Myc inhibition is effective against glioma and reveals a role for Myc in proficient mitosis

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Gliomas are the most common primary tumours affecting the adult central nervous system and respond poorly to standard therapy. Myc is causally implicated in most human tumours and the majority of glioblastomas have elevated Myc levels. Using the Myc dominant negative Ommyc, we previously showed that Myc inhibition is a promising strategy for cancer therapy. Here, we preclinically validate Myc inhibition as a therapeutic strategy in mouse and human glioma, using a mouse model of spontaneous multifocal invasive astrocytoma and its derived neuroprogenitors, human glioblastoma cell lines, and patient-derived tumours both in vitro and in orthotopic xenografts. Across all these experimental models we find that Myc inhibition reduces proliferation, increases apoptosis and remarkably, elicits the formation of multinucleated cells that then arrest or die by mitotic catastrophe, revealing a new role for Myc in the proficient division of glioma cells.
gliomas are the most frequent primary tumours affecting the adult human central nervous system. The World Health Organization defines four brain tumour classes (I, II, III, IV) on the basis of their morphological features and predicted clinical behaviour. The most lethal is grade IV glioblastoma (GBM), with a median survival of only 15 months. However, all grades respond poorly to conventional therapy. Gliomas are presumed to arise from mature glia or neural stem cells and diffusely infiltrate the surrounding tissue, making surgical resection very difficult. Gliomas may be astrocytic, oligodendritic or of mixed lineage and are all characterized by refractoriness to apoptosis and marked genomic instability, both of which are thought to contribute to their poor response to chemo- and radiotherapy. Recurring alterations observed in gliomas include amplification and activating mutations of EGFR, precocious receptor tyrosine kinase signalling, deletion of NF1 and elevated levels of p21 signalling (reviewed in ref. 3). In particular, signalling through the Ras network is essential for both proliferation and angiogenesis of human malignant astrocytomas. Myc is a bHLHZip transcription factor, causally implicated in most human cancers. Myc controls diverse cellular functions, including cell growth and proliferation, differentiation and programmed cell death. Its expression in normal cells is tightly regulated by mitogen availability but this control is compromised in tumour cells, either as a consequence of constitutive upstream oncogenic signals or direct mutation in the myc genes themselves. Myc expression correlates with glioma grade and some 60–80% of GBM exhibit elevated Myc levels. In addition, transgenic Myc expression in the astrocytic lineage of mice is sufficient to cause gliomas resembling the human disease. Increased c-Myc activity downstream of p53 and Pten mutations is also causally associated with impaired neuronal differentiation and enhanced self-renewal capacity of GBM tumour-initiating cells. Consistently, Myc knockdown in Pten−/−/p53−/− double null neurospheres reduces their tumorigenic potential. Others have proposed a central role for Myc also in the progression of gliomas driven by various different mutations suggesting that Myc inhibition could be effective in multiple types of glioma.

To assess the therapeutic potential of Myc inhibition in vivo we previously employed a dominant negative mutant of the Myc dimerization domain, termed Omomyc. Omomyc has an altered dimerization specificity, allowing it to sequester Myc away from its obligate partner Max and so inhibiting Myc-dependent dimerization specificity, allowing it to sequester Myc away from the dimerization domain, termed Omomyc. Omomyc has an altered previsely employed a dominant negative mutant of the Myc kinase signalling, deletion of NF1 and elevated levels of p21

Results

Myc inhibition increases survival of GFAP-V12Ha-Ras mice.

To determine the therapeutic impact of systemic Myc inhibition in both progression and maintenance of glioma, we combined our switchable TRE-Omomyc;CMVrtTA mouse with the well-characterized GFAP-V12Ha-Ras (ref. 19), a spontaneous mouse model of multifocal invasive astrocytoma in which the activated form of Ha-Ras is driven by the glial fibrillary acidic protein promoter (GFAP; Fig. 1a). The molecular and pathological progression of disease in GFAP-V12Ha-Ras mice resembles that of diffuse astrocytomas in humans, exhibiting reproducible kinetics of tumour progression, from astrogial hyperplasia (starting from 1–3 weeks of age) through to low- and high-grade gliomas.

Figure 1 | Myc inhibition confers a survival advantage in GFAP-V12Ha-Ras mice. (a) To obtain a mouse model of spontaneous glioma in which Omomyc expression could be activated upon doxycycline treatment, TRE-Omomyc;CMVrtTA mice were crossed with GFAP-V12Ha-Ras mice, giving the triple transgenic TRE-Omomyc;CMVrtTA;GFAP-V12Ha-Ras. (b) Kaplan–Meier curve showing that Omomyc expression confers a significant survival advantage to V12Ha-Ras expressing mice. Eight-week-old mice, untreated or treated with Omomyc, were monitored for symptom-free survival up to 57 weeks of age. (c) Representative pictures of GFAP immunostaining from the brains of mice included in the survival curve above. The panels on the right are higher magnification images of regions indicated by the black boxes. Control mice present dense GFAP-positive cells compared with Omomyc-treated mice.
Omomyc was induced in TRE-Omomyc;CMVrtTA;GFAP-V12Ha-Ras mice from postnatal week 8 with doxycycline and the animals then monitored to determine symptom-free survival. Strikingly, at 57 weeks of age, all Omomyc-expressing mice (8/8) were asymptomatic with no evidence of disease progression (Fig. 1b,c). By contrast, at the same time point only 25% of the untreated control GFAP-V12Ha-Ras mice were still alive, the majority having been euthanized by this time point due to the
appearance of progressive ataxia and neurological symptoms, associated with astrocytic hyperplasia and increased intracranial pressure (Fig. 1c).

Of note, mice subjected continuously to Myc inhibition showed no sign of any distress or discomfort, confirming the previously reported well-tolerated and mild side effects elicited by Myc inhibition in normal tissue maintenance and homeostasis.6

Myc inhibition limits growth of transformed neuroprogenitors

To understand how Myc inhibition prevents glioma formation, we first investigated the cellular compartment previously identified in different glioma models as the putative cell of origin of the tumour: the neuroprogenitor (NPG) cells.21,22 Recent reports demonstrate that glioma cells share features with neural stem and progenitor cells, including a high proliferation rate, and self-renewal and migration abilities.3,23,24 As Myc has a demonstrated role in normal and neoplastic stem cell biology, we assessed the impact of Omomyc expression in primary NPG cells derived from our TRE-Omomyc;CMVrtTA;GFAP-V12Ha-Ras mouse.

Myc inhibition impairs NPG self-renewal capacity

To investigate whether Myc inhibition impairs these characteristics, we employed a neurosphere self-renewal assay, measuring the number of cells able to reform a secondary neurosphere from a single cell. In this assay, NPGs are seeded at clonal density (500 single cells per well), then cultured with or without doxycycline for 21 days, at which point the number of spheres in each well is determined. Notably, Omomyc expression impairs the self-renewal ability of both the Ras-transformed and the control cells (Fig. 2g and Supplementary Fig. 1b), consistent with the crucial role ascribed to Myc proteins in promoting and maintaining self-renewal and stemness of both neoplastic and normal progenitor cells.25,26

Myc inhibition reverses the symptoms of GFAP-V12Ha-Ras mice

To test the therapeutic utility of Myc inhibition in advanced gliomas, we evaluated the impact of Omomyc expression in TRE-Omomyc;CMVrtTA;GFAP-V12Ha-Ras mouse presenting already clear neurological symptoms. Due to the variable tumour latency of our colony (see Fig. 1b, untreated–Omomyc animals), such symptoms appeared stochastically: the animals were initially hyperactive and often aggressive, but eventually became almost catatonic and unresponsive to external stimuli. At this point, they were treated with Omomyc. After 7 days of Myc inhibition, the therapeutic effect was dramatic. As documented by the movie...
(Supplementary Movie 1), mice were typically lethargic at the commencement of treatment, failed to groom and lacked appetite. After 7 days of Myc inhibition however, treated mice were active, responded again to external stimuli and were able to move, eat, drink and groom (Supplementary Movie 2).

Histological analysis of treated versus untreated samples clearly showed that untreated symptomatic mice exhibited regions with high density, GFAP-positive, pleomorphic and infiltrative astrocytes, typical histopathological features of human malignant astrocytomas (Fig. 3, middle versus upper panels). By contrast, the improved health of treated animals was associated with markedly reduced astrocytic (GFAP positive) cell density (Fig. 3, lower panels). Further immunohistochemical analysis of the residual GFAP-positive regions in Myc-inhibited symptomatic mice revealed a significant decrease in the number of proliferating cells compared with untreated mice (3.63% versus 7.83% positivity for the proliferation marker Ki67; Fig. 4a,b). In addition, we noted a small but significant increase in the number of apoptotic (TUNEL-positive) cells in Omomyc-expressing mouse brains when compared with Omomyc-negative controls (1.74% versus 0.48%; Fig. 4a,c), indicating that cell death contributes—at least to some degree—to the reduced cellular density observed upon Myc inhibition. A notable reduction in total cell number after Omomyc expression was confirmed by determining the number of nuclei present in five whole macroscopic fields (see total number of nuclei in treated versus untreated animals in Fig. 4b,c).

Intriguingly, we observed that in the GFAP-positive regions remaining after Omomyc treatment, there was a significant increase in multinucleated GFAP-positive cells compared with untreated mice (3.63% versus 7.83% positivity for the proliferation marker Ki67; Fig. 4a,b). In addition, we noted a small but significant increase in the number of apoptotic (TUNEL-positive) cells in Omomyc-expressing mouse brains when compared with Omomyc-negative controls (1.74% versus 0.48%; Fig. 4a,c), indicating that cell death contributes—at least to some degree—to the reduced cellular density observed upon Myc inhibition. A notable reduction in total cell number after Omomyc expression was confirmed by determining the number of nuclei present in five whole macroscopic fields (see total number of nuclei in treated versus untreated animals in Fig. 4b,c).

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Myc inhibition triggers mitotic crisis in human GBM cells. To ascertain whether the antitumorogenic effect of Myc inhibition in the mouse model might translate into an analogous therapeutic impact in a human system, we investigated the effect of Omomyc expression in cell lines derived from human grade IV gliomas. U87MG cells were infected with retroviruses directing the expression of either GFP (control) or a GFP-Omomyc fusion protein. Consistent with our in vivo results in the GFAP-V12 Ha-Ras mouse model, the total U87MG cell number was reduced on Omomyc induction, whereas GFP expression alone had no such effect (Fig. 5a). In addition, we observed a significant increase in cell death in U87MG cells expressing Omomyc, compared with control cells (Fig. 5b). Using a doxycycline-switchable lentiviral vector to drive expression of Omomyc, we also observed an increase in the proportion of flattened, senescent-like cells that stained positively for β-galactosidase (Fig. 5c). Broadly similar observations were made in Omomyc-expressing U373MG cells, which notably are mutant for p53 (Supplementary Fig. 2), although the cell flattening seen in U87MG cultures after Omomyc expression was not recapitulated (Supplementary Fig. 2c).

Cytological examination of fixed Omomyc-expressing U87MG cells stained for tubulin and DNA showed that Omomyc expression causes a reduction in the mitotic index (0.96% versus 1.86% in control cells), but does not affect mitotic progression (the prophase + metaphase/anaphase + telophase ratio was 0.317 in Omomyc-expressing cells and 0.326 in GFP-expressing cells). A fraction of the dividing cells in Omomyc-expressing cultures (14.7%) displayed mitotic defects including multipolar spindles, aberrant chromosome segregation, chromatin bridges in ana-telophase and an irregular central spindle/midbody (Fig. 6a (control cells) versus Fig. 6b (Omomyc-expressing cells)). The final outcome of these defective mitoses was the formation of multinucleated and micronuclei-containing cells (13.6% after 72 h Omomyc expression; Fig. 6c,d, and Supplementary Fig. 3a). Similar results were obtained in U373MG cells with a doxycycline-switchable RFP-Omomyc-expressing vector (Supplementary Fig. 3b and c), where time-lapse imaging over the course of 72 h revealed that multinucleated cells enter mitosis but are unable to complete the mitotic process and may eventually die (Supplementary Movie 3).

Myc inhibition alters SAE1 expression and PTP1B phosphorylation. Loss of SUMO activating enzyme (SAE) expression was recently found to exhibit synthetic lethality together with Myc overexpression. One intriguing phenotypic outcome of SAE knockdown in Myc overexpressing tumour cells was the appearance of mitotic abnormalities and induction of mitotic catastrophe. Interestingly, SAE1 is a direct target of OMOMYC.
Myc\textsuperscript{29}. To verify whether SAE1 could be altered in response to Omomyc expression, we performed western blot analysis on GBM cell lines. We observed a clear reduction of SAE1 expression in U87MG (Fig. 7a). Hence, reduced SAE activity could be responsible for the aberrant nuclei and mitotic catastrophe observed in this cell line.

On the other hand, in U373MG cells we observed no downregulation of SAE1 (Fig. 7a). Instead, phosphorylation of...
Myc inhibition limits growth of patient-derived tumours. To determine whether the antitumorigenic capacity of Myc inhibition extends to primary human tumour samples and, consequently, might be clinically applicable in human glioma, we used a lentiviral vector to drive doxycycline-dependent Omomyc expression in patient-derived GBM neurosphere cultures derived from a surgically resected human GBM (Supplementary Fig. 4a). In culture, Myc inhibition for 2 weeks reduced the overall number of neurospheres (Fig. 8a) and inhibited self-renewal of spheres after their dissociation (Fig. 8b). No effect of doxycycline treatment was observed in neurospheres lacking the Omomyc lentiviral vector (Supplementary Fig. 4b).

To investigate whether this potentially therapeutic effect might translate into an objective therapeutic impact, disaggregated neurospheres were orthotopically inoculated into the brains of NOD/SCID mice. Animals transplanted with neurospheres harbouring the Omomyc expression cassette were treated continuously with either doxycycline from 3 weeks after transplant (so that Omomyc was expressed throughout tumour development) or with sucrose in control mice. As expected, Myc inhibition significantly potentiated overall survival (Fig. 8c). Importantly, once again, we observed the appearance of aberrant nuclei in nestin-positive cells after Omomyc treatment (Fig. 8d,e).

Discussion

We previously demonstrated in preclinical mouse models that systemic Myc inhibition is a promising strategy for eradicating lung cancer16,17 and insulinoma18, inducing profound regression of tumours, eliciting only very mild side effects in normal tissues and circumventing the problems with resistance encountered in other targeted therapies. However, whether or not these conclusions were applicable to other types of cancer and to human cancers remained to be established. Here, we extend our previous studies by showing that Myc inhibition has a similar effective therapeutic potential for treating glioma, a disease with an especially dismal prognosis and in urgent need of new therapies. To do this, we made use of several experimental models, encompassing both mouse and human systems, including a Ras-driven mouse model of astrocytoma and NPG cells derived from it, human GBM cell lines and, importantly, a patient-derived neurosphere xenograft model. All these studies, both in vitro and in vivo, indicate that Myc inhibition suppresses glioma formation, inhibits glioma cell proliferation and survival and triggers regression of established disease. Unexpectedly, we also noted that Myc inhibition elicits profound aberrations in glioma cell mitosis, with the appearance of multinucleated cells, micronucleation and also multipolar and disorganised spindles. Time-lapse microscopy confirmed that such aberrant mitotic cells are unable to complete cell division and many subsequently die.

Although the suppression of Myc might be expected to induce replicative arrest—given its intimate role in coordinating cell proliferation—the mechanisms underlying glioma cell mitotic dysfunction and death are particularly intriguing. Interestingly, SUMO pathway components have been shown to be required for cell division and implicated in the control of mitotic chromosome structure, cell cycle progression, kinetochore function and cytokinesis31. Here we show that SAE1, a direct target of Myc29, is downregulated in response to Omomyc expression in U87MG cells (Fig. 7a), suggesting that a reduction in SAE activity is responsible for the aberrant nuclei and mitotic catastrophe observed in this cell line. In contrast, U373MG cells display no such downregulation of SAE1 levels but Myc inhibition is instead associated with the phosphorylation of PTP1B, a modification described to trigger cell death during mitotic catastrophe. The phosphatase PTP1B has been recently shown to exert antitumour effects in GBM32, however, it displays tumour-promoting effects in other cell types; opposing functions may be controlled by posttranslational modifications and substrate specificity. For example, PTP1B is negatively regulated by sumoylation upon entry into mitosis33, whereas its phosphorylation by Cdk1 and Plk1 during mitotic arrest promotes its activation and tumour suppressing activity30. Details of how these opposing modifications interact to determine cell fate remain unknown.

In this study we provide evidence to support the hypothesis that Myc inhibition blocks protein sumoylation (via decreased SAE1 expression) leading to defective mitosis, and ultimately the induction of mitotic catastrophe. Failure to satisfy the spindle assembly checkpoint (SAC) results in protracted M-phase arrest and sustained activation of mitotic kinases, leading to death during mitosis. In addition, enhanced PTP1B activity as a result
of Omomyc-dependent loss of PTP1B sumoylation and consequent gain in its phosphorylation may also directly contribute to mitotic cell death.

Our results demonstrate that Myc holds a central role in proficient mitotic programs, the precise molecular mechanism of which could vary in different cell types depending on the status of other cell cycle or genome integrity regulators, such as p53. It is not surprising that the pleiotropic nature of Myc is reflected in the degeneracy of its effectors, some of which control programs necessary for efficient cell division.

Whether or not the effects of Myc inhibition on mitotic aberrations are peculiar to GBM cells or also hold in other types of cancer remains to be established. The mode of action of a Myc inhibitor—in our case Omomyc—could vary with the context-specific role of Myc, whose function ramifies throughout all aspects of tumorigenesis both at the intracellular and the extracellular levels. Our data demonstrate that systemic Myc inhibition appears to be a very promising strategy for treating glioma, seemingly irrespective of its driving oncogenic lesions. While direct inhibition of Myc is currently not a pharmacological option, it may in the future prove possible to inhibit Myc function, either directly as recently shown using peptides or indirectly, as demonstrated for bromodomain inhibitors.

**Methods**

**Study design.** All the animal studies were performed in accordance with ARRIVE guidelines and following the three R’s rule of Replacement, Reduction and Refinement principles.

Mice were housed and treated in accordance with protocols approved by the Institutional Animal Care and Use Committee at the UCSF (University of California, San Francisco) and by the CEEA (Ethical Committee for the Use of Experimental Animals) at the VHIO (Vall d’Hebron Institute of Oncology), Barcelona.

The anonymized human sample employed was part of the tissue biological material stored in the tumour bank (TB) at the Hospital Vall d’Hebron. The sample had been previously collected with a signed patient consent form and its use had been approved by the Ethics Committee of the Hospital. The sample was a random one among the GBM patient samples available.

**Generation and maintenance of mice.** TRE-Omomyc;CMVrtTA mice were previously described. GFAP-V12Ha-Ras (TgGFAP-Hras1A12gus) mice were generated and kindly provided by Dr Abhijit Guha (Toronto Hospital Western Division-UHN) and Dr David H. Gutmann (WUSM) laboratories. The resulting triple transgenic TRE-Omomyc:CMVrtTA;GFAP-V12Ha-Ras mice were of a mixed CD1;FVB/N;C57BL/6 background. Doxycycline was dissolved in drinking water (2 mg ml−1 with 5% sucrose) and administered to the animals. Control animals received 5% sucrose.

**Tissue preparation and histology.** Mouse brains were removed and either frozen in OCT or fixed overnight in Zinc-buffered formalin and processed for paraffin embedding. For histological analysis, tissue sections (5 μm) were stained with haematoxylin and eosin.

**Immunohistochemistry.** For immunohistochemical analysis, tissue sections were de-paraffinized, rehydrated and subjected to high temperature antigen retrieval in 10 mM citrate buffer (pH 6.0). The following primary antibodies were used: rabbit monoclonal anti-Ki67 (clone SP6, Lab Vision, 1:100) rabbit polyclonal anti-GFAP (Novus Biologicals, 1:100), anti-nestin (Millipore, 1:100). These primary antibodies were applied for 2 h in blocking buffer (2.5% BSA, 5% goat serum, 0.3% Triton X-100 in PBS), followed by species-appropriate secondary Alexa Fluor 488 dye conjugated antibodies (Amersham) or Vectastain ABC kit and DAB reagents.
NPG isolation and culture. NPGs were isolated from the SVZ of adult mice.
Brains were dissected and washed in Hank’s buffer (Gibco). Tissue samples were minced, digested for 40 min with 0.05% trypsin, triturated and passed through a 45 μm cell strainer (BD Biosciences) to obtain a single-cell suspension. Cells were seeded in serum-free neural medium (Gibco) supplemented with 20 ng/ml of human bFGF (Gibco), 1 μg/ml of human EGF (Sigma), pen/strep and glutamine. Cells were grown at 37°C in 5% CO2.

NPG colony-forming assay was used to measure the proportion of cells that were able to form single-cell-derived NPGs. Single-cell-derived NPGs were seeded at 50 to 60 cells per 6-well plate. The day before transfection, 1.5 ml of medium containing 10% fetal bovine serum, glutamine (2 mM), penicillin (50 units ml⁻¹) and 12% precast gels (Life Technologies), transferred to PVDF membranes (Millipore) and incubated with antibodies against SAE1 (Novus Biologicals, 1:1000), β-actin (Sigma, 1:10000), and Open Lab 3.5.1 software. Patient-derived neurosphere culture and orthotopic inoculation.

Neurosphere cells were collected and fixed in 4% paraformaldehyde. Fixed cells were blocked with 5% BSA–0.1% Triton and then incubated with rabbit monoclonal anti-Ki67 (clone SP6, Lab Vision, 1:100) and rabbit polyclonal anti-GFAP (Novus Biologicals, 1:500) overnight and counterstained with Hoechst. Images were collected with an Axiovert S100 TV inverted fluorescence microscope (BD Biosciences). N-Galactosidase staining was performed according to the manufacturer’s instructions. Cells were then counterstained with propidium iodide and analysed with a FacsCanto flow cytometer (BD Biosciences). Analysis was performed using FlowJo software.

NPG self-renewal assay. To evaluate the self-renewal capacity of NPGs, a clonal colony-formation assay was used to measure the proportion of cells that were able to make single-cell-derived NPGs. Single-cell-derived NPGs were seeded at 50 to 60 cells per 6-well plate. The day before transfection, 1.5 ml of medium containing 10% fetal bovine serum, glutamine (2 mM), penicillin (50 units ml⁻¹) and streptomycin (50 μg/ml) at 37°C in 5% CO2. For growth curves and cell viability assays, 10⁴ cells were seeded in triplicate in 24-well plates. Cells were counted daily using a Neubauer haemocytometer for 3 days, and cell death was estimated by Trypan blue dye exclusion assay. On the third day, cells were incubated for 5 h with 0.4% Trypan blue (C176; DAKO fluorescent mounting medium containing 1 Hoechst counterstain. HRP-conjugated secondary antibodies were visualized by DAB staining (Vector Laboratories). Apoptotic cells were detected with ApopTag Detection kit (Chemicon International). Images were obtained with an Axiovist S100 TV inverted fluorescence microscope (Zeiss) and Open Lab 3.5.1 software, or with an Axiovert 100 inverted microscope (Zeiss) equipped with a Hamamatsu Orca digital camera.

For mitosis analysis, cells were seeded on coverslips in a six-well plate and fixed 72 h after the infection with retroviruses producing either GFP or the fusion protein GFP-Omomyc. Genomic DNA was visualized by DAPI staining (Vector Laboratories). Mitotic cells were stained with rabbit monoclonal anti-phospho histone H3 (Ser10) (Upstate, 1:200), mouse monoclonal anti-β-tubulin (Sigma, T9026, 1:100) and a Cy3 secondary antibody (Jackson Immunoresearch, 1:100). After immunostaining, cells were mounted on microscope slides with DAPI-containing Vectashield mounting solution (Vector Laboratories). For both Omomyc-expressing and control cells, images of 48 different mitoses were acquired using a fluorescence microscope (Zeiss) with a ×63 objective.

Viral vectors and infections. Omomyc DNA was cloned into the pEGFP retroviral vector (Clontech). A total of 2.5 × 10⁷ 293T cells were seeded on 60-mm plates and co-transfected with packaging plasmids PipL, PipLp, PipVSIG (Invitrogen) and pEGFP or pEGFP-Omomyc retroviral constructs using CaPO4 method. Supernatant was collected twice during the 2 days following transfection. The day before transfection, 1.5 × 10⁶ U87MG cells were seeded on 35-mm plates. Cells were then infected with 293T cells-derived supernatant containing retroviruses and polybrene (4 μg/ml). Omomyc was cloned into the pTRIPZ lentiviral vector (Open Biosystems, Thermo Scientific) and the shRNA sequences were removed. For infections, 293T cells were seeded at 70% confluence and the following morning 25 μM chloroquine added. Two hours later, 293 cells were transfected with pTRIPZ-Omomyc plus the lentiviral vectors pMD2G and psPAX2 by the CaPO4 method. The medium was changed the following day and soybean trypsin added at 5 μM. Viral supernatants were harvested on the subsequent 2 days, filtered and added to target cells with polybrene (0.8 μg/ml).

Statistical analysis. Data are shown in bar graphs as means ± standard deviation (s.d.), or as standard error (s.e.) if indicated. P-values were calculated using Student’s t-test to compare groups, or log-rank test for Kaplan–Meier curves.

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**Author contributions**

D.A. performed most of the studies with the GFAP:V12Ha-Ras model and derived NPGs (Figs 1–4) and initiated the writing of the paper. J.R.W. performed the experiments in Figs 7 and 8 and contributed to Figs 4 and 5. E.F. and M.P.S. performed the analysis of U87MG cells (Figs 5 and 6), and T.J. of U373MG cells. E.S. and N.M.S. were involved in the maintenance and care of the mouse colonies, respectively at the VCHO and UCSF animal facilities. J.C., S.R.-C., G.F., A.G.-J. and J.S. provided the patient-derived neurospheres and helped with the establishment of the xenograft model. D.M.-V. and M.-E.B. helped with tissue culture and cell imaging. I.B.S. helped with acquisition and maintenance of the GFAP:V12Ha-Ras model. M.M.M.G. helped with discussions regarding mitotic catastrophe and associated reagents. S.N. intellectually contributed to the human GBM cell line studies. G.I.E. participated in the experimental design related to Myc inhibition of the GFAP:V12 Ha-Ras model and in writing the manuscript. Finally, L.S. designed, coordinated, supervised and directed all the studies herein described, and wrote the manuscript with J.R.W.

**Additional information**

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

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