SUPPLEMENTAL TABLES

Table S1 Antibody information for the current study, Related to Figure 1.

Antibody	Vendor	Category #	Dilution
Primary antibodies			
Goat anti-Aldolase C	Santa Cruz	sc-12065	1:50
Mouse anti-APC-CC1	CalBioChem	OP80	1:50
Mouse anti-BrdU	BD Biosciences	347580	1:500
Rat anti-BrdU	Accurate Chemical & Scientific Corporation	OBT0030	1:500
Rat anti-CD9	eBioscience	14-0091	1:100
Goat anti-c-Myc	Novus Biologicals	NB600-338	1:500
Mouse anti-GFAP	Millipore	MAB360	1:400
Rabbit anti-GFAP	Dako	Z0334	1:500
Chicken anti-GFP	Aves Labs	GFP-1020	1:200
Mouse anti-Ki67	BD pharmingen	556003	1:400
Rabbit anti-Ki67	Novocastra Lab	NCL-Ki67p	1:500
Mouse anti-MBP	Covance	SMI-99P	1:1000
Mouse anti-Nestin	Millipore	MAB353	1:125
Mouse anti-NeuN	Millipore	MAB377	1:250
Chicken anti-Neurofilament M	Covance	Covance	1:1000
Chicken anti-Neurofilament L	Covance	PCK-308P	1:2500
Rabbit anti NG2	Millipore	AB5320	1:500
Mouse anti-O4	Millipore	MAB345	1:100
Rabbit anti-Olig2	Millipore	AB9610	1:1000
Rat anti-PDGFR-alpha	eBioscience	14-1401	1:200
Rabbit anti-Sox2	Millipore	AB5403	1:1000
Secondary antibodies			
Donkey anti-chichen Cy2	Jackson ImmunoResearch	703-225-155	1:250
Donkey anti-chichen Cy5	Jackson ImmunoResearch	703-175-155	1:250
Donkey anti-goat Alexa Fluore 555	Invitrogen	A21432	1:500 (1:1000 for TC)
Donkey anti-goat Cy5	Jackson ImmunoResearch	705-175-147	1:250
Donkey anti-mouse Alexa Fluore 488	Invitrogen	A21202	1:250 (1:500 for TC)
Donkey anti-mouse Alexa Fluore 555	Invitrogen	A31570	1:500 (1:1000 for TC)
Donkey anti-mouse Alexa Fluore 647	Invitrogen	A31571	1:250 (1:500 for TC)
Donkey anti-mouse Biotin	Jackson ImmunoResearch	715-065-151	1:250
Donkey anti-Rat Alexa Fluore 488	Invitrogen	A21280	1:250 (1:500 for TC)
Donkey anti-Rat Cy3	Jackson ImmunoResearch	712-165-150	1:500 (1:1000 for TC)
Donkey anti-Rat Cy5	Jackson ImmunoResearch	712-175-153	1:250 (1:500 for TC)
Donkey anti-rabbit Alexa Fluore 555	Invitrogen	A31572	1:500 (1:1000 for TC)
Donkey anti-rabbit Alexa Fluore 647	Invitrogen	A31573	1:250 (1:500 for TC)
Donkey anti-rabbit Biotin	Jackson ImmunoResearch	711-065-152	1:250
Streptavidin Alexa Fluore 405	Invitrogen	S-32351	1:200
Donkey anti-mouse Mono-Valent fragment	Jackson ImmunoResearch	715-007-003	1:10
Goat anti-Rat non-conjugated	Jackson ImmunoResearch	112-005-167	

Note: TC, tissue culture staining.

Table S2. Summary of tumor formation in mutant-MADM glioma model induced by *NSC*-Cre, Related to Figure 4.

ID	Age	Malignant glioma?	MADM-labeled?	Location	Symptom
Induced by hGFAP-Cre					
8172	209	Yes	Yes	Ctx	Seizure
8474	181	Yes	Yes	Ctx/Mid	Moribund
9921	171	Yes	Yes	Str	No Symptom
9922	171	Yes	Yes	Ctx	No Symptom
8788	165	Yes	Yes	Ctx/Cc	No Symptom
8785	161	Yes	Yes	Ctx	Seizure
8165	154	Yes	Yes	Str	Seizure
8790	154	Yes	Yes	Ctx/Cc	Ataxia
7706	152	Yes	Yes	Cc	No Symptom
7702	152	Yes	Yes	VF	No Symptom
8784	152	Yes	Yes	Ctx/Cc	Paralysis
8789	152	Yes	Yes	F	Hydrocephalus
8173	149	Yes	Yes	Ctx/Cc	Moribund
9923	146	Yes	Yes	Ctx/Cc	Moribund
9838	143	Yes	Yes	VF	Moribund
8168	137	Yes	Yes	F	Twisting
8791	125	Yes	Yes	VF	Moribund
8876	176	Yes	Yes	Str	No Symptom
5086	149	Yes	Yes	Cc	Seizure
10064	143	Yes	Yes	VF	Moribund
10084	142	Yes	Yes	Cc	Moribund
10078	139	Yes	Yes	OB	Moribund
10242	137	Yes	Yes	Hyp/Sub	Moribund
10326	136	Yes	Yes	Ctx	No Symptom
10079	128	Yes	Yes	Hyp/Sub	Moribund
10083	126	Yes	Yes	VF	Moribund
7691	68	Normal			No Symptom
7692	68	Normal			No Symptom
Induced by Nestin-Cre					
7584	151	Yes	Yes	F	No Symptom
8653	142	Yes	Yes	OB/Cc	Moribund
7874	139	Yes	Yes	VF	No Symptom
6256	135	Yes	Yes	Ctx	Ataxia
9013	170	Yes	Yes	F	No Symptom
9016	158	Yes	Yes	Ctx/Cc	No Symptom
9662	156	Yes	Yes	VF	No Symptom
9916	150	Other tumor	Yes	Intestine	Moribund
9578	149	Yes	Yes	OB	Moribund
9918	145	Normal	No		
10085	139	Yes	Yes	OB, Hyp/Sub	Moribund
10086	118	Yes	Yes	F	Moribund
10250	139	Normal	No		

Abbreviations:
Ctx: Neocortex; Mid: Midbrain; Str: Striatum; Cc: Corpus callosum; VF: Ventral forebrain; F: Entire forebrain; OB: Olfactory Bulb; Hyp/Sub: Hypothalamus with subarachnoid space invasion.

Table S3. Summary of tumor formation in mutant-MADM glioma model induced by *NG2*-Cre, Related to Figure 6.

ID	Age	Malignant glioma?	MADM-labled?	Location	Symptom
Induced by NG2-Cre (Continued)					
12204	305	Yes	Yes	F	Moribund
11186	297	Yes	Yes	Cc, Hyp/Sub	No Symptom
10530	288	Yes	Yes	OB, Str	Moribund
10576	279	Yes	Yes	OB, Hyp/Sub	No Symptom
12897	244	Yes	Yes	Hyp/Sub	Moribund
11725	236	Yes	Yes	Hyp/Sub	Moribund
11726	226	Yes	Yes	Hyp/Sub	Moribund
11723	225	Yes	Yes	VF	No Symptom
12609	220	Yes	Yes	F	Moribund
12090	217	Yes	Yes	Hyp/Sub	Ataxia
11577	212	Yes	Yes	Str	Paralysis
11184	186	Yes	Yes	Hyp/Sub	Moribund
10526	164	Normal	No		
11727	157	Normal	No		

Abbreviations:
Ctx: Neocortex; Mid: Midbrain; Str: Striatum; Cc: Corpus callosum; VF: Ventral forebrain; F: Entire forebrain; OB: Olfactory Bulb; Hyp/Sub: Hypothalamus with subarachnoid space invasion.

Table S4. Summary of orthotopic allograft assay, related to Figure 7.

ID	Primary tumor ID	Cell number injected	Malignant glioma?	Dpi*	Symptom	Cells injected
Primary tumors were from hGFAP-Cre induced						
10562	8173	100,000	No tumor	138	No symptom	(a)
10563	8173	100,000	Yes	47	No symptom	(a)
10564	8173	100,000	Yes	55	No symptom	(a)
10565	8173	100,000	Yes	49	No symptom	(a)
10566	8173	100,000	Yes	26	No symptom	(a)
10567	8206	100,000	Yes	66	Moribund	(a)
10568	8206	100,000	Yes	30	No symptom	(a)
10569	8206	100,000	No tumor	88	No symptom	(a)
10570	8206	100,000	Yes	17	No symptom	(a)
11358	9922	100,000	Yes	23	Mild symptom	(b)
11359	9922	100,000	Yes	29	No symptom	(b)
11360	9922	100,000	Yes	29	No symptom	(b)
11461	9923	100,000	Yes	16	No symptom	(a)
11462	9923	100,000	ND	17	Died before tissue collected	(a)
11463	9923	100,000	Yes	19	No symptom	(a)
Primary tumors are from Nestin-Cre						
11458	9226	100,000	Yes	21	No symptom	(a)
11459	9226	100,000	Yes	21	No symptom	(a)
11460	9226	100,000	Yes	21	No symptom	(a)
12466	8654	100,000	Yes	40	Moribund	(a)
12467	8654	100,000	Yes	38	Moribund	(a)
12468	8654	100,000	Yes	27	Moribund	(a)
Primary tumors are from NG2-Cre						
13127	11183	150,000	Yes	13	No symptom	(c)
13277	11183	150,000	Yes	45	Moribund	(c)
13278	11183	150,000	Yes	22	No symptom	(c)
13284	11183	150,000	Yes	42	Moribund	(c)
13279	11723	150,000	Yes	36	Moribund	(c)
13280	11723	150,000	Yes	36	Moribund	(c)
13487	11723	150,000	Yes	30	Moribund	(c)
13357	11725	150,000	Yes	30	Moribund	(c)
13358	11725	150,000	Yes	15	No symptom	(c)
13721	11725	150,000	Yes	39	Moribund	(c)
13285	11726	150,000	Yes	40	Moribund	(c)
13289	11726	150,000	Yes	34	Moribund	(c)
13290	11726	150,000	Yes	40	Moribund	(c)

Notes:

*Dpi: Days between initial tumor cell injection and sacrifice.

(a) Tumor cells used for injection were from the cell lines that were initially collected by the PDGFRα immunopanning method and were then maintained in Neurobasal/B27 medium.

(b) Tumor cells used for injection were from non-cultured, freshly purified tumor cells by PDGFRa-immunopanning method.

(c) Tumor cells used for injection were from non-cultured, freshly dissociated tumor cells.

EXTENDED EXPERIMENTAL PROCEDURES

Mouse lines

Mouse lines used for *NSC-Cre* glioma model: a pair of MADM mice (TG11, GT11) (Hippenmeyer et al., 2010), *hGFAP-Cre* (Zhuo et al., 2001) or *Nestin-Cre* (Petersen et al., 2002), *p53KO* (Jacks et al., 1994) and *NF1flox* (Zhu et al., 2001). Mouse lines used for NG2-Cre glioma model: a pair of MADM mice (TG11ML, GT11ML, unpublished lines), *NG2-Cre* (Zhu et al., 2008), *p53KO* (Jacks et al., 1994) and *NF1flox* (Zhu et al., 2001). TG11ML and GT11ML mouse lines have three mutually exclusive loxP variants in MADM cassettes, instead of single loxP sites in TG11 and GT11 mouse lines. This design was used to increase the efficiency of inter-chromosomal recombination induced by Cre transgenes. Detailed information is available upon request from H.Z..

Genotyping

For genotyping methods, primer sequences, and PCR conditions see below:

MADM11_Eif (for all MADM cassettes):

Chr11_CS1: 5-TGGAGGAGGACAAACTGGTCAC-3

Rosa4: 5-TCAATGGGCGGGGGTCGTT-3

Chr11_CS2: 5-TTCCCTTTCTGCTTCATCTTGC-3

PCR products: Knock-in (KI) band, 230bps; WT band, 350bps.

In MADM mouse model, only KI band can be detected.

Cre:

Upper primer 5-CACCCTGTTACGTATAGCCG-3;

Lower primer 5-GAGTCATCCTTAGCGCCGTA-3.

PCR product: KI band, 300bps.

P53 KO allele (Jacks et al., 1994)*:*

Neo tail: 5-ACCGCTATCAGGACATAGCGTTGG-3

p53 TJW5: 5-CACAGCGTGGTGGTACCTTATG-3

p53 TJW3: 5-GGTATACTCAGAGCCGGCCTG-3

PCR products: KI band, 700bps; WT band: 450bps.

NF1flox and recombination(KO) allele:

We identify flox(Neo) and KO alleles in the same PCR reaction with four primers, among which three were described previously(Zhu et al., 2001) and one was designed in our lab:

NF01: 5-ACCTCTCTAGCCTCAGGAATGA-3;

NF02: 5-CTTCAGACTGATTGTTGTACCTGA-3;

NF03: 5-TGATTCCCACTTTGTGGTTCTAAG-3 and

P2: 5-CATCTGCTGCTCTTAGAGGAACA-3

PCR products: WT band: 479bps; Flox (Neo) band, 350bp; KO band, 280bp.

PCR conditions

For Eif, Cre and NF1 PCRs: one cycle at 94 ^{o}C for 3minutes; 32 cycles at 94 ^{o}C for 15 seconds; 58°C for 25 seconds; 72 ^{o}C for 45 seconds; and then followed by 72 ^{o}C for 5 minutes. For p53KO PCR, the annealing temperature was increased to 60 ^{o}C.

5-Bromo-2'-deoxyuridine (BrdU) labeling

BrdU (Sigma) was administered by i.p. injection (50mg/kg body weight) for short term labeling (1.5hours) or by drinking water (1mg/ml) for long term labeling (7days). Mice were sacrificed right after the treatment and brain tissue was collected for the further analysis.

Pathology

Tissues used for pathological analysis were collected from O.C.T.-embedded brains. The tissue was thawed and the O.C.T. was removed by washing with cold PBS. The tissues were then preserved in 4% PFA until being processed for paraffin embedding and H&E staining.

Immunohistochemistry

Antibody combination and staining design

Most immunohistochemistry in our work involved MADM staining (for GFP and RFP), which occupies the commonly used green (488nm laser) and red (543 nm laser) channels in confocal imaging. Considering this, marker staining other than MADM was applied in either the Far-red channel (635 nm laser) or ultraviolet (UV) channel (405 nm laser), or both. If only a single marker was stained along with MADM (which we named three-channel staining, see below), we usually stained this maker with the appropriate secondary antibody to visualize it in the Far-red channel (such as Alexa Fluor 647 or Cy5 conjugated secondary antibodies). If more than one marker besides GFP and RFP was used (we named it as four-channel staining), the fourth would be the UV-channel which was visualized by using appropriate biotin-conjugated secondary antibody to recognize the primary antibody and then Alexa Fluor 405 conjugated streptavidin to develop the fluorescent signal.

Occasionally, we performed staining for three markers along with GFP and RFP (such as those in Figures 3 and S4, we named it as "4+1" channel staining). In this way, we borrowed the red channel from RFP for visualizing the fifth marker. Although this fifth marker staining cannot be visualized in either red WT or double-color cells, it helps us identify marker expression in green mutant cells, especially within

tumor region or among the pre-transforming mutant OPC population where cells are almost exclusively green or colorless.

For brain tissue containing grafted tumor cells (secondary tumors) the red channel was saved for staining cell markers other than RFP because no RFP labled WT cells existed in the tumor.

The detailed staining procedures are as following:

Three-channel staining with MADM and one more marker

Tissue sections were air-dried at room temperature for at least 1 hour after cryosectioning. Tissue sections were then re-hydrated and washed three times (10 minutes each) with phosphate-buffered saline (PBS) to remove sucrose prior to incubation with blocking/ permeablizing buffer (5% normal donkey serum and 0.3% Triton X-100 in PBS) for 30 minutes at room temperature to prevent non-specific binding. After this step, the sections were incubated overnight at 4°C with appropriate primary antibodies that were diluted in blocking/ permeablizing buffer according to Table S1.
The sections were washed three times (10 minutes each) with PBT (0.3% Triton X-100 in PBS) before incubation overnight at 4°C with appropriate fluorophore-conjugated secondary antibodies that had been diluted in PBT. Sections were washed three times in PBT (10 minutes each) and followed by one wash in PBS (5 minutes) before being mounted with anti-fade mounting gel mounting medium (Electron Microscopy Sciences, PA, #17985-10). To visualize nuclei, the slides were incubated with DAPI solution (25ng/ml in 1X PBS) for 10~30min before the last PBS wash.

Four channel staining with MADM and two more markers

The glass containers and slide racks used during the whole procedure were separated from those used for DAPI staining to avoid cross contamination.

Overall, the staining procedure is the same as for three-channel staining, excepting for the visualization of the UV-channel signal by fluorophore Alexa Fluor 405 (invitrogen). Alexa Fluor 405-conjugated donkey antibody was not commercially available when this work was performed. Alternatively, we incubated the sections with Biotin conjugated species-specific donkey secondary antibody (For the UV channel) together with the fluorophore-conjugated secondary antibodies for the other three channels overnight at 4°C. After a brief wash in PBT (10 minutes), the sections were incubated with Alexa Fluor 405-conjugated Streptavidin (1:200, Invitrogen) in PBT for 2 hours at room temperature. The slides were mounted in anti-fade gel mounting medium after three washes in PBT (10 minutes each) and one wash in PBS (5 minutes).

Staining including BrdU as a marker

Slides used for BrdU detection were treated with HCl prior to primary antibody incubation. Briefly, after three washes in PBS, the sections were submerged in 2N HCl (in PBS) for 30 minutes at 37°C. The

sections were then washed three times with PBS to remove HCl prior to incubation with the blocking solution.

Staining including Nestin as a marker

In the current study, we found that Mouse anti-Nestin monoclonal antibody (Millipore) generated high background signal in mouse tissue. To remove the background signal, we used Donkey-anti-mouse monovalent Fab fragment to block the tissue sections prior to primary antibody incubation. Briefly, after incubation in blocking solution for 1 hour, the slides were washed three times in PBT (2 minutes each) before incubation with Fab Donkey-anti-mouse IgG (H+L) (1:10 diluted in 1X PBT, Jackson Immunoresearch, # 715-007-003) overnight at 4°C. The slides were then washed three times in PBT (10 minutes each) prior to incubation with anti-Nestin primary antibody.

Tissue culture staining

Cells used for immunohistochemistry were cultured on poly-D-lysine (Sigma) coated 12mm glass coverslips. For staining, after gently removing the medium from the tissue culture plates, 0.5ml 4% PFA was added to each well and the cells were fixed at room temperature for 15 minutes. The coverslips were then washed one time with 1ml PBS, dried at room temperature for 30min and were kept in 1X PBS until used for staining. To stain surface antigen O4 (see *Table S1* for working condition), Triton X-100 was omitted during the entire process. Otherwise, 0.1% Triton X-100 was used for permeabilization.

Cells were incubated in blocking solution (10% NDS in PBS with or without 0.1% Triton X-100) for 1 hour at room temperature, and then incubated with primary antibody in blocking solution for 1 hour prior to three washes with PBS (with or without 0.1% Triton X-100, 10 minutes each). Cells were then incubated with appropriate secondary antibody (10% NDS in PBS with or without 0.1% Triton X-100) for 45 minutes at room temperature followed by 3 washes in PBS (with or without 0.1% Triton X-100, 10 minutes each). Before being mounted with anti-fade gel mounting medium, cells were incubated with DAPI for 5 minutes and washed one more time with PBS.

Quantification

Brain regions sampled for quantification

In the present study, we define 23 distinct adult brain regions in the nonneurogenic parenchyma of sagittal brain sections for cell quantification. Selected regions cover major representative brain structures encompassing both gray and white matter (see also Figure S3), including the cerebral cortex (four random fields from rostral to caudal), the corpus callosum (four random fields from rostral to caudal), the striatum (two random fields), the ventral forebrain (ventral to striatum, two random fields), the thalamus (two random fields), the hypothalamus (two random fields), the fornix, the anterior commissure, the olfactory

bulb (two random fields) and the midbrain (two random fields). Cerebellum was not included in the counting scheme because no tumor was found in this region.

We also define 5 regions close to the lateral ventrical wall of adult P60 brains used for counting both slow and fast dividing cells in neurogenic SVZ (see also Figure S3), including the dorsal lateral angle SVZ (S1) and the lateral wall SVZ (S2-S5).

Quantification of G/R ratios from P5 to P60 Mutant-MADM mouse model presented in Figure 2

Fixed brains were cryosectioned saggitally. Three sections (20μm in thickness) at least 200μm apart from each other (N=3 brains for each age) were stained and processed for data collection. To quantify MADM labeled cells and BrdU+ cells in the same brain, we stained the slides with MADM together with BrdU and DAPI. For each brain section, 9 regions were collected, including three in the cerebral cortex (1A-1C), three in the corpus callosum (2A, 2B and 2D), and three in the ventral side of the brain (3A, 4A/7A and 4B/7B) (see also Figure S3 for the location of each region). Each image was taken by a 40X objective with 1X digital zoom (0.1mm^2 each image) by 2μm optical sectioning (scanning depth 6 μm). Only the cells with clearly visible nuclei were counted.

Quantification of neurons, astrocytes, OPCs and oligodendrocytes in nonneurogenic parenchyma of P60 Mutant-MADM mouse brains presented in Figure 3A

To count the cell number and G/R ratios of four distinct cell types in Figure 3A, P60 brains (N=3 brains, 4 slices from each brain) were sectioned sagittally in 14μm (for neurons, oligodendrocytes and OPCs) or 30μm (for astrocytes) slices. Cell types were identified by MADM-labeling and cellular markers (NeuN for neurons, CC1 for oligodendrocytes and PDGFRα for OPCs) except for astrocytes. Because Myc (to visualize RFP, which was fused with a Myc-tag at the C-terminal (Hippenmeyer et al., 2010)) and Aldolase C (for astrocytes) antibodies were both raised from goat, we omitted the Myc antibody in such staining. We found that the live color of RFP was stable for several months and strong enough in its fluorescence intensity even without antibody staining. Therefore omitting myc staining did not affect the identification of red or double color cells.

We quantified neurons from 14 regions; and astrocytes and oligodendrocytes from 16 regions (see also Figure S3). Only cells with clearly visible cell bodies were counted. OPC quantification was performed together with BrdU long-term labeling quantification (see below). Each image was taken using a 40X objective with 1X digital zoom (0.1mm^2 area) by 1μm optical sectioning (scanning depth 10 μm). The number of red and green cells for each marker was summed together from all the regions of the four slices to calculate the average G/R ratio for the respective cell type in that brain. This value was averaged from three brains and presented as ± SEM.

Quantification of BrdU positive OPCs in the nonneurogenic parenchyma of P60 Mutant-MADM brains

Brain tissues were sectioned sagittally in 14µm thick slices and stained with MADM plus PDGFRα (Far-red channel) and BrdU (UV channel). We collected images from 21 regions as indicated in Figure S3. We processed each image in two ways for different purposes. First, we counted all the PDGFRα+ cells, including red, green, yellow and non-labeled cells. This count was used to calculate the G/R ratio of OPCs and the percentage of BrdU labeling among OPCs with distinct genotypes. Second, we focused on BrdU+ cells. BrdU+ cells will be either PDGFRα+ or PDGFRα- and present one of four possible colors. To confirm that PDGFRα- BrdU+ mutant cells also belong to the OPC-oligodendrocyte lineage, we co-stained adjacent slices with MADM plus PDGFRα (far-red channel), BrdU (UV channel) and Olig2 (Red channel) (See Figure 3).

Quantification of BrdU+ cells in the neurogenic subventricular zone (SVZ) of P60 Mutant-MADM brain. To identify the SVZ, we sectioned brains coronally. Four sections (30µm in thickness, at least 200µm apart) from each brain (three brains in total) were counted. As indicated in Figure S3, the SVZ was divided into dorsal lateral angle SVZ (Region S1) and the lateral wall SVZ (region S2-S5). We used a 40X objective with 2X digital zoom to collect each image (imaging area is 0.025mm^2 each). Bilateral SVZs in each brain section were scanned. Therefore, ten images were collected from each brain slice. The cell number from 40 images derived from a single brain was summed to calculate the G/R ratio and the percentage of mutant, WT and heterozygous cells among all BrdU positive cells in that brain. These values were averaged from N=3 brains and presented as ± SEM.

Characterization of NG2-Cre MADM mice

NG2-Cre mediated MADM-labeling in the SVZ was analyzed in coronal cryosections (20µm) at P360 (N=4). Non-adjacent brain sections separated by 60µm were collected from the rostral to caudal axis in the forebrain (i.e. collected one from every four sections), and stained with GFAP and DAPI. The SVZ was defined on each section based on DAPI staining (see Figure S6B) until the SVZ region could not be clearly identified. We used a 40X objective with 2X digital zoom to scan for MADM labeled cells within the SVZ and then performed multiple optical layer confocal sectioning (0.5 µm for each step) to examine whether the MADM-labeled cells expressed GFAP.

To examine MADM-labeled cells in the olfactory bulb (OB), bilateral OBs were coronally sectioned. 100 continuous sections were collected and immunostained with anti-NeuN antibody. The granule cell layer (GCL) was identified by DAPI staining. Four fields in the GCL of each OB section were randomly chosen using a 40X objective with 2X digital zoom. If any MADM labeled cells were found, further digital zoom (up to 4X) combined with multiple optical sectioning (0.5 µm for each step) was performed

to carefully examine whether these cells co-localized with NeuN staining. 800 fields were scanned from the bilateral OBs of a single mouse (N=4 mice).

Enrichment of OPC-like glioma cells

OPC-like tumor cells were purified from fresh glioma tissues as previously described (Cahoy et al., 2008) with minor modification. Detailed procedures see below.

Panning plate preparation:

BSL-1 plates: Two Petri dishes (100 x 10 mm, BD Biosciences-Falcon 351058) were incubated with BSL-1 solution (Vector Laboratories, Burlingame, CA, L-1100, 25μg BSL-1 in 10ml DPBS for each plate). The solution was swirled carefully to ensure that each whole Petri dish was completely coated with solution prior to incubation overnight at 4 ^{o}C. The next morning, the BSL-1 solution was removed from the plates and 20ml 0.2% BSA (in DPBS) was added to block the plates at room temperature until panning was performed (usually more than 4 hours).

PDGFRα immunopanning plates: Two Petri dishes (100 x 10 mm) were each incubated with 10 ml of a secondary antibody solution containing Tris buffer solution (50 mM pH8.5 Tris-Cl) and 75 μg (30μl) goat anti-rat IgG H+L chain (112-005-167) overnight at 4 ^{o}C. The next morning, the secondary antibody solution was removed and the plates were washed three times with 1X DPBS. Subsequently, a primary antibody solution containing 10μl Rat-anti-PDGFRα antibody (CD140a clone APA5, BD Biosciences-Pharmingen, 558774) in 10ml DPBS with 0.1% BSA was added to each plate. The plates were then incubated at room temperature until used and washed directly before immunopanning.

Cell dissociation

All dissection tools were sterilized by soaking in Cidex (Allegromedical, Cat 189980) for 10 minutes and then rinsed with autoclaved distilled water prior to dissection. Tools were kept in 70% ethanol between usages. Tumor-carrying mice were euthanized by cervical dislocation, sprayed down with 70% ethanol and then wiped with Betadine to remove loose fur. The head was cut off with a pair of large dissection scissors and then soaked in 70% ethanol for 45 seconds to 1 minute. The skin down the midline of the head was sliced through with a razor blade. The skull was cut open using a pair of small dissection scissors. Brain tissue was then lifted from the skull and washed once with sterilized cold PBS before being embedded into chilled but still liquid 4% low-melting temperature agarose (prepared in 1X PBS) on ice. After the agarose became solid, the brain was sliced into 1mm thick coronal sections and kept in cold SMEM (Invitrogen, #11380) in a Petri Dish on ice. Under a fluorescence dissection scope, tumors were easily identified because of the strong MADM fluorescence. The tumor region was dissected out with a 22.5^{o} straight microsurgical knife (Sharpoint, #72-2201). Adjacent brain slices containing some

tumor tissue were preserved in 4% PFA immediately after dissection. These slices were used for immunohistochemistry and H&E staining for tumor pathology.

The remaining dissected tumor tissue was transferred to a 60 mm Petri dish containing 5ml Papain solution and diced into ~1 mm^3 pieces. The Papain solution includes 1X EBSS (Sigma E7510), 0.5mM EDTA (Sigma E7510), 10mM HEPES (Gibco 15630-080), 26mM NaHCO3 (Sigma S5761-500g, cell culture grade), 0.16mg/ml L-Cysteine (Sigma C-7477, 25g), 20Unit/ml Papain, 1mg/ml DNase (Sigma, DN-25) and was carefully balanced in 5% CO_2 at 37°C for at least 30 minutes before use.

The tumor tissue was incubated in Papain solution at 37°C 5% CO_2 for 90 minutes (gently agitated every 15 minutes). Afterwards it was transferred into a sterile 15ml Conical polystyrene tube (BD BioScience #352095) and allowed to settle to the bottom of the tube (we used a 10ml serological pipette to transfer the tissue to avoid losing the cells at this step). The Papain solution was then pipetted off and 2ml Diluted trypsin inhibitor buffer was added. Diluted trypsin inhibitor includes 1X EBSS, 10mM HEPES, 26mM $NaHCO_3$, 1mg/ml ovomucoid (Sigma T9253-1G), 1mg/ml BSA (Sigma A-9418) and was carefully balanced in 5% CO_2 at 37°C for at least 30 minutes before use. After the tissue settled down (~2 minutes), the diluted trypsin inhibitor buffer was replaced by 2ml fresh diluted trypsin inhibitor buffer. The tissue was then triturated 8 times with a 5ml Pasteur pipette. After the tissue settled, 1ml of supernatant (containing dissociated cells) was carefully transferred to a new 15ml conical polystyrene tube and set aside. 1ml fresh diluted trypsin inhibitor buffer was added to the original tissue, and the trituration procedure was repeated. After the tissue settled, 1ml of supernatant was removed and combined with the other set aside supernatant. The trituration was repeated until all the diluted trypsin inhibitor buffer was used (10ml total). The last two rounds of trituration were performed with a 1ml pipette tip to ensure all the visible tissue chunks were fully dissociated into single cells.

Once the tissue was dissociated, 4ml standard trypsin inhibitor solution (10mg/ml BSA in diluted tripsin inhibitor buffer) was placed in a new 15ml conical polystyrene tube. Dissociated cell suspension was carefully layered on top of standard trypsin inhibitor solution. The layered solution was centrifuged at 220g for 15 minutes followed by immediate removal of the supernatant. Cells were re-suspended in 6ml panning solution (0.02% BSA, 1mg/ml DNase in 1X DPBS, Invitrogen 14040-182) using a 5ml pipette and centrifuged at 220g for 10 minutes. Cells were then re-suspended in 12 ml Panning buffer and passed through a 70µm cell strainer (Fisher 08-771-23) before performing immunopanning.

Immunopanning

The first BSL-1 coated plate was washed 2X with PBS and 1X with DPBS. 100µl of single cell suspension was set aside for cell number counting and cell culture. The remaining cell suspension was poured onto the first BSL-1 plate and incubated at room temperature for 15 minutes. During that incubation time, we counted the living cell number using the Trypan Blue method and a hemacytometer.

The second BSL-1 coated plate was washed 2X with PBS and 1X with DPBS before the supernatant from the first plate was transferred to it.

During the second BSL-1 panning plate, one primary antibody coated plate was washed 3X with 20ml DPBS. The supernatant from the second BSL-1 plate was transferred to the washed primary antibody plate and incubated for 45min, and then the supernatant was transferred to the second primary antibody coated. During the second primary antibody panning plate, the first primary antibody panning plate was washed 6-8 times with DPBS to remove the non-binding cells. At the end of the last wash the plate was checked under the microscope to visualize floating cells. Additional washes were performed until there were almost zero and certainly less than 5% floating cells.

Cells were collected by incubating the first primary antibody panning plate with 2ml pre-balanced TrypLE (Invitrogen 12605-010) in 5% CO_2 at 37°C for 7 minutes. The reaction was then stopped by adding 2ml trypsin inhibitor solution (2mg/ml Ovomucoid, 0.4% BSA in 1X DPBS). Tumor cells were dislodged from the plate surface by squirting with a 1 ml pipette tip. We collected the cells and also the supernatant from the second primary antibody plate and transferred them separately to new 15ml conical polystyrene tubes which were pre-coated with 0.4% BSA at 37C for 1 hour to prevent adherence of cells to the tubes. After centrifugation at 220g for 10min, the cells were washed one time with NSC medium (see cell culture section for recipes) before re-suspension in the same medium for further use (RNA extraction or allografting, see below).

WT neurosphere culture

The fetal NSCs were enriched from two independent litters of E15.5 WT embryonic brains according to the method described previously (Pollard et al., 2006) with minor modification. The forebrains (removed cerebella, olfactory bulbs and midbrains) were dissociated with Papain solution (as described above) and triturated into single cells. The dissociated cells were adjusted to 100,000 cells/ml and plated onto Poly (2-hydroxyethyl methacrylate) (Sigma 192066-1G) pre-treated 6-well tissue culture plates. The cells were cultured in NSC medium containing Neurobasal medium (Invitrogen 21103-049) with B27 supplement (50X, Invitrogen 17504-044) plus Penicillin/ Streptomycin solution (100X, Invitrogen 15140122), GlutaMAX (100X, Invitrogen, 35050-061), 50ng/ml EGF and 10ng/ml FGF (PeproTech 100-18B).

After three days of culture in 5% CO2 at 37°C, the primary neurospheres were pooled together and passaged twice as follows. We collected neurospheres in a 15ml conical polystyrene tube and let them settle for 10 minutes before removing the supernatant. The neurospheres were washed once with DPBS and incubated with pre-warmed 1X TrypLE for 20 minutes at 37°C. TrypLE was inactivated with equal volume 2X trypsin inhibitor solution (2mg/ml Ovomucoid, 0.4% BSA in DPBS) and the spheres were then dissociated into single cells by triturating 40 times with a 1ml pipette tip. Cells were centrifuged at

220xg for 10 minutes. The supernatant was removed and the cells were washed once with NSC medium. Cells were passed through a cell strainer and plated onto a new plate and cultured to form secondary neurospheres. Three to four days later secondary neurospheres were dissociated into single cells as above. Dissociated cells were expanded in NSC medium using 1X adherent $25cm^2$ PDL-coated flask for 4 days and passaged one more time in 3X $25cm^2$ PDL-coated flasks. The expanded cells were trypsinized off the flasks, washed, and frozen in 15% DMSO (in NSC medium) in liquid nitrogen for further use or directly lysed in TRIzol for RNA analysis.

Realtime quantitative PCR

Total RNA was extracted by TRIzol (Invitrogen) followed by phenol/chloroform extraction and ethanol precipitation(Miller et al., 2009). cDNA was then synthesized using Superscript III and Oligo (dT) Primers (Invitrogen #18080-044 and #AM5730G). qRT-PCR primers were designed using MacVector v10.0 and Primer 3 Plus (*www.bioinformatics.nl*) to cover two exons flanking a >500bp intronic region. Gene amplification was tested by at least two distinct functional primer sets. Only primer sets with the best performance for each gene were used for further analysis.

qPCR was done using an ABI 7900HT Real-Time PCR System for 40 cycles by denaturing at 95 °C for 15 seconds, annealing at 58 °C for 30 seconds and elongating at 72 °C for 30 seconds, followed by the default dissociation curve program. PCR amplification was performed in RT2 Real-Time SYBR Green/Rox PCR Master Mix (SABiosciences). Relative levels of cDNA were measured by using control primers for β-Actin (ActB) and/or Glyceraldehyde-3-Phosphate Dehydrogenase (Gapdh) to normalize gene expression for each sample. CT values were measured within the geometric amplification phase and averaged for triplicate reactions. Reactions with a CT value of 35 or higher were considered null. Values for individual genes were normalized by subtracting the average of the internal control gene(s) Gapdh and/or ActB.

Primers used include: Sox10: 5'-ATGTCAGATGGGAACCCAGA-3' and 5'-GGTCCAGCTCAGTCACATCA-3; NG2(CSPG4): 5'- AGCAAGGAAGTGCAGAGGAG-3' and 5'-CATCGAAAGACACCATCACG-3'; PDGFRα: 5'- GACGAGACCATCGAGGACAT-3' and 5'-GCCTCGGGAACTTTCTCTCT-3'; CD9: 5'- TGTCTCAGTCGGTTGTCGAG-3' and 5'-GCTCGAAGATGCTCTTGGTC-3'; Nestin: 5'- GATCGCTCAGATCCTGGAAG-3' and AGAGAAGGATGTTGGGCTGA; Gapdh: 5'-CGTCCCGTAGACAAAATGGT-3' and 5'-GAATTTGCCGTGAGTGGAGT-3', and ActB: 5'- TTGCTGACAGGATGCAGAAG-3' and 5'-AGTCCGCCTAGAAGCACTTG-3'.

Microarray and gene expression analysis

44K Mouse Development Oligo Microarrays from Agilent Technologies were used for microarray analysis. For each experiment, total RNA was fluorescently labeled and hybridized directly against a common reference sample generated from the RNA pool of four WT P17 mouse brain neocortexs. After scanning the images by using a GenePix 4000B scanner, the intensity values were extracted by using the Agilent Feature Extraction Software. In this study, we only focused on the channel of the tumor samples to extract their intensity values, but ignored the reference channel signals. Probe sequences were downloaded from the Agilent website and aligned to a transcript sequence database consisting of 49,040 *Mus musculus* genes from Ensembl57. After removing probes that did not align, or aligned with more than 5 mismatches (n=19,194), or probes that aligned to multiple genes (n=2,283), 23,006 probes representing 17,585 genes remained. Expression profiles were quantile normalized and log transformed. Expression values for genes represented by multiple probes were collapsed by taking the mean value of the set of probes.

Data from Cahoy et al (Cahoy et al., 2008) were downloaded and pre-processed as described previously(Verhaak et al.). The data set contains 38 mouse samples, which clustered into 8 clusters after selecting 2,000 variably expressed genes. Gene sets were defined by selecting the 250 genes with the highest absolute t-test statistic value, except for the OPC cluster with only two cluster members, where fold change values were used. The clusters used in the present study contained the following samples: (1) Postnatal day 16 myelin oligodendrocytes (n=4); (2) Postnatal day 6 to 7 OPCs (n=2); (3) Postnatal day 7 neurons (n=4);. (4) Postnatal day 17-30 astrocytes (n=6). Gene sets for four previously described GBM subtypes(Verhaak et al.) were defined by selecting the top 250 marker genes as provided, and by mapping the human genes to mouse genes using Ensembl57.

Single sample GSEA has been described elsewhere (Barbie et al., 2009; Verhaak et al.). In short, genes were ranked by their expression values. The empirical cumulative distribution functions (ECDF) of both the genes in the signature as well as the remaining genes were calculated. An enrichment score was obtained by a sum of the difference between a weighted ECDF of the genes in the signature and the ECDF of the remaining genes. This calculation was repeated for all signatures and samples. A positive score indicates a high ranking of up-genes in the signature, and low ranking of down-genes in the signature. A negative value does not indicate the opposite, but rather a lack of effect.

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