Function of Oncogene Mycn in Adult Neurogenesis and Oligodendrogenesis

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Abstract

Human MYCN is an oncogene amplified in neuroblastoma and many other tumors. Both human MYCN and mouse Mycn genes are important in embryonic brain development, but their functions in adult healthy nerve system are completely unknown. Here, with Mycn-eGFP mice and quantitative RT-PCR, we found that Mycn was expressed in specific brain regions of young adult mice, including subventricular zone (SVZ), subgranular zone (SGZ), olfactory bulb (OB), subcallosal zone (SCZ), and corpus callosum (CC). With immunohistochemistry (IHC), we found that many Mycn-expressing cells expressed neuroblast marker doublecortin (DCX) and proliferation marker Ki67. With Dcx-creER and Mki67-creER mouse lines, we fate mapped Dcx-expressing neuroblasts and Mki67-expressing proliferation cells, along with deleting Mycn from these cells in adult mice. We found that knocking out Mycn from adult neuroblasts or proliferating cells significantly reduced cells in proliferation in SVZ, SGZ, OB, SCZ, and CC. We also demonstrated that the Mycn-deficient neuroblasts in SGZ matured quicker than wild-type neuroblasts, and that Mycn-deficient proliferating cells were more likely to survive in SVZ, SGZ, OB, SCZ, and CC compared to wild type. Thus, our results demonstrate that, in addition to causing tumors in the nervous system, oncogene Mycn has a crucial function in neurogenesis and oligodendrogenesis in adult healthy brain.

Keywords Mycn · Adult · Neurogenesis · Oligodendrogenesis

Introduction

MYCN, the human gene encoding N-Myc, was first identified as an oncogene amplified in human neuroblastoma [1], a tumor characterized as having undifferentiated neuroblasts [2]. The expression level of MYCN correlates with the prognosis of neuroblastoma [3], and overexpression of human MYCN gene produces neuroblastoma in mice [4]. MYCN overexpression is also observed in other nervous system tumors such as medulloblastoma, glioblastoma, retinoblastoma, and spinal ependymoma, as well as tumors outside of the nervous system such as neuroendocrine prostate cancer, nephroblastoma (Wilms’ tumor), and many others [5–7]. The nucleotide sequence of MYCN is very similar to that of oncogene MYC [8].

In addition to causing tumors, MYCN is also expressed in human fetal brain [9], and heterozygous MYCN mutation causes Feingold syndrome characterized as reduced brain size and learning disability [10]. Similarly, mouse Mycn gene is expressed in embryonic and neonatal forebrains and hindbrains [11], and in embryonic eye, spinal cord, dorsal root ganglion (DRG) (spinal ganglia) and trigeminal ganglia (cranial ganglion) [12]. A homozygous Mycn-deficient mouse dies between 10.5 and 12.5 days of gestation, with significant developmental abnormalities in central and peripheral nervous systems [13]. Conditional deletion of Mycn from Nestin-expressing neuronal progenitor cells (NPCs) in embryo results in dramatic reduction of NPC proliferation and disruption of neuronal differentiation, leading to a substantial decrease in brain mass and disorganization of both the cortex and cerebellum [14]. Mycn also plays an important role in the proliferation and survival of embryonic sympathetic neuroblasts [15]. Finally, Mycn is highly functional in pluripotent cells as well, because overexpression of Mycn, along with Oct4, Sox2, and Klf4, transforms mouse embryonic fibroblasts into induced pluripotent stem cells [16].

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As *Mycn* was initially found to be not expressed in adult brain [11], the function of *Mycn* in the nervous system of a healthy adult has never been revealed. With *Mycn-EGFP* reporter mice [17], we found that *Mycn* is expressed in restricted regions in adult brain, such as subventricular zone (SVZ), subgranular zone (SGZ) of hippocampal dentate gyrus (DG), the two major sites for adult neurogenesis [18], and corpus callosum (CC) and subcallosal zone (SCZ),
**Fig. 1** Mycn is expressed in specific sites in adult mouse brain. a–c IHC of GFP and DAPI in the subventricular zone (SVZ) and corpus callosum (CC) (a), subgranular zone (SGZ) and subcallosal zone (SCZ) (b), and olfactory bulb (OB) (c) of young adult Mycn-eGFP reporter mice. The arrowheads point to the GFP-positive cells in CC (a) and SCZ (b), d IHC of DAPI, DCX, and GFP in the SVZ of Mycn-eGFP reporter mice. The enlarged image shows the co-localization of GFP and DCX in SVZ. e–g IHC of DCX and GFP in the SGZ (e), SCZ (f), and olfactory bulb (g) of Mycn-eGFP reporter mice. The arrowheads point to the DCX and GFP double-positive cells (e, f), and the enlarged images show the GFP and DCX double-positive cells. h Quantification of d–g showing the percentage of DCX/GFP double-positive cells among GFP (+) cells in the SVZ, SGZ, SCZ, and OB of young adult Mycn-eGFP reporter mice. i Quantification of d–g showing the percentage of DCX/GFP double-positive cells among DCX (+) cells in the SVZ, SGZ, SCZ, and OB of young adult Mycn-eGFP reporter mice. j IHC of GFP and NeuN in the dentate gyrus of young adult Mycn-eGFP reporter mice. The arrow points to the GFP and NeuN double-positive cells, and the enlarged figure shows the NeuN and GFP double-positive cells. k Quantification of j showing the percentage of NeuN/GFP double-positive cells among GFP (+) cells in the SGZ of Mycn-eGFP reporter mice. l IHC of GFP and Olig2 in the CC above SVZ of Mycn-eGFP reporter mice. The dashed line shows the boundary between SVZ and CC, and the arrows point to the GFP and Olig2 double-positive cells, with the enlarged figure showing the Olig2 and GFP double-positive cells. m Quantification of l showing the percentage of Olig2/GFP double-positive cells among GFP (+) cells in the CC of young adult Mycn-eGFP reporter mice.

A site of adult oligodendrogenesis [19]. Our results further demonstrate that Mycn plays important roles in adult neurogenesis and oligodendrogenesis.

**Materials and Method**

**Animals**

All animal procedures were performed according to protocols approved by Institutional Animal Care and Use of Laboratory animals. Animals were housed in groups of five in a pathogen-free barrier facility. Young adult (8–12-week-old) male and female mice were used in all experiments. Tamoxifen or hydroxytamoxifen was injected in doses of 100 mg per kg intraperitoneally.

Mycn-EFGP mouse, STOCK Tg(Myecn-EFEGP)ET250Gsat/Mmucl, RRID:MMRRC_010553-UCD, was obtained from the Mutant Mouse Resource and Research Center (MMRRC) at University of California at Davis, an NIH-funded strain repository, and was donated to the MMRRC by Nathaniel Heintz, Ph.D. at Rockefeller University to GENSAT.

Mycn fl/fl mice were obtained from Jackson Laboratory (B6.129-Mycn tm1Pak/J, stock No. 006933); Mki67-creER (Mki67 tm2.icreERT2tLE/J, stock No. 029803). Dcx-creER was a gift from professor Zhiqi Xiong. Rosa26-TdTOMATO-loxp-STOP-loxp mice were obtained from Jackson Laboratory (B6. Cg-Gt (ROSA)26Sortm14(CAG−TdT−Tomato)Hze/J, stock No. 007914).

**Immunohistochemistry**

Animals were sacrificed and then intracardially perfused with ice-cold PBS and 4% paraformaldehyde (PFA; Thermo Scientific, AC416785000). Brains were dissected, post-fixed in 4% paraformaldehyde overnight, and embedded in OCT compound (Tissue-Tek, Thermo Fisher, 4585). Coronal brain sections (20 µm thick) were collected by cryostat. The sections were washed with 1 × PBS and incubated with blocking buffer with 10% fetal bovine serum (FBS) (Thermo Fisher) and 0.3% Triton X-100 in PBS for 30 min at room temperature. Primary antibodies diluted in 1% FBS and 0.3% Triton X-100 in PBS were added to the slices and incubated overnight at 4 °C. Subsequently, conjugated secondary antibodies of Alexa Fluor 488, Alexa Fluor 555, or Alexa Fluor 647 (1:1000; Invitrogen) were added, and DAPI (1:1000; Invitrogen) was used for showing nucleuses.

The following primary antibodies and their dilutions were used: GFAF (rabbit, 1:1000; Dako, #20334), Ki67 (rabbit, 1:1000; Abcam), doublecortin (DCX) (rabbit, 1:500; Abcam), NeuN (mouse, 1:500; Millipore), oligodendrocyte transcription factor 2 (Olig2) (rabbit, 1:500; Millipore, #AB9610), and Tbr2 (rabbit, 1:500; Abcam). Images were captured using a Zeiss LSM700 confocal microscope as a single plain and were processed with Fiji/ImageJ (NIH).

**Cell Counting**

For EdU + cell number counting, we chose three regions to count the EdU + cell number, including SVZ, hippocampus, and olfactory bulb. For each region, we cut the brain from the same region and collected 20-µm cryosections of each brain region from three animals per group. In order to get the information in the whole region, we mounted, immunostained, and counted the EdU + cells in every sixth section of the brain region. To quantify the Td-tomato + cells in the targeted regions, we counted Td-tomato + cells from 3 to 4 animals per group in slices with the same region. We use the number of cells per field to measure the density of cells within SVZ, CC, SGZ, SCZ, and olfactory bulb (OB), with the field defined as the specific brain regions under 20× magnification, and count the Td-Tomato + cell number in that specific region. To analyze the distribution of the newborn cells in dentate gyrus, we divided the granule cell layer into three layers equally, as shown in Fig. 7b, counted the Td-tomato–positive cells in each layer, and calculated the percentage of the cell number in each layer versus the whole Td-tomato + cell number in the dentate gyros. To quantify the Td-tomato–positive cells that co-express Olig2,
Fig. 2 *Mycn* is enriched in *Dcx*-expressing cells. a–c In *Mycn-eGFP* reporter mice, qRT-PCR study shows that the GFP (+) cells sorted by FACS from SVZ, dentate gyrus (DG), and SCZ had higher *Dcx* mRNA levels than GFP (−) cells. Data are shown as mean±SEM, and the analysis performed was unpaired two-tailed Student’s *t* test. d IHC of GFP, Td-Tomato, and DCX in SVZ and CC from a *Dcx-creER/+; Td-Tomato/+; Mycn-eGFP* mouse 1 day after hydroxytamoxifen treatment. In these mice, the cells expressing *Dcx* in the last 24 h were labeled with Td-Tomato. The dashed lines show the boundary between SVZ and CC. Virtually, all the Td-Tomato (+) cells in SVZ and CC were GFP (+). Most Td-Tomato (+) cells in SVZ and some Td-Tomato (+) cells in CC were also DCX (+). Enlarged figures show the GFP/Td-Tomato double–positive cells and the GFP/DCX double–positive cells. Scale bar=100 µm. e Quantification of showing the percentage of DCX/Td-Tomato double–positive cells among Td-Tomato (+) cells in the SVZ and CC of a *Dcx-creER/+; Td-Tomato/+; Mycn-eGFP* mouse 1 day after hydroxytamoxifen treatment. Data are shown as mean±SEM, and the analysis performed was unpaired two-tailed Student’s *t* test.
we counted Td-tomato–positive cells in three sections in each area per animal and quantified the double-labeled Td-tomato/Olig2–immunoreactive cells.

**EdU Staining**

EdU solution (10 mg/kg; Invitrogen, C10337) was injected intraperitoneally 24 h before tissue collection. Click-iT reaction cocktail (1 x Click-iT reaction buffer, CuSO4, Alexa Fluor azide, and 1 x reaction buffer additive) was prepared and applied to slides for 30 min and washed with PBS for 10 min three times, followed by first antibody incubation overnight. Then, the slides were incubated with secondary antibody for 1 h at room temperature and washed with PBS for 10 min, followed by DAPI staining.

**Flow Cytometry**

The brain region such as SVZ was freshly microdissected and homogenized. The homogenized tissue was filtered by using the Foxp3 transcription factor staining buffer kit (Thermo Fisher) for intracellular and intranuclear staining and was washed twice with Perm/Wash (0.01% sodium azide, 0.5% saponin, and 2% BSA in PBS). Cells were then resuspended in the desired antibody mix in PBS and stained for 30 min at 4 °C. After washing with PBS, cells were fixed and permeabilized using the FACS Aria III. The following fluorochrome-conjugated monoclonal antibodies were used: Ki67 (clone SOLA15, 1:5000) and DCX (anti-rabbit, 1:100; Abcam). Dead cells and duplets were excluded for analysis using SSC-A/H, FSC-A/H, and a fixable viability kit (near-IR Dead cells and duplets were excluded for analysis using staining; BioLegend).

**qRT-PCR**

For qRT-PCR, cDNA synthesis was performed using a SuperScript III First-Strand Synthesis System (Invitrogen, 18,080–051). Quantitative PCR was performed using PerfeCTa SYBR Green FastMix Rox (Quanta Biosciences) in a qPCR system (Bio-Rad). β-Actin was used as the housekeeping gene for normalization. The following primers were used: 

- Actb (β-actin), CCACACCGCACCAGTTCG/TAC AGCCCGGGAGCATCGT; 
- Mycn, AGCGTTCAACTA GCAGACCAT/CGAAAGGCCAGATTGTTGG; 
- Dcx, CGACCAAGACGCAAATGGAAC/CAATGACAG CGGCAGGTACA.

**Experimental Design and Statistical Analysis**

The experiments were conducted with 3–4 animals each group, the minimal number of animals to obtain results with statistical significance. For immunohistochemistry (IHC) studies, at least 3 sections from each animal were immunostained, and the average value of all the sections from the same animals was used to represent the value of that particular animal. For flow cytometry studies, one experiment was performed in one animal. For qRT-PCR, three duplicates were performed for each primer pair, and the average value of all three duplicates was used to represent the value of that particular animal. No data was excluded, and data are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism, version 8.0. Unpaired two-tailed Student’s t tests were used for single comparisons between two groups. Other data were analyzed using one-way or two-way analysis of variance (ANOVA).

**Results**

**Mycn Is Expressed in Adult Healthy Brain of Mice**

To investigate if Mycn is expressed in adult brain, we studied young adult (8–12 weeks old) Mycn-eGFP reporter mice generated by GENSAT [17], in which eGFP is expressed under the control of bacterial artificial chromosome containing the promoter and the enhancers of mouse Mycn gene. We found that GFP was expressed in SVZ (Fig. 1a), SGZ (Fig. 1b), and olfactory bulb (OB) (Fig. 1c). We also found GFP (+) cells in CC (Fig. 1a, arrowheads), as well as in SCZ (Fig. 1b, arrowheads), a thin region between the hippocampus and CC that contains neural stem cells [19]. We then dissected the SVZ and hippocampus from Mycn-eGFP reporter mice and sorted out the GFP (+) cells from the SVZ, dentate gyrus, and SCZ with fluorescence-activated cell sorting (FACS). With qRT-PCR, we confirmed that Mycn gene was enriched in the GFP (+) cells in SVZ (Supplementary Fig. 1a), dentate gyrus (Supplementary Fig. 1b), and SCZ (Supplementary Fig. 1c).

Because Mycn is associated with neuroblastoma, we were particularly interested in finding out whether Mycn is expressed in the neuroblasts of adult healthy mice. Our IHC studies of brain sections of young adult Mycn-eGFP reporter mice with DCX, which is expressed in adult neuroblast and neuronal intermediate progenitor cells (nIPCs) [20], revealed the co-localization of DCX with GFP in...
**Fig. 3** Mycn-expressing cells can proliferate. a–d IHC of GFP and Ki67 in the SVZ and CC (a), SGZ (b), SCZ (c), and OB (d) of a young adult Mycn-eGFP mouse. The dashed line in a shows the boundary between SVZ and CC. The arrows point to the GFP and Ki67 double–positive cells, with the enlarged figures showing the Ki67 and GFP double–positive cells. e The quantitative analysis of a–d shows the percentage of Ki67 (+) cells in GFP (+) cells in different brain regions of a Mycn-eGFP reporter mouse. f–h IHC of GFP and TdTomato in a Mycn-eGFP reporter mouse in SVZ and CC (f), SGZ (g), SCZ (h), and OB (i) after tamoxifen treatment for 5 days. In these mice, the proliferating cells and the cells proliferated in the past 5 days were labeled with TdTomato. The dashed line in f shows the boundary between SVZ and CC, and the dashed lines in h show the boundary of SCZ. The arrows and arrowheads show the GFP and TdTomato double–positive cells, and the enlarged figures show the TdTomato and GFP double–positive cells. j The quantitative analysis of f–i shows the percentage of GFP and TdTomato double–positive cells among GFP (+) cells in the SVZ, CC, SGZ, SCZ, and OB of a Mycn-eGFP mouse after 5-day tamoxifen treatment. Scale bar = 100 µm

Moreover, our analysis of the published single-cell RNA-Seq (scRNA-Seq) dataset from perinatal, juvenile, and adult dentate gyrus [20] shows that Mycn is expressed in radial glia-like (RGL) cells, nIPCs, neuroblasts, and immature granule cells (GCs), as well as in some mature GCs (Supplementary Fig. 2a), consistent with our IHC study that some GFP (+) cells in the dentate gyrus of Mycn-eGFP mice expressed the mature neuron marker NeuN (Fig. 1j, k). Interestingly, the expression pattern of Mycn is very similar to that of Dcx in dentate gyrus, which is also expressed in RGLs, nIPCs, neuroblasts, immature GCs, and some mature GCs (Supplementary Fig. 2b). In contrast, the neuroblast marker Eomes is expressed more specifically in nIPCs and neuroblasts (Supplementary Fig. 2c). On the other hand, our analysis of published scRNA-Seq dataset from oligodendrocyte lineage cells of juvenile and adult CNS [24] shows that Mycn is expressed in some oligodendrocyte precursor cells (OPCs), differentiation-committed oligodendrocyte precursors (COPs), and certain myelin-forming oligodendrocytes (MFOLs) and mature oligodendrocytes (MOLs) (Supplementary Fig. 3a). In comparison, Olig2 gene is expressed in every oligodendrocyte lineage cells (Supplementary Fig. 3b).

**Mycn-Expressing Cells Can Proliferate**

As one major character of adult neurogenesis and oligodendrogenesis is the proliferation of RGLs, nIPCs, neuroblasts, and OPCs [20, 21], we investigated if proliferation occurred in Mycn-expressing cells. Our IHC studies of brain sections from young adult Mycn-eGFP reporter mice with the proliferation marker Ki67 [25] showed that 4% of the GFP (+) cells in SVZ, 28% of the GFP (+) cells in CC, 3% of the GFP (+) cells in SGZ, 13% of the GFP (+) cells in SCZ, and 8% of the GFP (+) cells in OB were Ki67 (+) (Fig. 3a–e). To further confirm that Mycn-expressing cells do proliferate, we crossed the Mycn-eGFP mouse with the Mycn-eGFP mouse along with the TdTomato reporter mouse. In young adult Mycn-eGFP/+; TdTomato/+; Mycn-eGFP/+ mice, tamoxifen treatment for 5 days fate labeled the proliferating cells and the cells recently proliferated within last 5 days with TdTomato. We found that in this animal, 33% of GFP (+) cells in SVZ, 38% of GFP (+) cells in CC, 21% of GFP (+) cells in SGZ, 45% of GFP (+) cells in SCZ, and 34% of GFP (+) cells in OB were TdTomato (+) (Fig. 3f–j). All together, these results suggest that Mycn-expressing cells in adult healthy brain proliferate in multiple brain regions.
**Mycn** is Essential for Cell Proliferation in Adult Neurogenesis and Oligodendrogenesis

To investigate if Mycn contributes to cell proliferation in adult neurogenesis, we crossed Dcx-creER line with Mycn flox line [14] to generate Dcx-creER/+; Mycn fl/fl littermates (Fig. 4a, b). We also crossed Mki67-creER line with Mycn flox line to generate Mki67-creER/+; Mycn fl/fl littermates, and daily tamoxifen treatment of young adult Mki67-creER/+; Mycn fl/fl mice for 5 days will delete Mycn from any cells that have expressed Mki67 in the past 5 days in Mki67-creER/+; Mycn fl/fl mice. With flow cytometry to analyze DCX (+) cells in SVZ (Supplementary Fig. 4a, b), we found that the DCX (+) cells with Mycn deletion had significant less proliferating Ki67 (+) cells compared to DCX (+) cells from control +/+; Mycn fl/fl littermates (Fig. 4a, b). We also crossed Mki67-creER line with Mycn flox line to generate Mki67-creER/+; Mycn fl/fl mice, and daily tamoxifen treatment of young adult Mki67-creER/+; Mycn fl/fl and control +/+; Mycn fl/fl mice for 5 days will delete Mycn from any cells that have expressed Mki67 in the past 5 days in Mki67-creER/+; Mycn fl/fl mice.

![Flow cytometry plots](image)

**Fig. 4** Mycn is required for the proliferation of DCX (+) cells in SVZ. a Representative flow cytometry scatter plots to assess the proliferation of DCX (+) cells in the SVZ of young adult Dcx-creER/+; Mycn fl/fl (control) versus Dcx-creER/+; Mycn fl/fl (cKO) mouse. b Quantification of a shows that the deletion of Mycn from Dcx-expressing cells significantly reduced the proliferation of DCX (+) cells. c Representative flow cytometry scatter plots to assess the proliferation of DCX (+) cells in the SVZ of young adult Mki67-creER/+; Mycn fl/fl (control) versus Mki67-creER/+; Mycn fl/fl (cKO) mouse. d Quantification of c showing that the deletion of Mycn from Mki67-expressing cells significantly reduced the proliferation of DCX (+) cells. Data are shown as mean ± SEM, and the analysis performed was unpaired two-tailed Student’s t test.
With flow cytometry (Supplementary Fig. 4c, d), we found that in SVZ, the deletion of Mycn from Mki67-expressing cells also resulted in significantly less proliferation of the DCX (+) cells compared to that in control +/+; Mycn flo/fl littermates (Fig. 4c, d). Of note, the proliferation rate of the total cell populations in SVZ also decreased in Mycn cKO animals compared with that in intact animals (Supplementary Fig. 5). These results suggest that Mycn contributes to the proliferation of DCX (+) cells in SVZ in adult.

To further study the functional role of Mycn in cell proliferation in brain regions of young healthy animals, we injected EdU, a nucleoside analog of thymidine that can be incorporated into DNA during DNA synthesis for cell proliferation [26], into young adult Mki67-creER/+; Mycn flo/fl and control +/+; Mycn flo/fl mice after daily tamoxifen treatment for 5 days. Consistent with our flow cytometry result (Fig. 4c, d), our IHC with EdU showed that deleting Mycn from Mki67-expressing cells significantly reduced the total number of EdU (+)-proliferating cells in SVZ compared to that in control +/+; Mycn flo/fl mice (Fig. 5a, b). Remarkably, although many EdU (+) cells were observed in SGZ, SCZ, and OB in control +/+; Mycn flo/fl mice, almost no EdU (+) cells were observed in these regions when Mycn was deleted from Mki67-expressing cells (Fig. 5c–f). These results suggest that Mycn is required for cell proliferation in the sites of adult neurogenesis (SVZ and SGZ) and oligodendrogenesis (SCZ).

**Mycn Inhibits the Maturation of Dcx-Expressing Cells in Adult Dentate Gyrus**

To investigate if Mycn is involved in maturation of neuroblast in adult, we crossed Dcx-creER line with TdTomato reporter line and Mycn flox line. Treating young adult and Dcx-creER/+; TdTomato/+; Mycn flo/fl mice and control Dcx-creER/+; TdTomato/+; +/+ mice with daily tamoxifen for 5 days fate labeled all the cells that have expressed Dcx in last 5 days with TdTomato, along with deletion of Mycn in TdTomato-labeled cells in Dcx-creER/+; TdTomato/+; Mycn flo/fl mice. We did IHC of dentate gyrus with DCX antibody 14 days after tamoxifen treatment and measured the percentage of TdTomato (+) cells that are still DCX (+). We found significantly less DCX (+) cells in the TdTomato (+) cells with Mycn deletion compared to the control TdTomato (+) cells with intact Mycn (Fig. 6a, b), suggesting that TdTomato (+) cells with Mycn deletion were more mature than TdTomato (+) cells with intact Mycn. In addition, in the same mice, we observed that more TdTomato (+) cells in the dentate gyrus of Mycn-deleted TdTomato (+) cells expressed the mature neuron marker NeuN compared to that of control TdTomato (+) cells with intact Mycn (Fig. 6c, d), further supporting that Mycn inhibits the maturation of Dcx-expressing cells.

Using the same type of animals, we also measured the distribution of TdTomato (+) cells in the granule layer of dentate gyrus, because the newly generated neurons get more and more mature when they migrate from inner layers to outer layers [20, 27]. We found that 14 days after tamoxifen treatment of young adult and Dcx-creER/+; TdTomato/+; Mycn flo/fl mice and control Dcx-creER/+; TdTomato/+; +/+ mice, more TdTomato (+) cells with Mycn deletion migrated away from the inner layer to the middle layer of dentate gyrus compared to the control TdTomato (+) cells with intact Mycn (Fig. 7). Taken together, these results indicate that Mycn inhibits the maturation of neuroblasts in adult dentate gyrus.

**Mycn Inhibits the Survival of Newly Generated Cells in Sites of Adult Neurogenesis and Oligodendrogenesis**

To monitor the fate of proliferated cells in the sites of adult neurogenesis and oligodendrogenesis, we crossed Mki67-creER line with TdTomato reporter line and Mycn flox line. Treating young adult Mki67-creER/+; TdTomato/+; Mycn flo/fl mice and control Mki67-creER/+; TdTomato/+; +/+ mice with daily tamoxifen for 5 days fate labeled all the cells that have expressed Mki67 in last 5 days with TdTomato, along with deletion of Mycn from TdTomato-labeled cells in Mki67-creER/+; TdTomato/+; Mycn flo/fl mice. Fourteen days after the last tamoxifen treatment in the control Mki67-creER/+; TdTomato/+; +/+ mice, 94% of TdTomato (+) cells in SVZ were DCX (+) but none of them were NeuN (+) (Supplementary Figs. 6a and 7), whereas in dentate gyrus, 65% of TdTomato (+) cells were DCX (+) and 72% of TdTomato (+) cells were NeuN (+) (Supplementary Figs. 6b and 7a, b), and in OB, 36% of TdTomato (+) cells were DCX (+) and 63% of TdTomato (+) cells were NeuN (+) (Supplementary Figs. 6c and 7a, b). These results suggest that most of the proliferating cells in SVZ, dentate gyrus, and OB develop into neuroblasts or mature neurons. On the other hand, 66% of TdTomato (+) cells in SCZ and 83% of TdTomato (+) cells in CC were Olig2 (+) (Supplementary Figs. 6d, e and 7c), suggesting that the cells which proliferated 2 weeks ago mainly developed into oligodendrocyte lineage cells in SCZ and CC. Very little, if any, of the TdTomato (+) cells in these regions were microglia or astrocytes (Supplementary Fig. 6).

We then compared the TdTomato (+) cells between Mki67-creER/+; TdTomato/+; Mycn flo/fl mice and control Mki67-creER/+; TdTomato/+; +/+ mice 14 days after the last tamoxifen treatment and found that animals with Mycn deletion in Mki67-expressing cells had significantly more TdTomato (+) cells than the control animals in SVZ and CC (Fig. 8a–c), SGZ and SCZ (Fig. 8d–f), and OB (Fig. 8g, h),...
suggesting that Mycn inhibits the survival of newly generated cells in these regions.

Discussion

Human MYCN is an oncogene involved in many tumors, including neuroblastoma, which is characterized as inappropriate proliferation of undifferentiated neuronal progenitor cells [5]. The copy numbers of MYCN are closely associated with the aggressiveness of neuroblastoma [28], with increased expression of MYCN that increases the proliferative potential of neuroblastoma cells [29]. Inhibition of MYCN function suppresses the proliferation and enhances the neuronal differentiation of MYCN-amplified neuroblastoma cells [30], and with different conditions, MYCN can either promote or inhibit apoptosis of neuroblastoma cells [5]. In addition to tumorigenesis, MYCN is also crucial for embryonic development, as it is expressed in human fetal brain [9], and heterozygous MYCN mutation causes microcephaly and learning disability in human [10]. Similarly, mouse Mycn is also expressed in embryonic central and peripheral nervous systems, and homozygous Mycn mutant mouse dies between 10.5 and 12.5 days of gestation [12, 13]. Deletion of Mycn from embryonic neuronal stem cells severely reduces the proliferation of embryonic neuronal progenitor cells and dramatically increases neuronal differentiation, with limited effect on apoptosis of embryonic neuronal progenitor cells [14]. On the other hand, overexpression of Mycn in mouse multipotent sympathoadrenal progenitors promotes proliferation, apoptosis, and neural lineage commitment [31]. In this study, we demonstrated that Mycn plays a crucial role for adult neurogenesis and oligodendrogenesis in mice.

Mycn Is Expressed in Adult Brain

With Mycn-EGFP reporter mice, we found that Mycn is expressed in SVZ (Fig. 1a) and SGZ (Fig. 1b), two major sites of adult neurogenesis, as well as in OB (Fig. 1c), CC (Fig. 1a), and SCZ (Fig. 1b). Our further analyses revealed that many Mycn-EGFP cells co-expressed the neuroblast marker DCX in SVZ, SGZ, SCZ, and OB (Fig. 1d–g), as well as the proliferation marker Ki67 in SVZ, SGZ, OB, SCZ, and CC (Fig. 3a–e). Some Mycn-EGFP cells were also found in the granular layer of dentate gyrus, and these cells were also NeuN (+) (Fig. 1j), suggesting that Mycn was expressed in certain hippocampal neurons, like those newly differentiated from neuroblasts. In addition, some Mycn-EGFP cells in CC also co-expressed the oligodendrocyte marker Olig2 (Fig. 1i, m), indicating that Mycn was expressed in oligodendrocyte lineage cells. Indeed, analysis of the published scRNA-Seq result from adult mouse dentate gyrus [20] showed that Mycn is mainly expressed in nIPCs, neuroblasts, and immature GCs and in small portions of the mature GDs (Supplementary Fig. 2a), similar to the expression pattern of Dcx (Supplementary Fig. 2b). Interestingly, although almost all DCX (+) cells in these regions co-expressed GFP in Mycn-EGFP reporter mice (Fig. 1j), not all GFP (+) cells were DCX (+) (Fig. 1i). As the DCX (+) cells were detected by DCX IHC in this study, some cells with a relatively low level of Dcx expression might not be detected by DCX IHC.

Mycn Is Required for the Proliferation of Adult nIPCs and Neuroblasts

By deletion of Mycn from adult Dcx-expressing cells or in adult Mki67-expressing proliferating cells, we demonstrated that Mycn is required for the proliferation of nIPCs and neuroblasts in SVZ, SGZ, and OB. Unlike in SVZ where the proliferation is mildly reduced in Mycn cKO animals (Fig. 5b), in SGZ and OB, the proliferation is almost completely prevented in Mycn cKO animals (Fig. 5d, f), suggesting that Mycn is absolutely required for the proliferation of nIPCs and neuroblasts in SGZ and OB, whereas the proliferation in SVZ has Mycn-independent mechanism. The effect of Mycn on the proliferation of adult nIPCs and neuroblasts is likely mediated by the axis of cyclin-dependent kinase (CDK)-retinoblastoma protein (RB)-E2F transcription factor family, a core transcriptional machinery that determines if a cell exits the G1 phase to enter the S phase of cell cycle [32], because CDK6, RB, and E2F1 are required for the proliferation of adult neuronal progenitors and immature newborn dentate granule cell neurons [33–35], and knockdown of MYCN resulted in downregulation of CDK6 and E2F1 in neuroblastoma cells [36]. Deletion of Mycn from embryonic neuronal progenitor cells or knockdown of MYCN from neuroblastoma cells also upregulates CDK inhibitor p27 [14, 36], which is known to modulate CDK6 kinase activity in adult hippocampus to control the expansion of neuronal progenitor cells [34].
**Mycn Inhibits the Maturation of Adult Neuroblasts and the Migration of Newly Generated Neurons**

With the fate mapping of Dcx-expressing cells in dentate gyrus, we found that the Dcx-expressing cells with Mycn deletion matured faster than the control Dcx-expressing cells (Fig. 6), and the newly generated granule neurons with Mycn deletion migrated further than the control neurons (Fig. 7), suggesting that Mycn inhibits the maturation of adult neuroblasts and the newly generated neurons. Similarly, knockdown MYCN promotes neuronal differentiation in neuroblastoma [37], and retinoic acid, which induces morphological differentiation of neuroblastoma cells [38], inhibits the expression of MYCN in these tumor cells [39].

**Mycn Inhibits the Survival of Adult Neuronal Progenitor Cells**

With the fate mapping of Mki67-expressing proliferated cells in SVZ and CC (Fig. 8a–c), SGZ and SCZ (Fig. 8d–f), and OB (Fig. 8g, h) 14 days after they had expressed Mki67, we found that Mycn deletion from Mki67-expressing cells increased the number of the proliferated cells. The phenomenon of increased number of the proliferated cells was not the result of more cells being proliferated, because Mki67-expressing cells with Mycn deletion proliferated significantly...
less (Fig. 5). These results suggest that Mycn inhibits the survival of adult neuronal progenitor cells. The mechanism on how Mycn regulates the survival of adult neuronal progenitor cells needs to be further studied.

**Mycn Is Required for the Genesis of Adult Oligodendrocytes**

In Mycn-EGFP reporter mice, we found GFP (+) cells in CC (Fig. 1a) and SCZ (Fig. 1b), a known adult oligodendrogenesis site between hippocampus and CC [19]. Our fate-mapping study of Dcx-expressing cells revealed that these GFP (+) cells in CC originate from the Dcx-expressing cells in SVZ (Fig. 2d), and our IHC study showed that some of the GFP (+) cells in SCZ co-expressed DCX (Fig. 1f). Some of these GFP (+) cells in CC and SCZ also co-expressed Ki67 (Fig. 3a, c), suggesting that they were proliferating cells, and our fate-mapping studies showed that the majority of Mki67-expressing cells in these regions developed into Olig2 (+) oligodendrocytes (Supplementary Figs. 6d, e and 7c). In fact, some of the GFP (+) cells in CC were Olig2 (+) (Fig. 1l). These results suggest that the Mycn-expressing cells in these regions developed into oligodendrocyte lineage cells. Indeed, analysis of published scRNA-Seq from cells of oligodendrocyte lineage [24] showed that Mycn is expressed in OPCs, CDPs, and certain MFOLs and MOLs (Supplementary Fig. 3a).

We found that deletion of Mycn from Mki67-expressing cells blocked cell proliferation in SCZ (Fig. 5c, d), suggesting that Mycn is required for the cell proliferation in this region. In addition, with fate mapping of proliferated cells, we found significantly more survived cells that have recently proliferated in CC and SCZ of Mycn cKO animals (Fig. 8c, f). Thus, like in adult neurogenesis, Mycn promotes...
cell proliferation and inhibits cell survival in adult oligoden-drogenesis sites. Interestingly, CDK4, rather than CDK6, is essential for the proliferation of adult oligodendrocyte progenitor cells [40], and knockdown of CDK4 in neuroblas-toma cells reduces their proliferation [41]. In addition, both E2F1 [42] and p27 [43] contribute to the regulation of oligoden-drocyte progenitor proliferation. Thus, it would be interesting to investigate the possible interaction between Mycn and CBK-BR-E2F pathway in adult oligoden-drogenesis.

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Author Contribution J.C. performed and analyzed the data of the experiments and wrote the manuscript. Z.G. designed the experiments, wrote the manuscript, and supervised all aspects of the study.

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Data Availability No data or material needs to be submitted.

Code Availability No code needs to be submitted.

Declarations 

Ethics approval and Consent to Participate All animal procedures were performed according to protocols approved by Institutional Animal Care and Use of Laboratory Animals. Consent to participate is not needed.

Consent for Publication All the data is suitable for publication.

Conflict of Interest The authors declare no competing interests.

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