RESEARCH COMMUNICATION

***Tsc1* mutant neural stem/progenitor cells exhibit migration deficits and give rise to subependymal lesions in the lateral ventricle**

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Subependymal nodules (SENs) and subependymal giant cell astrocytomas (SEGAs) are common brain lesions found in patients with tuberous sclerosis complex (TSC). These brain lesions present a mixed glioneuronal phenotype and have been hypothesized to originate from neural stem cells. However, this hypothesis has not been tested empirically. Here, we report that loss of *Tsc1* in mouse subventricular zone (SVZ) neural stem/progenitor cells (NSPCs) results in formation of SEN- and SEGA-like structural abnormalities in the lateral ventricle, the consequence of abnormal migration of NSPCs following *Tsc1* loss.

Supplemental material is available for this article.

Received April 6, 2011; revised version accepted July 1, 2011.

Tuberous sclerosis complex (TSC) is a multisystem genetic disease characterized by benign tumors in the brain, kidney, and other vital organs. The genetic cause of TSC is a loss-of-function mutation in either the *TSC1* or *TSC2* genes, which encode the interacting proteins hamartin (*TSC1*) and tuberin (*TSC2*), respectively (Kwiatkowski and Manning 2005).

Brain lesions, including cortical tubers, subependymal nodules (SENs), subependymal giant cell astrocytomas (SEGAs), and white matter abnormalities, develop in 90% of TSC patients and are the most common and debilitating aspect of the disease (Marcotte and Crino 2006; Rosser et al. 2006; Napolioni et al. 2009). These abnormalities are believed to be at the root of neurological manifestations including epilepsy, mental retardation, and autism. SENs present as multiple small nodules along the lateral ventricle walls, giving a characteristic “candle dripping” appearance. SEGAs are histologically indistinguishable

from SENs, but are larger and tend to arise near the foramen of Monro. Neuroimaging studies have suggested that SEGAs arise from SENs (Morimoto and Mogami 1986; Fujiwara et al. 1989). Clinically, SEGAs present as benign, slow-growing tumors with a low mitotic index. However, SEGAs often obstruct cerebrospinal fluid flow, causing hydrocephalus and increasing intracranial pressure. SEGAs are initially characterized by strong immunoreactivity to astroglial cell markers, such as glial fibrillary acidic protein (GFAP) and S100 β (Burger et al. 2002; Lopes et al. 2007). However, both dysmorphic glial cells and neural cell types are present within the tumor mass. Many cells in SEGAs are also found to be reactive to neuronal markers, such as neurofilaments and synaptophysin (Burger et al. 2002; Lopes et al. 2007). Given this mixed glioneuronal phenotype, the astrocytoma nature of SEGAs has been challenged, and they are now more often referred to as subependymal giant cell tumors (SGCTs) (Marcotte and Crino 2006; Buccoliero et al. 2009; Napolioni et al. 2009). Furthermore, this mixed glioneuronal phenotype and the recent finding that SEGAs contain cells that express glial and neural progenitor markers have led to the hypothesis that the developmental origin of SENs and SEGAs might be neural stem/progenitor cells (NSPCs) (Ess et al. 2005). However, to date, this hypothesis has not been tested experimentally.

Although TSC1/2 function in the brain has been studied in several murine models (Uhlmann et al. 2002; Meikle et al. 2007; Feliciano et al. 2011), the role of TSC1/2 in the NSPC population is not clear. In rodents and humans, postnatal neurogenesis occurs mainly in the subgranular zone (SGZ) of the dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle (Ming and Song 2005). Throughout adulthood, these stem cell niches continuously produce new neurons. In particular, SVZ stem cells differentiate into neuroblasts as they migrate through the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into olfactory interneurons. The SVZ–RMS–OB pathway therefore provides an excellent system for studying TSC1/2 function in NSPC migration and differentiation.

In this study, we used genetic tools to ablate *Tsc1* in postnatal SVZ NSPCs. The resultant mice develop nodular protrusions on the brain lateral ventricle walls and small tumors near the interventricular foramen (IF) that recapitulate many features of human SENs and SEGAs. Further study revealed that development of these SEN- or SEGA-like structures resulted from abnormal aggregation and migration of NSPCs after *Tsc1* loss. Our data provide experimental evidence that TSC1 is involved in NSPC migration, and that *Tsc1* ablation in these cells leads to formation of SENs and SEGAs.

Results and Discussion

Tsc1^{Nestin} conditional knockout (cKO) mice develop structural abnormalities in the lateral ventricle

We previously generated a tamoxifen (TMX)-inducible *Nestin-CreER*^{T2} transgenic mouse line that enables targeting of NSPCs at various developmental stages (Supplemental Fig. S1; Chen et al. 2009). To study the function of the TSC1/2 complex in NSPCs, we crossed *Tsc1*^{loxP}

[**Keywords:** neural stem/progenitor cells; subependymal giant cell astrocytomas (SEGAs); subependymal nodules (SENs); tuberous sclerosis complex (TSC); lateral ventricle (LV)]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.16750211>.

mice (Meikle et al. 2007) with *Nestin-CreER^{T2}* mice. Progeny were induced with TMX at postnatal day 7 (P7) or 1 mo and examined at 3 mo and 6–7 mo, respectively (Supplemental Fig. S2A). For each cohort, TMX-treated *Nestin-CreER^{T2};Tsc1^{loxP/loxP}* mice (*Tsc1^{Nestin}* cKO mice) were compared with TMX-treated littermate wild-type mice and heterozygotes (*Nestin-CreER^{T2};Tsc1^{loxP/+}*). The *Tsc1^{Nestin}* cKO mice exhibited enlarged and heavier brains at both time points (Supplemental Fig. S2B,C). No body weight differences were observed between control and cKO groups.

Further analysis revealed that the enlarged *Tsc1^{Nestin}* cKO brains were accompanied by hydrocephalus, and to a lesser extent, an enlarged hippocampus (Fig. 1A; Supplemental Fig. S3A, left panels). Close examination of the dilated ventricles revealed the presence of abnormal structures in the lateral ventricles, specifically near the IF between the lateral and third ventricles (Fig. 1A,B; Supplemental Fig. S3). In the *Tsc1^{Nestin}* cKO brain, the IF was noticeably dilated, in contrast to the narrow channel connecting the lateral and third ventricles on comparable sections from normal brain (Fig. 1A, arrowheads indicate the narrow connection between ventricles). Upon H&E staining, we also noticed that, next to the normal IF, a neural stem cell (NSC)-rich subventricular region was visible as a thick layer of cells with dense, hyperchromatic

nuclei just beneath the ependymal cell layer (Fig. 1A, asterisk). This region displayed intense Cre activity upon TMX induction (Supplemental Fig. S1, right panels). However, in *Tsc1^{Nestin}* cKO brain, this stem cell-rich region was not obvious, and instead we observed the presence of abnormal structures inside the ventricle. More often, these abnormal structures that developed near the IF contained a small but well-circumscribed tumor with distinct histological features (Fig. 1A; Supplemental Fig. S3A), which was sometimes accompanied by multiple cell masses nearby (Supplemental Fig. S3A, arrows). Among the mice examined, 93.3% (28 of 30) of cKO mice induced at P7 and 100% (19 of 19) of cKO mice induced at 1 mo were found to have abnormal structures in the ventricles close to the IF. Additionally, in cKO mice, we also frequently observed small nodular protrusions on the wall of the lateral ventricle on the side of the striatum. These structures were made up of discrete clusters of cells with distinctive “candle dripping” morphology (Fig. 1B; Supplemental Fig. S3B).

The location and morphology of these small nodular structures and tumors are reminiscent of idiopathic SENs and SEGAs that develop in TSC patients. The small tumors near the IF were well circumscribed with an outer layer of densely packed cells that contained hyperchromatic nuclei surrounded by scanty cytoplasm. These features resemble those of NSPCs that are normally located in the SVZ just beneath the ependymal cell layer. The inner cells within these tumors did not resemble any type of normal brain cell but had similarity to the histopathology of TSC-associated SENs and SEGAs. When examined under higher magnification, the inner tumor mass was found to be composed of a heterogeneous cell population containing small spindle-like cells, as well as cells that resembled differentiated neurons, with prominent nuclei and abundant eosinophilic cytoplasm (Fig. 1C). These various cell types were loosely gathered in a highly fibrillary background. More interestingly, among the cell mass, some cells were unusually large (Fig. 1C, inset). Further study revealed that the proliferative index among the inner tumor mass was low, as measured by Ki67 staining (Fig. 1D, arrows). Although there were some Nestin-positive cells, consistent with the presence of stem/progenitor cells, most of the cells stained with either mature neuronal markers (MAP2 or NeuN) or astrocytic markers (S100 β or GFAP) (Fig. 1D,E). Processes from both MAP2-positive and GFAP-positive cells contributed to the fibrillary matrix (Fig. 1E), indicating a mixed glioneuronal composition. Thus, our tumors present several key histological features of human SEGA, including the anatomic location, a mixed glioneuronal composition, and the presence of “giant” cells. However, we did not observe multinucleated cells within these tumor masses, as is typically seen in human SEGAs.

Tsc1 mutant SVZ stem/progenitor cells exhibit aberrant migration

To investigate how these ventricular nodules and small tumors developed, we induced *Tsc1* recombination in a group of mice at P7 and examined them at 1 and 2 mo of age. We observed that in *Tsc1^{Nestin}* cKO mice, SVZ cells began to aggregate as they migrated to the lateral ventricle (Fig. 2A). The nodular protrusions on the lateral ventricle wall could be detected as early as 1 mo of age.

Figure 1. *Tsc1^{Nestin}* cKO mice develop SEN- and SEGA-like abnormalities. (A) *Tsc1^{Nestin}* cKO mice develop hydrocephalus and small tumors in the lateral ventricle, as shown by H&E staining on mice induced at P7 and examined at 3 mo of age. (Arrowheads) Interventricular foramen (IF); (*) stem cell-rich subventricular region; (LV) lateral ventricle; (3rdV) third ventricle. Bars: for whole forebrain pictures, 2 mm; for 4 $\times$ pictures, 500 μ m; for 10 $\times$ pictures, 200 μ m. (B) *Tsc1^{Nestin}* cKO mice develop small nodular excrescences in the lateral ventricle (mice induced at P7, examined at 3 mo of age). (St) Striatum. Bar, 200 μ m. (C) Histological features of the inner tumor cells near the IF. Bar, 40 μ m. Inset shows a giant cell within the tumor mass. Bar, 10 μ m. (D) Inner tumor cells are mostly cells immunoreactive for either neuronal (MAP2 and NeuN) or astrocytic (S100 β and GFAP) markers. (Arrows) Ki67⁺ cells. Bar, 50 μ m. (E) MAP2 (red) and GFAP (green) double-staining reveals mixed glioneuronal phenotype of cells inside the tumor and their fibrillated processes. Bar, 50 μ m.

Figure 2. Development of small nodular structures and tumors in the lateral ventricle involves aberrant aggregation and migration of SVZ NSPCs. (A) *Tsc1^{Nestin}* cKO mice progressively develop abnormalities in the lateral ventricle, as shown by mice induced at P7 and examined at 1 and 2 mo of age. (Arrows) Clusters of cells show aberrant aggregation and migration from the SVZ to the lateral ventricle. Bar, 200 μ m. (B) Both nodular structures and early tumor masses are composed of cells in which Cre activity has been induced, as visualized by X-gal staining on brain sections of *Tsc1^{Nestin}* cKO mice bearing the *R26-lacZ* reporter allele. Bar, 200 μ m. (C) PCR assay detected the recombined *Tsc1* allele ($\Delta Tsc1$ allele) in tumor samples but not in controls (SVZ samples of untreated *Nestin-CreER^{T2}*; *Tsc1^{loxP/loxP}* mice and ear samples of TMX-treated *Nestin-CreER^{T2}*; *Tsc1^{loxP/loxP}*). *Tsc1* loxP is the floxed allele. (D) Phospho-S6 staining indicates strong mTOR signaling in the tumor mass. Bar, 50 μ m. (E) Nodular structures and tumors that develop in the ventricle originate from NSPCs. Bar, 200 μ m. (F) Neuroblasts retained in the lateral ventricle give rise to neurons, as shown by DCX (green) and NeuN (red) double-staining. (Arrows) Cells that are double-positive for NeuN^{dim} and DCX. Bar, 50 μ m.

By 2 mo of age, it was common to see multiple nodular structures present (Fig. 2A, top panels), with some nodular structures appearing to have detached from the ventricle wall and floating inside the ventricle (Fig. 2A, arrowhead). In the IF of mutant mice, we observed SVZ cell clusters forming as they migrated to the lateral ventricles at 1 mo of age (Fig. 2A, arrows), and by 2 mo of age, we often detected bigger cell masses at the same location (Fig. 2A, bottom panels). We believe that these initial tumor masses give rise to the SEGAs-like structures seen later at 3 mo of age.

Given the restricted activity of the Cre transgene to stem/progenitor cells and the absence of phenotype in TMX-treated controls, we concluded that the abnormal nodules and tumors originated from SVZ NSPCs that had lost the *Tsc1* gene through Cre-mediated recombination. To confirm this, we examined Cre activity in the cells that comprise these abnormal structures using the *R26-lacZ* reporter line (Soriano 1999). We found that the nodular structures and the initial tumors were positive

for X-gal staining, indicating that Cre activity had been induced in those cells (Fig. 2B). Indeed, as a consequence, we detected mainly recombined *Tsc1* LoxP allele ($\Delta Tsc1$ allele) in the tumor sample, demonstrating that in tumor cells the *Tsc1* gene had been disrupted by Cre-mediated recombination (Fig. 2C). In addition, consistent with human SEGAs studies, we observed high levels of phospho-S6 expression within the tumor masses, indicating activated mTOR signaling, as a consequence of TSC1/2 complex loss of function (Fig. 2D; Kwiatkowski and Manning 2005). All of these data suggest that both the nodules and the tumors are likely derived from Nestin-positive NSPCs that have lost *Tsc1*.

Tsc1-null stem/progenitor cells undergo differentiation in the lateral ventricle

We next sought to evaluate how *Tsc1*-null NSPCs give rise to cell types that exhibit the histological features of human SENs and SEGAs. By immunostaining, we found that both the nodular structures and the initial tumors shared similar cell components (Fig. 2E). Both contained a subset of stem/progenitor marker-positive cells (Fig. 2E, Ki67 and Nestin staining), consistent with a stem/progenitor cell of origin. However, the majority of cells were Doublecortin (DCX)-positive, a marker for neuroblasts that derive from Nestin-positive NSPCs. Some of the inner cells of the initial tumor clearly displayed differentiated cell morphology. This was frequently observed in 2-mo-old cKO mice that were induced at P7. The more differentiated cell morphology was consistent with the reduced proliferation among the inner cell population of the tumor, as evidenced by Ki67 staining (Fig. 2E, Ki67 immunostaining in the bottom panels). Indeed, some of those cells expressed NeuN even when the cell mass was very small, suggesting that differentiated neurons were present in the cell mass. Interestingly, as shown by staining on adjacent sections of a small early tumor, there was some overlap of NeuN-positive and DCX-positive cells (Fig. 2E, NeuN and DCX immunostaining in the bottom panels). This was further confirmed by NeuN and DCX double-staining on many other tumor samples collected at 3 mo of age (Fig. 2F). Within the tumor mass, we clearly observed some dim NeuN-positive cells that retained DCX staining (Fig. 2F, arrows), suggesting that the DCX-positive neuroblasts retained in the lateral ventricle undergo further differentiation and give rise to neurons. These data provide evidence that the neuronal cell component of the tumors is derived from NSPCs, and not from entrapped nearby neurons.

Additionally, we observed GFAP-positive cells within the inner mass of the early tumors (Fig. 2E, GFAP immunostaining in the bottom panels). We hypothesized that those cells may have directly differentiated from Nestin-positive stem cells, as we observed Nestin-positive cells within the cell mass (Fig. 2E, Nestin immunostaining in the bottom panels). To evaluate whether loss of *Tsc1* affects the ability of NSCs to differentiate into different neuronal lineages, we isolated *Tsc1^{loxP/loxP}* SVZ NSCs from P7 pups and generated *Tsc1*-null SVZ NSCs by infecting the cells with Cre-GFP-expressing adenovirus. Loss of *Tsc1* was confirmed by Western blot analysis (Supplemental Fig. S4A). In cultured NSCs, loss of *Tsc1* did not result in obvious changes in morphology or proliferation (Supplemental Fig. S4B). Upon removal of growth factors, *Tsc1*-null NSCs were able to differenti-

ate into various neural lineages, including neurons, astrocytes, and oligodendrocytes (Supplemental Fig. S4C). Therefore, *in vitro*, loss of *Tsc1* does not affect the ability of NSCs to give rise to different neural lineages.

Impaired migration of OB granule neuron precursors

In wild-type mice, SVZ stem cells continuously generate DCX-expressing neuroblasts that migrate through the RMS to the OB, where they differentiate into granule or periglomerular interneurons (Ming and Song 2005). In the *Tsc1^{Nestin}* cKO mice, we observed that the NSPCs aggregated in the lateral ventricle and differentiated into neuroblasts, resulting in the formation of nodular structures and small tumors in the ventricles. We therefore predicted that this should have repercussions on the state of interneurons in the OB. Indeed, although the overall structure of the OB remained intact in the *Tsc1^{Nestin}* cKO mice, the cell density of the granule cell layer was significantly reduced compared with wild-type and heterozygote controls (Fig. 3A,B). This was not due to increased cell death, as shown by cleaved caspase-3 staining, although we observed a few apoptotic cells within the nodular structures, there was no obvious increase in apoptotic cells in either the SVZ or the OB of the *Tsc1^{Nestin}* cKO mice (Supplemental Fig. S5A,B). We reasoned that the reduced number of granule neurons in the OB was due to the decrease in new neurons coming from the SVZ. To visualize all of the new OB neurons generated, we included the *R26-LacZ* allele in heterozygous and *Tsc1^{Nestin}* cKO mice. Although β -gal-positive cells were present in the OB of *Tsc1^{Nestin}* cKO mice, they were significantly reduced compared with the heterozygous controls (Fig. 3C,D). Thus, the reduction in granule neuron

number coincides with the reduction in new neurons and is consistent with the observation that neuroblasts are retained in the lateral ventricle, where they form abnormal structures, rather than migrating to the OB.

The presence of β -gal-positive granule neurons in the OB of *Tsc1^{Nestin}* cKO mice indicated that not all *Tsc1*-null neuroblasts migrate to the lateral ventricle. Some mutant neuroblasts were able to migrate to the OB and differentiate into mature neurons (Supplemental Fig. S6A). This is not likely to be due to incomplete recombination of the *Tsc1* flox allele for two reasons. First, most of these β -gal-positive cells demonstrated higher phospho-S6 staining, a downstream marker of *Tsc1* inactivation; and second, the cells were noticeably larger, a known consequence of TSC1/2 complex downstream hyperactivation (Supplemental Fig. S6B). These data indicate that some fraction of *Tsc1*-null neuroblasts do not exhibit migration deficits. Thus, either the precise timing of *Tsc1* loss is critical to the cell, or other pathways are also involved in regulating neuroblast migration.

Loss of *Tsc1* in transit-amplifying neural progenitors results in abnormal growths in the ventricle

During our analysis of the *Nestin-CreER^{T2}-R26* mice, we noted that Cre activity was also present in the ependymal cells upon TMX treatment, as demonstrated by S100 β and YFP double-staining (Fig. 4A, left panel). Ependymal cells are highlighted with S100 β staining, and Cre activity upon TMX induction is reflected by YFP expression from the *R26-YFP* reporter allele (Srinivas et al. 2001). We believe that the Cre activity in ependymal cells reflects the nature of the *Nestin* promoter used in our *Nestin-CreER^{T2}* mouse line, as other reported transgenic lines using the same *Nestin* promoter also display transgene expression in ependymal cells (Yamaguchi et al. 2000; Imayoshi et al. 2008).

Ependymal cells and SVZ stem cells share the same lineage, both being derived from radial glial cells that exist during early developmental stages (Merkle et al. 2004; Spassky et al. 2005). Therefore, it is likely that in human patients, these two cell types could share the same somatic mutations. Additionally, it is known that ependymal cells provide an important niche for stem/progenitor cells to grow. We therefore wanted to determine whether, in our mouse model, loss of *Tsc1* in the ependymal cells contributed to the migration deficit. To address this question, we crossed *Tsc1* flox mice with the *Ascl1-CreERTM* transgenic line, which drives *Cre* expression in SVZ transit-amplifying neural progenitors but not in ependymal cells (Fig. 4A,B; Kim et al. 2007). Additionally, with the *Ascl1-CreERTM* line, only transit-amplifying neural progenitors are targeted, but not NSCs. In this way, using acute TMX treatment, we could temporally target a wave of neurogenesis. To target as many neural progenitors as possible, we performed the initial induction at P7 and boosted it at P9 and P11. Examination of mice at 1 mo of age with X-gal staining indicated successful targeting of a small percentage of SVZ forebrain progenitors, compared with the *Nestin-CreERTM* line (Fig. 4A; Supplemental Fig. S1). We then examined the *Ascl1-CreERTM*; *Tsc1^{loxP/loxP}* cKO mice (herein referred to as *Tsc1^{Ascl1}* cKO mice) at 6 mo of age along with littermate controls. Gross examination of *Tsc1^{Ascl1}* cKO mice revealed no overt brain abnormalities and no obvious deficits in the SVZ or most of the ventricle region (Fig. 4C, left panels).

Figure 3. *Tsc1^{Nestin}* cKO mice have fewer granule cells in the OB due to reduced migration. (A) *Tsc1^{Nestin}* cKO mice have less granule cells in the OB. Bars: left panels, 500 μ m; right panels, 100 μ m. (B) Quantification of cell density in medial granule cell layer (GCL) of OB. $n = 3$ mice were examined for each genotype. Data are plotted by mean $\pm$ SEM and analyzed by Student's *t*-test; (***) $P < 0.01$. (C) Fewer newly generated granule cells in the OB were observed in *Tsc1^{Nestin}* cKO mice. Bar, 50 μ m. (D) Quantification of β -gal-positive cells in the medial granule cell layer. $n = 3$ pairs of mice were examined. Data are plotted by mean $\pm$ SEM and analyzed by Student's *t*-test; (***) $P < 0.01$.

Figure 4. *Tsc1* loss in transit-amplifying neural progenitors results in a neuroblast migration deficit. (A) Cre activity was induced in both SVZ NSPCs and ependymal cells in *Nestin-CreER^{T2}* mice, while in the *Ascl1-CreERTM* mice, only a limited number of SVZ progenitor cells are targeted upon TMX induction. Ependymal cells are marked by S100β (red) staining. Cre activity is marked by YFP (green) staining. *Nestin-CreER^{T2};Tsc1^{loxp/+};R26-YFP* mice and *Ascl1-CreERTM;Tsc1^{loxp/+};R26-YFP* mice were treated with TMX at P7 (for *Nestin-CreER^{T2}* mice) or at P7, P9, and P11 (for *Ascl1-CreERTM* mice) and examined at 1 mo of age. Bar, 50 μm. (B) Diagram illustrating *Ascl1* expression in transit-amplifying neural progenitor cells. (C) Gross examination revealed that *Tsc1^{Ascl1}* cKO mice develop small abnormal growths in the lateral ventricle near the IF. Bars: for whole forebrain pictures, 2 mm; for 4× pictures, 500 μm; for 10× pictures, 200 μm. (D) Abnormal growths in the lateral ventricle of *Tsc1^{Ascl1}* cKO mice are composed of Cre-positive cells. Cre activity is visualized by X-gal staining on brain sections of *Tsc1^{Ascl1}* heterozygous and cKO mice bearing the *R26-lacZ* reporter allele. Bar, 200 μm.

However, more than half of the *Tsc1^{Ascl1}* cKO mice (eight of 14, 57.1%), had small abnormal growths in the lateral ventricle near the IF (Fig. 4C, boxed area); four of these eight mice had developed obvious hydrocephalus. We examined Cre activity using the *R26-lacZ* reporter line and saw many blue cells retained in the SVZ of *Tsc1^{Ascl1}* cKO mice. More importantly, the abnormal growths that developed in the ventricle were found to be mainly composed of cells that were X-gal-positive (Fig. 4D), indicating that the abnormal growths originated from the subset of transit-amplifying neural progenitor cells that had lost *Tsc1*. Additional examination demonstrated that these abnormal growths were similar to the tumors in the *Tsc1^{Nestin}* cKO mice that developed in the same location (Supplemental Fig. S7). We interpret the lower frequency and smaller size of the abnormal growths in the *Tsc1^{Ascl1}* cKO mice, as compared with *Tsc1^{Nestin}* cKO mice, to reflect the limited number of neural progenitor cells that were targeted. By the TMX pulse, we only induced *Tsc1* recombination in a small proportion of transit-amplifying cells present at induction. Nevertheless, our data indicate that loss of *Tsc1* in transit-amplifying neural progenitors is sufficient to cause a migration deficit in neuroblasts. Therefore, loss of *Tsc1* in ependymal cells is not required for the abnormal migration behavior of NSPCs seen in the *Tsc1^{Nestin}* cKO mice.

Considering the many TSC-associated brain lesions, the observation that loss of *Tsc1* in the NSPC population alone is sufficient to cause aberrant neuroblast migration

may have larger implications. Migration deficits following *Tsc1* loss may be a general underlying mechanism for other TSC-related brain lesions as well. For example, cortical tubers, the most common TSC-associated brain lesion, are characterized by disrupted cortical lamination and the presence of aberrant glia and neurons within the tuber, possibly due to a migration problem of the NSCs during formation of the cortical layers. This idea is supported by a recent study in which inducing *Tsc1* loss in a small subset of cortical progenitor cells by in utero electroporation at embryonic day 15 (E15) resulted in tuber-like structure formation (Feliciano et al. 2011). However, while we have demonstrated that *Tsc1* loss in ependymal cells is not required for the *Tsc1*-null NSPC migration deficits, we do not exclude the possibility that loss of *Tsc1* in ependymal cells can facilitate tumor formation or contribute to the development of hydrocephalus in our model.

While our tumors recapitulate several key features of human SEGAs, we did not observe pleomorphic multinucleated tumor cells that are frequently seen in human SEGAs. We note that mouse models of cortical tubers also possess some, but not all, histological features of tubers (Meikle et al. 2007; Feliciano et al. 2011). This may reflect differences between species, or it may be that some histological features take a longer time to develop, and in our mouse model, we have only captured the initial stages of SEGA development.

One interesting question is why SEGAs preferentially develop near the foramen of Monro. In our mouse model, nodular structures that developed on the lateral ventricle wall did not become very large and tended to “fall” into the ventricle,

whereas similar cell masses that formed near the IF grew bigger and developed into small tumors. We believe this ventricular region might be advantageous for tumor development due to its stem cell-rich environment and the narrow ventricular space, which could facilitate formation of initial cell masses and provide a supportive growth environment. It is possible that multiple cell masses develop simultaneously and then merge into one large mass. This is supported by the observation of individual cell masses in this area (Fig. 2A, arrows; Supplemental Fig. S3A, arrows). This idea has also been proposed in human SEGA development; it is believed that SEGAs originate from SENs near the foramen of Monro (Morimoto and Mogami 1986; Fujiwara et al. 1989).

In summary, we show that *Tsc1* loss specifically in NSPCs can drive the development of SEN- and SEGA-like structures in the ventricles, due to aberrant aggregation and migration of SVZ NSPCs following *Tsc1* loss. Our study offers direct genetic evidence that the cells of origin of human SENs and SEGAs are the NSPCs, thus providing new insight into TSC1/2 function in the nervous system.

Materials and methods

Animals and TMX treatment

Nestin-CreER^{T2} and *Ascl1-CreERTM* mice have been described previously (Kim et al. 2007; Chen et al. 2009). *Tsc1^{loxp}* mice were a gift from

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Dr. David Kwiatkowski (Harvard Medical School, Boston) (Meikle et al. 2007). TMX (Sigma) was dissolved in sunflower seed oil (Sigma). To activate *CreER^{T2}* in *Nestin-CreER^{T2}* mice, 0.5 mg of TMX was intraperitoneally delivered to P7 mice, and 10 mg/20 g body weight of TMX was orally administered to 1-mo-old mice once a day for two consecutive days. To induce Cre activity in *Ascl1-CreERTM* mice, 0.5 mg of TMX was intraperitoneally delivered to P7 mice, and the induction was boosted at P9 and P11.

Microdissection and PCR assay for detecting recombined *Tsc1* allele

Genomic DNA from tumor samples was isolated from paraffin sections by microdissection under the microscope. Tumor location was determined by methyl green staining on the section prior to microdissection. Genomic DNA from both the microdissected SVZ sample of untreated *Nestin-CreER^{T2};Tsc1^{loxP/loxP}* mice and the ear sample of TMX-treated *Nestin-CreER^{T2};Tsc1^{loxP/loxP}* served as control.

Histology and immunohistochemistry

Histology and immunohistochemistry are described in the Supplemental Material.

Acknowledgments

We thank Dr. David Kwiatkowski for the *Tsc1^{loxP}* mice, and Drs. Dawen Zhao and Jian Chen for helpful discussion. This work was supported by the Simons Foundation (to L.F.P.). L.F.P. is an American Cancer Society Research Professor.

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Genes Dev. 2011, **25**:

Access the most recent version at doi:[10.1101/gad.16750211](https://doi.org/10.1101/gad.16750211)

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