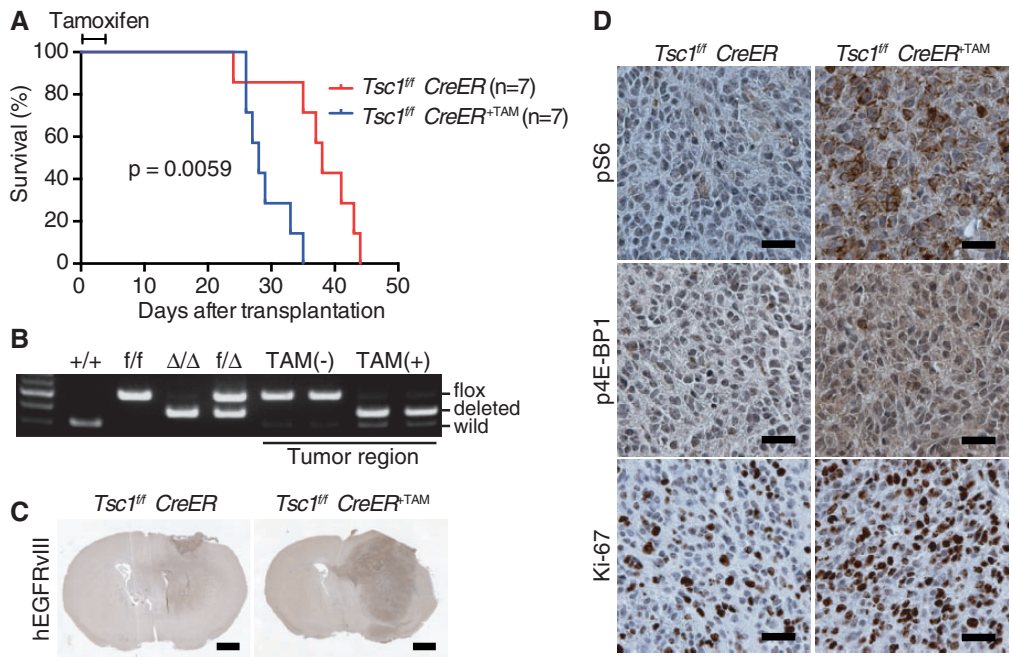

Fig. 3 Acceleration of EGFRvIII-driven glioma development by *Tsc1*-deficiency. (A) Effect of *Tsc1* deletion on survival of mice bearing EGFRvIII-driven gliomas. The mice were intracranially inoculated *with* EGFRvIII-transduced $Tsc1^{f/f}$ $p16^{Ink4a-/-}p19^{Arf-/-}CreER$ NSPCs and then administered TAM. Kaplan–Meier analysis and the log-rank test were used to assess the effect of *Tsc1* deletion on tumour latency. (B) Deletion efficiency of *Tsc1* by TAM in glioma tissues. Genomic DNA was isolated from glioma tissues and evaluated by PCR analysis. Plus symbol, f, and triangle symbol indicate wild-type, floxed, and deleted allele of *Tsc1*, respectively. (C–D) Immunohistological analysis of control and *Tsc1*-deficient (+TAM) gliomas. Tissue sections of gliomas were stained with antibodies against the indicated molecules. Scale bars = 1 mm (C), 50 μm (D).

mTORC1 hyperactivation causes malignant phenotypes in Tsc1-deficient gliomas

Gliomas lacking *Tsc1* showed obvious hemorrhagic regions (Fig. 4A and B). Because robust angiogenesis is an important histological hallmark of GBM, we used a CD34 antibody to examine microvasculature formation. *Tsc1* deficiency increased the number of $CD34^+$ cells with a microvasculature appearance (Fig. 4C) and the expression of VEGF (Fig. 4D), suggesting that up-regulation of VEGF promoted microvasculature formation in the gliomas. Thus, by enhancing the microvasculature, mTORC1 hyperactivation may promote cell proliferation and accelerate glioma development in combination with oncogenic signals *in vivo*.

To investigate the link between mTORC1 hyperactivation and the malignant phenotypes of $Tsc1^{f/f}CreER^{+TAM}$ gliomas, we administered rapamycin, an allosteric mTORC1 inhibitor, to mice with $Tsc1^{f/f}CreER$ and $Tsc1^{f/f}CreER^{+TAM}$ gliomas. Rapamycin significantly prolonged survival in mice with both types of glioma, with no significant difference in survival between the groups (Fig. 5A). Rapamycin

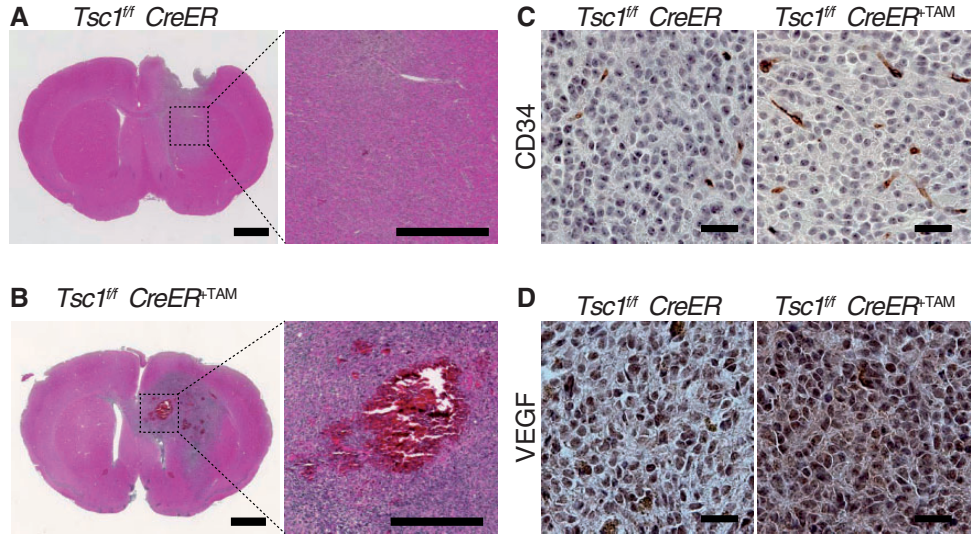

Fig. 4 Enhancement of intratumour haemorrhage through increased microvascular formation due to *Tsc1* deficiency. (A, B) H&E staining of control (A) and *Tsc1*-deficient (B) gliomas. Scale bar = 1 mm (left), 0.5 mm (inset). (C, D) Immunohistological analysis of CD34 (C) or VEGF (D) in control and *Tsc1*-deficient gliomas. The sections were counterstained with haematoxylin. Scale bars = 50 μm.

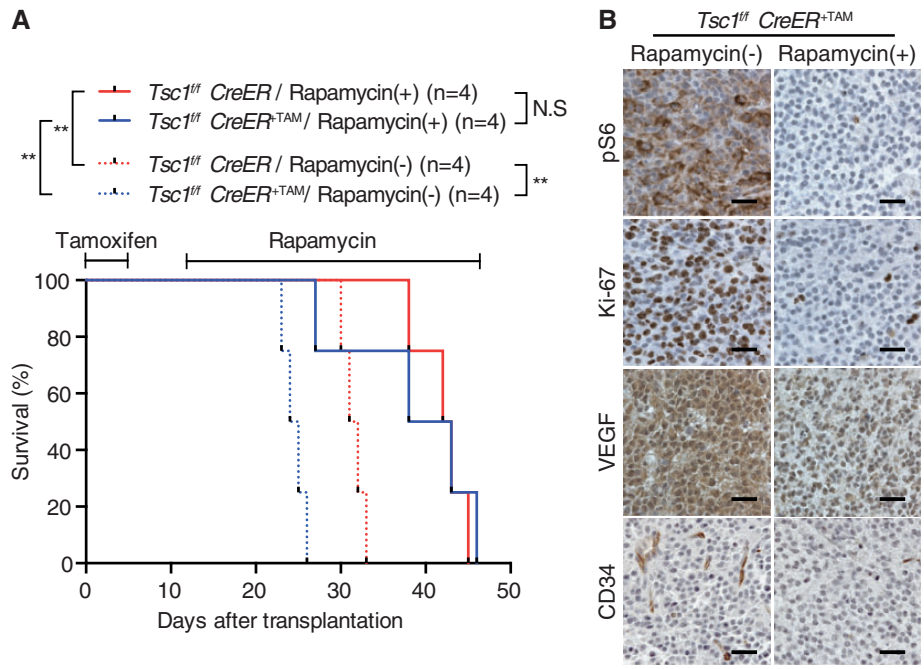

Fig. 5 Reversal of malignant phenotypes in *Tsc1*-deficient gliomas by rapamycin. (A) Effects of rapamycin on the latency of $Tsc1^{f/f}CreER$ and $Tsc1^{f/f}CreER^{+TAM}$ gliomas. Mice were treated with rapamycin (20 mg/kg) or vehicle from Day 12 until death. Cre activity was induced by TAM injection. $^{**}P < 0.01$ (log-rank test). (B) Representative immunohistochemistry of tumour regions from $Tsc1^{f/f}CreER^{+TAM}$ gliomas. Sections were stained for pS6, Ki-67, VEGF, and CD34 (brown) and counterstained with haematoxylin (blue). Scale bars = 50 μm.

treatment decreased S6 phosphorylation and expression of Ki-67 and dramatically diminished VEGF expression and the $CD34^+$ microvasculature in *Tsc1*-deficient gliomas (Fig. 5B). These results demonstrate that mTORC1 hyperactivation due to *Tsc1* deficiency accelerates gliomagenesis through enhanced microvasculature formation.

Discussion

One of the scientific questions about malignant gliomas is their cell of origin. Experiments with genetic engineering techniques using mouse models have clearly shown that gliomas can be derived from fully differentiated astrocytes and neurons, as well as from neural stem cells and oligodendro progenitor cells (*14–16*). On the other hand, glioma cells exhibit stem-like features and have properties in common with NSPCs. Therefore, it is worth investigating the roles of signalling molecules in the self-renewal of normal NSPCs and gliomagenesis.

Although one previous study reported that *Tsc1* deletion did not affect NSPC proliferation (*7*), in another study *Tsc1* deletion resulted in transient expansion of

NSPCs followed by premature differentiation and reduced self-renewal (*6*). Furthermore, a recent study reported that mTORC1 activation by RHEB overexpression promoted the differentiation of neural stem cells to transient-amplifying cells at the expense of their self-renewal (*17*). Although these inconsistent results may be due to differences in assay methodology, it remains unclear how mTORC1 activity regulates the behavior of normal NSPCs. Our data suggest that mTORC1 activates the $p16^{Ink4a}$ and $p19^{Arf}$ tumour suppressors in NSPCs, causing defects in self-renewal. Because the defective phenotypes of *Tsc1*-deficient NSPCs affected secondary or tertiary, but not primary, sphere formation, loss of *Tsc1* may cause serious damages to the self-renewal capacity of neural stem cells, but not to the proliferation of progenitors.

In GBM, mutations in genes encoding molecules upstream of mTORC1 are presumed to induce its hyperactivation (*2*). Glioma patients with high mTORC1 activity show poor prognosis (*18*, *19*). However, mTOR inhibitors (rapamycin and its analogs) have failed to show significant beneficial effects in patients with GBM (*20*, *21*). Therefore, it has not been clear whether mTORC1 activation is a bona fide driver of gliomagenesis. Although Pten deficiency or Ras activation, which induce mTORC1 activation, cause glioma development in mouse models (*22*, *23*), these signals also stimulate signal transduction pathways other than mTORC1. In this study, we demonstrated that, although mTORC1 hyperactivation accelerates glioma formation and enhances the malignant phenotype in a glioma model, it is not sufficient for gliomagenesis. In this experimental setting, the immunocompromised-recipient mice occasionally died 6 months after transplantation for reasons not associated with gliomas (presumably infections). Therefore, it is possible that the mice would develop gliomas at later time points, but this would likely require additional aberrant signals caused by gene mutations that occur with long latency. Thus, mTORC1 hyperactivation is a potent modifier, but not an initiator, of malignant gliomas.

Aberrant vasculature associated with enhanced angiogenesis is one of the histological hallmarks of GBM, because a sufficient supply of nutrients and oxygen is required for rapidly growing tumours (*24*). Rapamycin inhibits angiogenesis and VEGF production in a mouse metastatic colon cancer model (*25*), and *Tsc2* deficiency induces VEGF production in fibroblasts (*26*), suggesting that hyperactivation of mTORC1 causes VEGF production in tumour cells, resulting in enhanced angiogenesis. Consistent with these data, we also found that mTORC1 hyperactivation promoted microvasculature formation, stimulating glioma proliferation. Thus, mTORC1 activation may support glioma development not only in a cell-autonomous manner but also by affecting the surrounding environment.

Although mTORC1 inhibition by rapamycin significantly suppressed gliomas, all mice in our study eventually died of glioma development. One reason is that the presence of rapamycin-insensitive mTOR substrates limits the ability of rapalogs to completely inhibit mTORC1 targets (*27*, *28*). ATP-competitive mTOR inhibitors have been developed and reportedly show more cytotoxic effects, and recently NVP-BEZ235, a PI3K/mTOR inhibitor, was found to be effective in a xenograft glioma model (*29*). However, it is still unclear whether complete inhibition of mTORC1 activity leads to eradication of glioma cells *in vivo*, because we have recently reported that leukemia stem cells can survive and proliferate in an mTORC1-independent manner (*30*). A detailed analysis of gliomas by using a genetic approach to inhibiting mTORC1 and mTORC2 would contribute to the identification of novel targets for GBM therapy.

Supplementary Data

Supplementary Data are available at *JB* Online.

Acknowledgements

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Conflict of interest

None declared.

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