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Embryonic cerebrospinal fluid influence in the subependymal neurogenic niche in adult mouse hippocampus

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ABSTRACT

The adult mouse hippocampal neurogenic niche is a complex structure which is not completely understood. It has mainly been related to the Subgranular layer of the dentate gyrus; however, as a result of differential neural stem cell populations reported in the subventricular zone of the lateral ventricle and associated with the hippocampus, the possibility remains of a multifocal niche reproducing developmental stages. Here, using a set of molecular markers for neural precursors, we describe in the adult mouse brain hippocampus the existence of a disperse population of neural precursors in the Subependymal Zone, the Dentate Migratory Stream and the hilus; these display dynamic behaviour compatible with neurogenesis. This supports the idea that the adult hippocampal niche cannot be restricted to the dentate gyrus subgranular layer. In other neurogenic niches such as the Subventricular Zone, a functional periventricular dependence has been shown due to the ability to respond to embryonic cerebro-spinal fluid. In this study, we demonstrate that neural precursors from the three areas studied (Sub-ependymal Zone, Dentate Migratory Stream and hilus) are able to modify their behaviour by increasing neurogenesis in a locally differential manner. Our results are compatible with the persistence in the adult mouse hippocampus of a neurogenic niche with the same spatial structure as that seen during development and early postnatal stages.

1. Introduction

Neurogenic niches in adult brain mammals are of great interest in neurosciences as a key tool for developing strategies for neuronal regeneration. Probably the best-known neurogenic niche in adult mammals is the "subventricular zone" (SVZ), which has been related with physiological neuroregeneration in the olfactory system (Alvarez-Buylla and Lim, 2004; Johansson et al., 2010; Kazanis et al., 2008). The main characteristic of the SVZ niche is the existence of a neural precursor population dependent of the brain's lateral ventricle surface, which preserves its ability to self-renew, migrate via a predetermined pathway (the Rostral Migratory Stream) to the olfactory bulb, and differentiate to replace interneurons.

Previous data reported experimental evidence of a functional relation between SVZ neural precursor activity and cerebrospinal fluid (Gato and Desmond, 2009; Lehtinen and Walsh, 2011). The influence of embryonic cerebrospinal fluid on neural precursor behaviour induces an increase in DNA synthesis and leads to an expansion of the neural precursor population, together with a significant enhancement of neurogenic differentiation from neural precursors (Alonso et al., 2017; Gato et al., 2020). Taking these studies together, we can define the subventricular niche as a "Periventricular Niche" because of its dependence on cerebrospinal fluid influence.

Another neurogenic niche has been recognised in the mammal adult brain, located in the dentate gyrus of the hippocampus and associated with neuronal replacement involved in short memory neuronal circuits (Seri et al., 2001, 2004).

The entire neurogenic hippocampal niche in adult mammals has been located at the subgranular layer of the dentate gyrus, where a reduced population of neural precursors with astrocytic characteristics, is able to migrate to the granular layer and differentiate into new neurons, which establish connections with the hippocampal CA3 area

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(Ihunwo et al., 2016; Zhao et al., 2008). Therefore, the hippocampal neurogenic niche can be considered a "non-periventricular neurogenic niche", and consequently outside the influence of the ventricular cavity and its content of cerebrospinal fluid.

The precursor population of the hippocampal neurogenic niche does not reveal full neuronal precursor characteristics (self-renewal and multipotentiality). Consequently, the local (subgranular layer) self-renewing capacity is not clearly demonstrated and these precursors are considered neuronally compromised precursors instead of genuine neural precursors (Clarke and van der Kooy, 2011; Seaberg and van der Kooy, 2002). In addition, a further neural precursor population has been located close to the dentate gyrus in the subependimal zone (SEZ), showing full neural precursor activity (Cuccioli et al., 2015), and the possibility of the involvement of both populations in a sole dispersed neurogenic niche has been suggested (Berg et al., 2018; Sachewsky et al., 2014).

This hypothesis is supported by the developmental origin of the hippocampal niche. During development, the hippocampal niche appears as a prosencephalic wall cell population, which, under the influence of an organizing centre called the "cortical Hem", comes into direct contact with the lateral ventricle and its content (Cerebrospinal fluid). This cellular population is able to generate several waves of neural precursors migrating from the lateral ventricle to the dentate gyrus, along a specific pathway, namely, the dentate migratory stream (DMS) located just beneath CA3 (Parisot et al., 2017), in order to differentiate into neurons. Finally, in adult age, this prosencephalic neural precursor population seems to disappears, and only the cells located at the granular layer of the dentate gyrus remain active (Hatami et al., 2018; Kriegstein and Alvarez-Buylla, 2009).

However, the existence of multifocal neurogenic niches, which remains in adult mammal brain, requires further research. Previous research have demonstrated that the (SVZ) neurogenic niche, both during embryonic and adult stages, is functionally dependent on cerebrospinal fluid content in the brain ventricles, which leads us to consider it a "periventricular niche" (Gato et al., 2020; Zappaterra and Lehtinen, 2012). In this study, we attempt to demonstrate that, in the adult mammal brain hippocampus also remains as a multifocal neural precursor population with the capacity to respond to embryonic cerebrospinal fluid signals with an increase neurogenetic activity.

2. Materials and methods

2.1. Animals

Adult Swiss mice were used in this study with the support of the animal research and welfare service of the University of Valladolid. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Valladolid and were in accordance with the European Community guiding principles on the care and use of animals for research.

2.2. Obtaining embryonic cerebrospinal fluid

This study focused on the neurogenic influence of e-CSF in adult mouse hippocampal neural precursors, similarly to what we previously described in those of the Subventricular Zone (Alonso et al., 2017). Embryonic CSF has specific characteristics (it is a powerful neurogenic inducer in embryonic and adult neural precursors) and is produced by the neuroepithelial cells in the developmental stages before choroid plexus evolution (Fame and Lehtinen, 2020). In mice, the choroid plexus develops functionally at 14.5 days, becoming responsible for Foetal CSF secretion and regulation (Kompaníková and Bryja, 2022). Consequently, we chose 13.5-day Embryonic CSF in our study.

Mice embryos of 13.5-day developmental stage were explanted and dissected from the extra-embryonic membranes. The Embryonic Cerebrospinal fluid (eCSF) was obtained by micro-aspiration from the mesencephalic cavity, as previously described (Gato et al., 2004). eCSF samples were kept at 4º C during handling and then aliquoted (20 μL), lyophilized and frozen at − 40º C until used. We used eCSF from 13.5-day mice embryos owing to the difficulty of obtaining a sufficient amount of eCSF at previous stages.

2.3. Brain Brdu incorporation

We used BrdU incorporation to DNA as a neural precursor lineage tracer (Landgren and Curtis, 2011). Three-month-old adult mice of both sexes were treated with bromodeoxyuridine (BrdU) in order to identify DNA synthesis neural stem cells activity in the adult mouse brain. Two 0.1 ml doses of BrdU (3 g/l) were injected intraperitoneally at an interval of 48 h. The choice of the BrdU administration protocol is aimed at labelling a sufficient number of cells in order to localize neural precursors in the Subependimal zone and their migratory pathway to the dentate gyrus. 7 days later from the last by BrdU injection, we sacrifice by an injection of a lethal sedative dose plus an analgesic (0.18 ml Imalgene®; 0.07 ml Rompun®).

In our study, we use a dose of BrdU (0.6 mg/mice) which is slightly lower than that normally employed (Farioli-Vecchioli et al., 2012; Taupin, 2007) in order to avoid the toxic effects of BrdU DNA incorporation in neural precursor viability (Taupin, 2007).

2.4. Organotypic culture of sections containing lateral ventricle SVZ

Brain sections organotypic cultures were performed according to current protocols (Schommer et al., 2017; Stoppini et al., 1991). Animals were housed and handled in accordance with Spanish animal welfare regulations (RD53/2013) and in agreement with the European community Council Directive (2010/63/EU). Adult mice brains from both sexes were obtained by surgery under deep anesthesia. Upon removal, each brain hemisphere was immersed in sterile saline at for 1.5 h 4 C, then placed in 3% liquid agar and leave at room temperature. Coronal 300-mm-thick sections from the hippocampal zone, were obtained with a Vibratome and carefully placed onto a Millipore filter paper (0.8 mm pore diameter). In each brain slice, under binocular microscope and with micro-forceps, a total of 5–6, latex micro-beads (50–100 μm diameter Sigma SD-91) were implanted close to the ventricular surface (SEZ). Latex micro-beads were previously soaked for 24 h at 4 C either in the culture medium or 13,5 days mice embryos e-CSF. Finally, brain sections were covered with a collagen layer (collagen 1% in DMEM-Fetal Bovine Serum 8:1:1). Brain sections were then cultured in a floating system, as previously reported (Gato et al., 2005). We used DMEM supplemented culture medium with 1% Penicillin/Streptomicyn + 25% of horse serum + 6 mg/ml Glucose + 25% HBSS, at 37 C and in a 5% CO2 atmosphere for 7 days. Following culture, the samples were fixed in Carnoy's solution for 1 h, embedded in paraffin and sectioned at 8 μm. The histological sections were processed either for histological staining (Nissl technique) or for BrdU immunolabelling, either alone or in combination with selected cytoplasmic and receptor markers for neural stem cells.

In this study, we use 8 animals, 4 for each experimental condition (Control and eCSF treated).

2.5. Immunocytochemistry

2.5.1. Sox-2/BrdU immunofluorescence labelling

Histological brain sections from BrdU-treated mice were dewaxed, rehydrated and treated with HCl (2 M) for 1 h at 37ºC, in order to denature the DNA for BrdU immunolabelling. To prevent unspecific labelling, endogenous peroxidase was inhibited with 3% H_2O_2 in methanol for 15 min at room temperature. Next, the sections were incubated with anti-BrdU primary antibody (Dako monoclonal mouse anti-BrdU, dilution 1:100) and anti-Sox-2 developed in rabbit (Sta. Cruz® Ref.: SC17319) at 1/100 dilutión, overnight at 4º C in a wet

chamber. After being washed, the sections were incubated for 30 min with secondary antibodies for anti-Sox-2 and anti-BrdU, we used anti-rabbit Ig G-Alexa Fluor® 594 (Invitrogen. Ref.: A11008) at 1/1000 dilución, and Anti-mouse Ig G-Alexa Fluor® 488 (Invitrogen. Ref.: 10680) at 1/1000Dilución, respectively. Both were incubated for 1 h in a dark humidity chamber.

2.5.2. Doublecortin/BrdU immunofluorescence labelling

A similar procedure to the previous one was followed with a monoclonal rabbit anti-Doublecortin antibody (Abcam, dilution 1:1000) and a monoclonal mouse anti-BrdU (Dako dilution 1:100). As secondary antibodies for anti-Doublecortin and anti-BrdU, we used Alexa Fluor 488 goat anti-rabbit (Invitrogen, dilution 1:1000) and Alexa Fluor 594 goat anti-mouse (Invitrogen, dilution 1:1000), respectively. Both were incubated for 1 h in a dark humidity chamber.

2.5.3. βIII-tubulin/BrdU immunofluorescence labelling

Histological brain sections from BrdU-treated mice were processed as previously described to denature DNA and then incubated with two primary antibodies: monoclonal rabbit anti-βIII-tubulin (Sigma, dilution 1:100) and monoclonal mouse anti-BrdU (Dako, dilution 1:100). Both were simultaneously incubated overnight at 4ºC in a humidity chamber. The secondary fluorochrome-labelled antibodies used were Alexa Fluor 488 goat anti-rabbit (Invitrogen, dilution 1:1000) and Alexa Fluor 594 goat anti-mouse (Invitrogen, dilution 1:1000) for anti βIII-Tubulin and anti-BrdU, respectively. Both were incubated for 1 h in a dark humidity chamber.

2.5.4. Calretinin/BrdU immunofluorescence labelling

A similar procedure to the previous one was followed with a monoclonal anti-Calretinin antibody (Milipore® AB5054, dilution 1:200) developed in rabbit and a monoclonal mouse anti-BrdU (Dako dilution 1:100). As secondary antibodies we used Alexa Fluor 488 goat anti-rabbit (Invitrogen, dilution 1:1000) and Alexa Fluor 594 goat anti-mouse (Invitrogen, dilution 1:1000), respectively. Both were incubated for 1 h in a dark humidity chamber.

2.6. Detection of neuroblasts and new neurons

To quantify the results, 16 half brains were studied, 8 from each experimental condition (eCSF treated and Controls). We chose 10 images at 40 × magnification from each preselected area (SEZ, DMS and hilus), and at least one from each control or eCSF treated half-brain. BrdU-positive NSC nuclei co-labelled with each one of the other cellular markers employed, were photographed with a Leica TCS SPE confocal laser microscope in order to identify and count the positive co-labelled cells.

2.7. Statistics

The total number of double-immunolabelled cells in each case was counted and plotted on the graph bars as the mean ± standard deviation. We used 10 different images to evaluate each double immune-histochemical label and area studied, and at least one of these came from each of the eight half-brain controls or the eCSF-treated ones (n: 8).

Statistical analyses of data from the three areas studied (Fig. 1) were conducted by one-way ANOVA analysis of variance, followed by a post-hoc Bonferroni test (the significance threshold was set at $p \leq 0.05$ *). In order to compare results between the Control and eCSF-treated samples (Fig. 2), we used a two-tailed Student's t-test (the significance threshold was set at $p \leq 0.001$ ** or $p \leq 0.05$ *).

2.8. TUNEL assay

We assessed apoptosis by means of the TUNEL technique on paraffin sections. Apoptotic cells were detected using the Apoptosis Detection System Fluorescein Kit (Promega) following the manufacture's instructions. Visualization was made with a confocal microscope (Zeiss LSM-310). We performed quantitative analysis by counting in SEZ, DMS and hilus, the number of stained nuclei in 6 microscopic images from 3 different samples. The statisticall significance was tested by a one-way ANOVA analysis of variance followed by a post-hoc Bonferroni test (the significance threshold was set to $p \leq 0.05$ *).

3. Results

3.1. Parahippocampal multifocal neurogenic niche related with the brain lateral ventricle

Firstly, we endeavoured to confirm the presence of active neural precursors in the nervous tissue close to the adult dentate gyrus of the hippocampus, which includes the subependimal zone of the lateral ventricle (SEZ), the dentate migratory stream (DMS) and the hilus. For this purpose, we used immune-detection of colabelled BrdU-Sox2 cells for the study in control brains. Our results show the presence of nuclear BrdU-Sox2 positive cells (compatible with undifferentiated neural precursors) in the three locations studied SEZ (Fig. 1A), DMS (Fig. 1B) and hilus (Fig. 1C) in mouse adult brain. In a quantitative study of co-labelled Brdu-Sox-2 precursors (Fig. 1 D), the number of BrdU-Sox 2 positive cells does not shows statistically significant differences between the SEZ and the two other studied areas. This result was compatible with the existence of a multifocal neurogenic niche with periventricular features close to the adult hippocampal brain, as occurs in developmental stages.

A second step in our study was the co-labelling of presumptive precursor cells with BrdU and DCX, a migratory precursor labeller. As shown in Fig. 1 E, F and G, presumptive precursors with migratory features can also be detected in the three areas studied. However, quantification of co-labelled cells (Fig. 1 H) reveals a paradoxical but statistically significant decrease in migratory precursors in DMS with respect to SEZ, and particularly with the hilus, suggesting that precursors from different locations behave in a specifically dynamic manner. Furthermore, the fact that the number of mature neurons did not increase progressively from the SEZ to the hylus, as occurs with newborn neurons (Fig. 1P), may reflect migratory restrictions, partial neuronal death, or the existence of other neuronal targets outside the hilus. We observed the presence of increased apoptosis in DMS by TUNEL technique and the study did not show any significant differences with respect to SEZ or hillus (data not shown).

Next, we attempted to detect how many of these neural precursors acquire molecular neuronal features, revealing ongoing neurogenesis. For this purpose, we carried out co-labelling with BrdU and β III tubulin (specific for newborn neurons). As can be seen in Fig. 1 I, J and K, newborn neurons from neural precursors were detected in all three areas studied (SEZ, DMS and hilus). These results suggest that each focal location may be able to support precursor neurogenesis, being compatible with self-sufficient neurogenic niches in the three different areas studied. Quantification of BrdU-β III tubulin positive cells (Fig. 1L) shows a progressive increase in neurogenesis from the SEZ to the Hilus, which could be explained by a summative effect of neurogenesis in migratory new-born neurons from each focal location, reaching maximal neurogenic values in the Hylus close to the Dentate Gyrus. This is compatible with the hypothesis of a persistent migratory way from the SEZ to the Dentate Gyrus in the adult brain.

Additionally, we assume that BrdU-Calretinin co-expression may be considered specific for mature neurons derived from neural precursors. As revealed in Fig. 1M, N and O, we detected BrdU-Calretinin co-labelled cells in all three locations studied without significant differences, which reinforce the idea that each of the locations investigated in this study, has a fully active functional neurogenic niche.

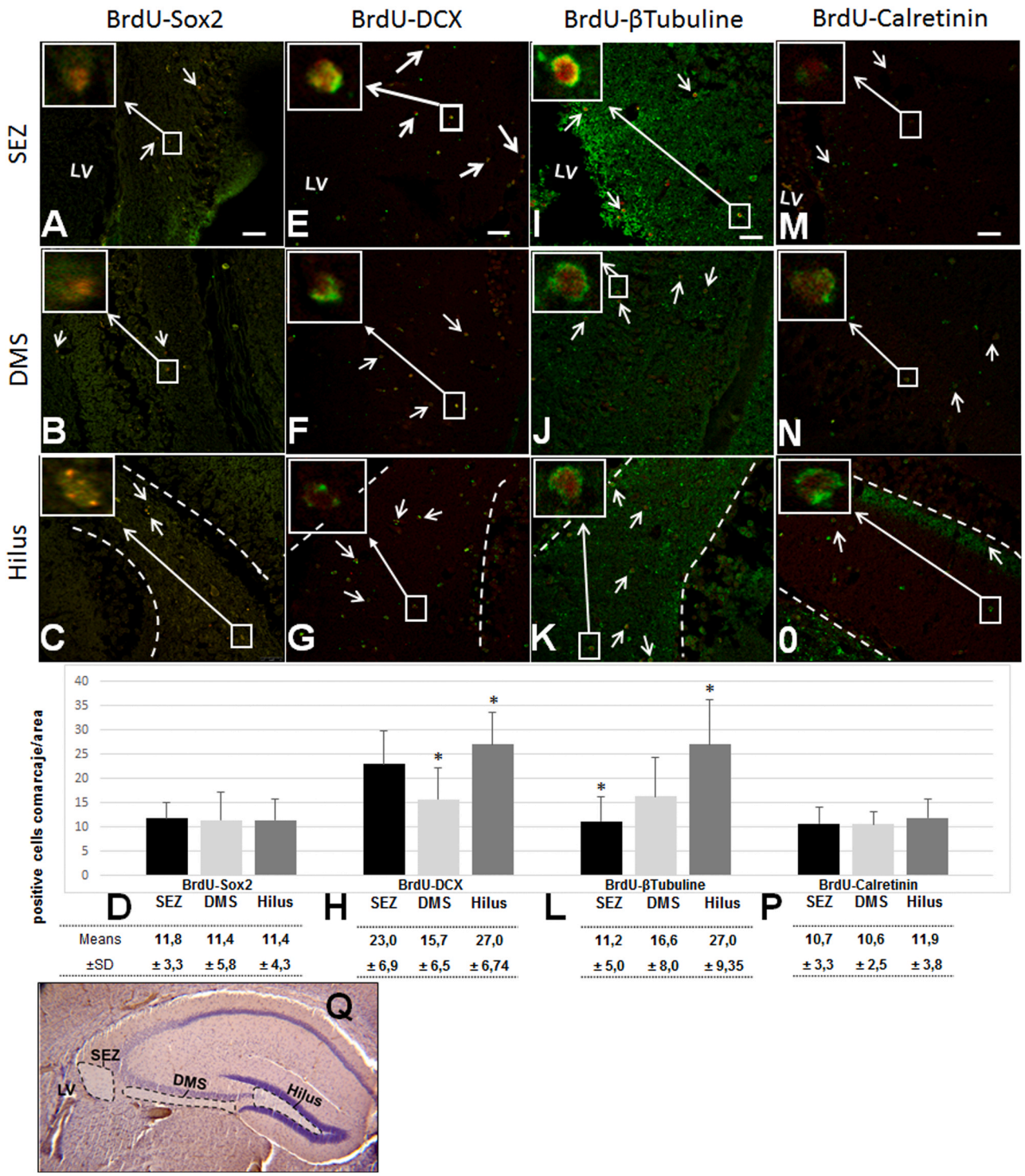

Fig. 1. Immunofluorescent images obtained by Laser Confocal Microscopy from adult mouse brain histological sections showing the hippocampal structure. Images show a view of the areas studied: Subependymal Zone (SEZ) close to the posterior horn of the Lateral Ventricle (LV) (Images A, E, I and M), the Dentate Migratory Stream (DMS) (Images B, F, J and N), and the hilus in the Dentate Gyrus (Images C, G, K and O). Image Q shows the limits and location of the studied areas. White arrows mark co-labelled cells. Inserts are high magnification showing co-labelled cells.Images A, B and C show identification of non-differentiated precursor cells by co-expression of BrdU (red) as a lineage tracer and Sox-2 (green) as a non-differentiation marker. Image D shows quantification of the number of co-labelled cells in the three differentiated areas. Graph bars express mean ± standard deviation. Images E, F and G show identification of migratory neural precursor by co-expression of BrdU (red) as a cell lineage tracer and Doublecortin (green) as a migratory neural precursor marker. Image H shows quantification of the number of co-labelled cells in the three differentiated areas. Graph bars express mean ± standard deviation. Images I, J and K show identification of young neurons deriving from Neural Stem Cells by co-expression of BrdU (red) as a cell lineage tracer and βIII Tubulin (green) as a newborn neuronal marker. Image L shows quantification of the number of co-labelled cells in the three differentiated areas. Graph bars express mean ± standard deviation. Images M, N and O show identification of neurons deriving from Neural Stem Cells by co-expression of BrdU (red) as a cell lineage tracer and Calretinin (green) as a mature neuronal marker. Image P shows quantification of the number of co-labelled cells in the three differentiated areas. Graph bars express mean ± standard deviation. Data were analyzed by means of a one way ANOVA post-hoc Bonferroni test. The significance threshold (*) was stablished at $p \leq 0.05$. $n = 8$. Scale bar in all figures: 25 μm.

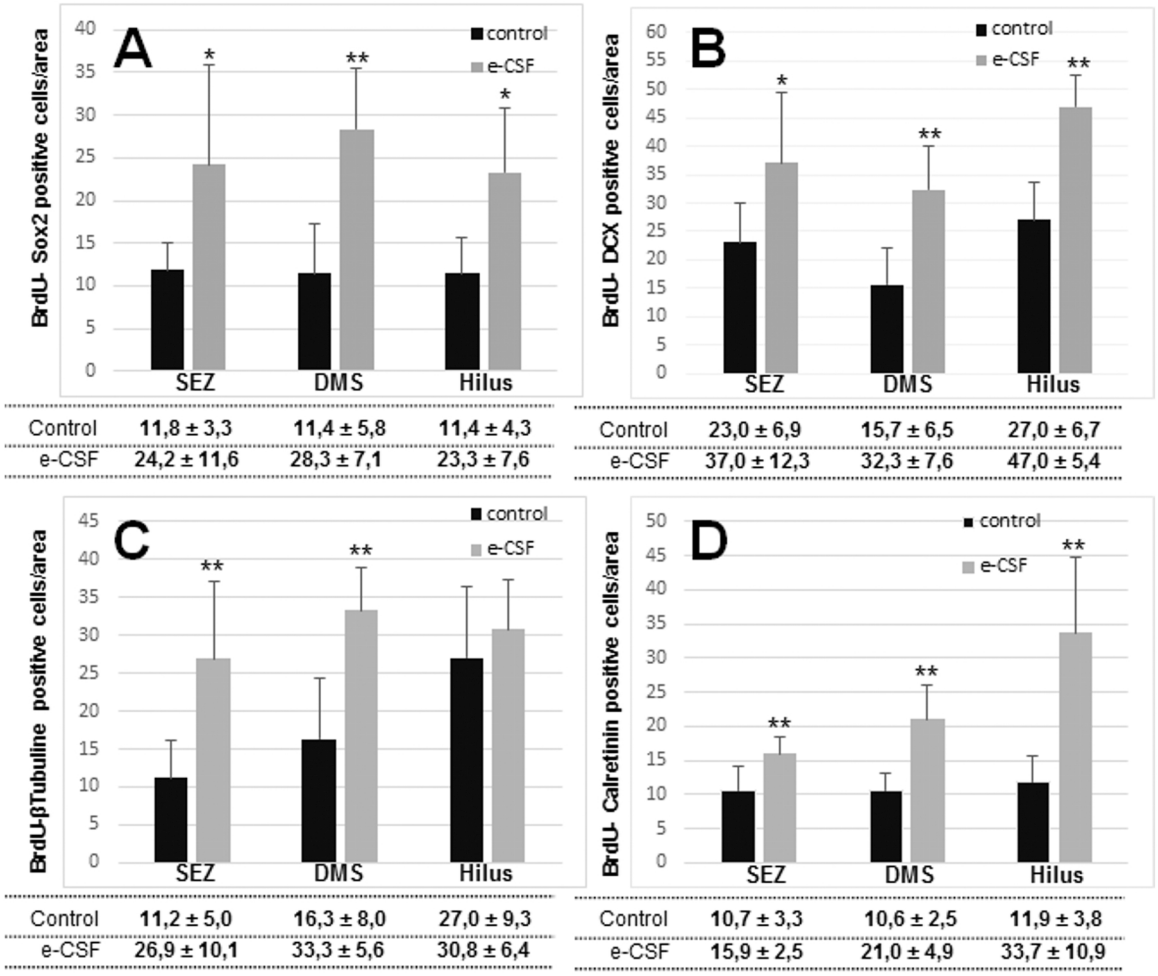

Fig. 2. Diagram bars showing the influence of embryonic cerebro-spinal fluid (eCSF) in the behaviour of adult mouse hippocampal neural precursors, detected in Subependymal zone (SEZ), Dentate migratory stream (DMS) and Hilus.Image A shows the quantitative differences of non-differentiated neural precursors (BrdU-Sox2 co-labelled cells), between Controls and eCSF-treated brain sections.Image B shows the quantitative differences of migratory ability (BrdU-DCX co-labelled cells) in the same neural precursor as in A between Controls and eCSF-treated brain sections.Image C shows comparative quantification of early neuronal differentiation (BrdU-βIII Tubulin co-labelled cells) between Controls and eCSF-treated brain sections. Image D shows comparative quantification of mature neurons (BrdU-Calretinin co-labeled cells) between controls and eCSF treated brain sections. Data were analyzed by means of a two-tailed student test in order to compare the results between control and e-CSF treated groups. The significance threshold were established at $p \leq 0.001$ (**) or $p \leq 0.05$ (*).

3.2. This multifocal neurogenic niche behaviour respond to cerebrospinal fluid neurogenis stimulus

The second part of our study was to verify to what extent this neural precursor population located between the SEZ and the hilus can be considered a "Periventricular system", maintaining the capacity to respond to CSF stimulus. To test this hypothesis, we exposed in vitro cultured adult mouse brain sections to 13.5-day mice embryos cerebrospinal fluid, which has been shown to increase precursor replication and neurogenesis in the other adult brain neurogenic niche, the SVZ neural precursors.

Our results show that neural precursors exposed to e-CSF undergo specific changes in their behaviour in the three areas studied, which hints at a persisting of the capacity to respond to specific signals from CSF with respect to neural differentiation and support periventricular niche features.

In Fig. 2 A, which corresponds to non-differentiated neural precursors (co-labelled with BrdU-Sox 2), a homogeneous but significant increase in the number of these cells can be appreciated in all three areas studied in the eCSF treated sections wuith respect to controls. This supports the idea that each focal area (SEZ, DMS and hilus) have a specific population of non-differentiated neural precursors capable of responding to CSF stimulus. This lends weight to the hypothesis regarding independent precursor niches in each of the areas investigated and leads us to consider this region a multifocal niche rather than a single one with several consecutive steps.

We also studied the influence of e-CSF in the migratory ability of neural precursors (co-labelling BrdU-DCX) in SEZ, DMS and the hilus. As can be appreciated in Fig. 2 B, e-CSF seems to exert a significant influence on the migratory potential of the precursors located in the three areas, yet the increase in the number of co-labelled cells was proportional to the number of migratory precursors detected in controls for each specific location. This finding supports the hypothesis that e-CSF is able to stimulate precursor activity.

In addition, we evaluated the influence of e-CSF on neuronal precursor differentiation. In a first approach, we attempted to ascertain this influence on early neuronal differentiation by BrdU-βIII tubulin co-localization (Fig. 2C). As we showed previously, Fig. 1, the number of new-born neurons from neural precursors increased progressively from the SEZ to the hilus. However, under eCSF influence (Fig. 2 C), we found a significant increase in the number of newborn neurons in the three locations, which were more evident in ZSE, and DMS (where the number of co-labelled cells was smaller in controls), leading to more homogenous values for early neurogenesis in the three locations. These data

suggest that neural precursor niche cells maintain dependence on CSF stimulus and suggest that in normal conditions there is a spatial regulatory neurogenic mechanism, which may determine the hilus as the zone with maximal neurogenesis.

Finally, following the normal evolution of the neurogenic process from neural precursors, we evaluated neuronal maturation by BrdU-Calretinin co-localization. We found, in controls, an equivalent number of mature neurons in the SEZ, DMS and Hylus. Under eCSF influence (Fig. 2 D), we detected a progressive increase in neuronal maturation from the SEZ to the hilus, which was remarkably high in the hilus. These findings are in accordance with the early neurogenesis values induced. The results strongly suggest that in normal conditions the maximal neurogenic potential is developed close to the dentate gyrus and in SEZ and DMS may be under restrictive control.

In view of our results as a hole, we conclude that the neural precursors dispersed from the SEZ, DMS and hilus, still remains in the adult mice brain under eCSF influence and, together with the subgranular zone could be considered as a complex periventricular niche as occurs during development.

4. Discussion

Our result support the previous described data (Clarke and van der Kooy, 2011; Urbán and Guillemot, 2014; Zhao et al., 2008) showing that, in the adult mouse hippocampal area, there is a cellular population with Neural Precursor characteristics outside the Subgranular zone (SGZ) of the Dentate Gyrus. According with ours results, cells with neural precursor features are located in a broad area, including the Subependimal zone (SEZ) close to the posterior end of the lateral ventricle, the Dentate Migratory Stream (DMS) located under CA3 and in the Hilus, resembling developmental stages (Berg et al., 2018; Hatami et al., 2018; Xu et al., 2015).

This Neural Precursor population seems to be active, with molecular evidence of being involved in neurogenic differentiation. Consequently, they carry out the main requirements for them to be considered Neurogenic niche.

The presence of neural precursors close to, yet outside, the dentate gyrus in adult mammal brains has been previously described (Seaberg and van der Kooy, 2002). Although evidence of their relationship with dentate gyrus neurogenesis has not been established to date, it has been proposed as part of the hippocampal niche (Chechneva et al., 2005; Li et al., 2015; Sachewsky et al., 2014).

The spatial pattern of the neural precursors we detected seems to be coincident with the pattern described during hippocampal development. In agreement with previous reports (Kriegstein and Alvarez-Buylla, 2009; Sugiyama et al., 2014; Xu et al., 2015), during development, neural precursors came from a specific area of the prosencephalic neuroepithelium, namely, the Dentate Neuroepithelium, which forms part of the lateral ventricle surface, in direct contact with embryonic cerebrospinal fluid. Several precursor migratory waves originate in this area (through the future dentate migratory stream), generating the cellular components of the adult dentate gyrus. The last migratory wave apparently depletes the precursor population in the dentate neuroepithelium and dentate migratory stream, and a residual neural precursor population remains, restricted to the subgranular zone of the dentate gyrus (Spalding et al., 2013).

SGZ, however, have been regarded as restricted "Neuronal Precursors" involved directly in dentate gyrus neurogenesis, instead of the "Neural Precursor" described in the canonical neurogenic niches (Hevner, 2016). Furthermore, the precursor replicative activity described in the SGZ seems to be scarce or not evident, which questions the self-renewing potential of this neurogenic niche (Hevner, 2016; Palmer et al., 2000), and supports the idea that other surrounding precursors may contribute to hippocampal neurogenic niche activity.

Our results support the presence of active neural precursors in the parahippocampal area, with migratory activity and neurogenic potential in adult brain mice hippocampal region, in the areas in which they were present during development. This suggests that hippocampal developmental patterning of the location and behaviour of neural precursors may not completely disappear in the mouse adult brain.

We detected a multifocal niche extending from the SEZ to the hilus through the DMS, and despite the precursors exhibiting migration and neuronal differentiation, their behaviour seemed to be locally differential. Consequently, neurogenic activity increases from the SEZ to the hilus, as occurs during development (Clarke and van der Kooy, 2011), this could be compatible with local expression of neurogenic inductors as Retinoic Acid (Goodman et al., 2012).

In relation with the final destination of the neurons generated in this multifocal niche, jet we have no evidence that these neural precursors are incorporated in the SGZ, contributing to dentate gyrus neurogenesis in the adult brain. This is a mayor question, which requires further research.

A significant difference between the adult SGZ niche and the developmental niche is their location with respect to the lateral ventricle surface. As we mentioned above, the dentate neuroepithelium is in direct contact with the brain ventricle cavity and its content, namely, embryonic cerebro-spinal fluid, whilst the SGZ is a long way from this cavity and consequently, they probably are outside cerebro-spinal fluid influence, which leads us to surmise a loss of "periventricular" property.

Several research studies have reported experimental evidence that the cerebro-spinal fluid, which fills the brain ventricles cavity, exerts a trophic influence on the cellular behaviour of periventricular niches, inducing neurogenesis (Alonso et al., 2017; Alonso and Gato, 2018; Fame and Lehtinen, 2020). In this context, we previously reported that embryonic cerebrospinal fluid was able to induce intense neurogenesis, both in the embryonic brain neuroepithelial cells and in adult mouse subventricular niche precursors (Alonso et al., 2017). Several growth factors are involved in this neurogenic effect (Fame and Lehtinen, 2020; Lamus et al., 2020; Martín et al., 2006), as well as Retinoic Acid (Alonso et al., 2011).

Here, our results show that the neural precursors detected in the SEZ, DMS and hilus significantly modify their behaviour in the presence of embryonic cerebrospinal fluid, increasing their neuronal differentiation. This data strongly suggests that these adult brain neural precursors preserve their ability to respond to the ventricular stimulus and support the hypothesis that they form part of a "periventricular" niche.

Finally, we conclude that the developmental pattern of the neurogenic niche in the hippocampus can also remain active in adults, which allows us to propose a common theory of the neurogenic niche based on its dependence on brain ventricles and their content, cerebro-spinal fluid.

Further research is required to clarify the contribution of these neural precursors to dentate gyrus neurogenesis in the adult brain and their relations with SGZ precursors.

Declaration of Competing Interest

The authors do not have any conflict of interest.

Data Availability

Data will be made available on request.

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References

Alonso, M.I., Gato, A., 2018. Cerebrospinal fluid and neural stem cell niche control. Neural Regen. Res. 13 (9), 1546–1547. https://doi.org/10.4103/1673-5374.237114.

Alonso, M.I., Lamus, F., Carnicero, E., Moro, J.A., de la Mano, A., Fernández, J.M.F., Desmond, M.E., Gato, A., 2017. Embryonic cerebrospinal fluid increases neurogenic activity in the brain ventricular-subventricular zone of adult mice. Front. Neuroanat. 11. https://doi.org/10.3389/fnana.2017.00124.

Alonso, M.I., Martín, C., Carnicero, E., Bueno, D., Gato, A., 2011. Cerebrospinal fluid control of neurogenesis induced by retinoic acid during early brain development. Dev. Dyn. 240 (7) https://doi.org/10.1002/dvdy.22657.

Alvarez-Buylla, A., Lim, D.A., 2004. For the long run: maintaining germinal niches in the adult brain. Neuron 41 (5), 683–686. https://doi.org/10.1016/s0896-6273(04)00111-4.

Berg, D.A., Bond, A.M., Ming, G.-L., Song, H., 2018. Radial glial cells in the adult dentate gyrus: what are they and where do they come from? F1000Research 7, 277. https://doi.org/10.12688/f1000research.12684.1.

Chechneva, O., Dinkel, K., Schrader, D., Reymann, K.G., 2005. Identification and characterization of two neurogenic zones in interface organotypic hippocampal slice cultures. Neuroscience 136 (1), 343–355. https://doi.org/10.1016/J.NEUROSCIENCE.2005.07.058.

Clarke, L., van der Kooy, D., 2011. The adult mouse dentate gyrus contains populations of committed progenitor cells that are distinct from subependymal zone neural stem cells. Stem Cells 29 (9), 1448–1458. https://doi.org/10.1002/STEM.692.

Cuccioli, V., Bueno, C., Belvindrah, R., Lledo, P.M., Martinez, S., 2015. Attractive action of FGF-signaling contributes to the postnatal developing. Hippocampus. Hippocampus 25 (4), 486–499. https://doi.org/10.1002/HIPO.22386.

Fame, R.M., Lehtinen, M.K., 2020. Emergence and developmental roles of the cerebrospinal fluid system. Dev. Cell 52 (3), 261–275. https://doi.org/10.1016/J.DEVCEL.2020.01.027.

Farioli-Vecchioli, S., Micheli, L., Saraulli, D., Ceccarelli, M., Cannas, S., Scardigli, R., Leonardi, L., Cinà, I., Costanzi, M., Ciotti, M.T., Moreira, P., Rouault, J.-P., Cestari, V., Tirone, F., 2012. Btg1 is required to maintain the pool of stem and progenitor cells of the dentate gyrus and subventricular zone (AUG). Front. Neurosci. 6, 124. https://doi.org/10.3389/fnins.2012.00124.

Gato, A., Alonso, M.I., Lamus, F., Miyan, J., 2020. Neurogenesis: a process ontogenically linked to brain cavities and their content, CSF. Semin. Cell Dev. Biol. *102*. https://doi.org/10.1016/j.semcdb.2019.11.008.

Gato, A., Desmond, M.E., 2009. Why the embryo still matters: CSF and the neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis. Dev. Biol. 327 (2), 263–272. https://doi.org/10.1016/j.ydbio.2008.12.029.

Gato, A., Martin, P., Alonso, M.I., Martin, C., Pulgar, M.A., Moro, J.A., 2004. Analysis of cerebro-spinal fluid protein composition in early developmental stages in chick embryos. J. Exp. Zool. Part A-Comp. Exp. Biol. 301A (4), 280–289. https://doi.org/10.1002/jez.a.20035.

Gato, Á., Moro, J.A., Alonso, M.I., Bueno, D., de La Mano, A., Martín, C., 2005. Embryonic cerebrospinal fluid regulates neuroepithelial survival, proliferation, and neurogenesis in chick embryos. Anat. Rec. Part A Discov. Mol. Cell. Evolut. Biol. 284 (1) https://doi.org/10.1002/ar.a.20185.

Goodman, T., Crandall, J.E., Nanescu, S.E., Quadro, L., Shearer, K., Ross, A., Mccaffery, P., 2012. Patterning of retinoic acid signaling and cell proliferation in the hippocampus. Hippocampus 22 (11), 2171–2183. https://doi.org/10.1002/HIPO.22037.

Hatami, M., Conrad, S., Naghsh, P., Alvarez-Bolado, G., Skutella, T., 2018. Cell-biological requirements for the generation of dentate gyrus granule neurons. Front. Cell. Neurosci. *12*. https://doi.org/10.3389/FNCEL.2018.00402.

Hevner, R.F., 2016. Evolution of the mammalian dentate gyrus. J. Comp. Neurol. 524 (3), 578–594. https://doi.org/10.1002/CNE.23851.

Ihunwo, A.O., Tembo, L.H., Dzamalala, C., 2016. The dynamics of adult neurogenesis in human hippocampus. Neural Regen. Res. 11 (12), 1869–1883. https://doi.org/10.4103/1673-5374.195278.

Johansson, P.A., Cappello, S., Götz, M., 2010. Stem cells niches during development–lessons from the cerebral cortex. Curr. Opin. Neurobiol. 20 (4), 400–407. https://doi.org/10.1016/J.CONB.2010.04.003.

Kazanis, I., Lathia, J., Moss, L., ffrench-Constant, C. (2008). The neural stem cell microenvironment. In StemBook. C. (MA) (Ed.), StemBook. Cambridge (MA).

Kompaníková, P., Bryja, V., 2022. Regulation of choroid plexus development and its functions. Cell. Mol. Life Sci. 79 (6) https://doi.org/10.1007/s00018-022-04314-1.

Kriegstein, A., Alvarez-Buylla, A., 2009. The glial nature of embryonic and adult neural stem cells. Annu. Rev. Neurosci. 32, 149–184. https://doi.org/10.1146/ANNUREV.NEURO.051508.135600.

Lamus, F., Martín, C., Carnicero, E., Moro, J.A., Fernández, J.M.F., Mano, A., Gato, Á., Alonso, M.I., 2020. FGF2/EGF contributes to brain neuroepithelial precursor proliferation and neurogenesis in rat embryos: the involvement of embryonic cerebrospinal fluid. Dev. Dyn. 249 (1) https://doi.org/10.1002/dvdy.135.

Landgren, H., Curtis, M.A., 2011. Locating and labeling neural stem cells in the brain. J. Cell. Physiol. 226 (1), 1–7. https://doi.org/10.1002/JCP.22319.

Lehtinen, M.K., Walsh, C.A., 2011. Neurogenesis at the brain–cerebrospinal fluid interface. Annu. Rev. Cell Dev. Biol. https://doi.org/10.1146/annurev-cellbio-092910-154026.

Li, D., Takeda, N., Jain, R., Manderfield, L.J., Liu, F., Li, L., Anderson, S.A., Epstein, J.A., 2015. Hopx distinguishes hippocampal from lateral ventricle neural stem cells. Stem Cell Res. 15 (3), 522–529. https://doi.org/10.1016/J.SCR.2015.09.015.

Martín, C., Bueno, D., Alonso, M.I., Moro, J.A., Callejo, S., Parada, C., Martín, P., Carnicero, E., Gato, A., 2006. FGF2 plays a key role in embryonic cerebrospinal fluid trophic properties over chick embryo neuroepithelial stem cells. Dev. Biol. 297 (2) https://doi.org/10.1016/j.ydbio.2006.05.010.

Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 4, 479–494. ⟨https://onlinelibrary.wiley.com/doi/epdf/10.1002/1096-9861%2820001002%29425%3A4%3C479%3A%3AAID-CNE2%3E3.0.CO%3B2-3⟩.

Parisot, J., Flore, G., Bertacchi, M., Studer, M., 2017. COUP-TFI mitotically regulates production and migration of dentate granule cells and modulates hippocampal Cxcr4 expression. Development 144 (11), 2045–2058. https://doi.org/10.1242/dev.139949.

Sachewsky, N., Leeder, R., Xu, W., Rose, K.L., Yu, F., van der Kooy, D., Morshead, C.M., 2014. Primitive neural stem cells in the adult mammalian brain give rise to GFAP-expressing neural stem cells. Stem Cell Rep. 2 (6), 810–824. https://doi.org/10.1016/J.STEMCR.2014.04.008.

Schommer, J., Schrag, M., Nackenoff, A., Marwarha, G., Ghribi, O., 2017. Method for organotypic tissue culture in the aged animal. MethodsX 4, 166–171. https://doi.org/10.1016/J.MEX.2017.03.003.

Seaberg, R.M., van der Kooy, D., 2002. Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. J. Neurosci. 22 (5), 1784–1793. ⟨https://www.ncbi.nlm.nih.gov/pubmed/11880507⟩.

Seri, B., García-Verdugo, J.M., Collado-Morente, L., McEwen, B.S., Alvarez-Buylla, A., 2004. Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. J. Comp. Neurol. 478 (4), 359–378. https://doi.org/10.1002/CNE.20288.

Seri, B., García-Verdugo, J.M., McEwen, B.S., Alvarez-Buylla, A., 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. J. Neurosci. 21 (18), 7153–7160. https://doi.org/10.1523/JNEUROSCI.21-18-07153.2001.

Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Boström, E., Westerlund, I., Vial, C., Buchholz, B.A., Possnert, G., Mash, D.C., Druid, H., Frisén, J., 2013. XDynamics of hippocampal neurogenesis in adult humans. Cell 153 (6), 1219. https://doi.org/10.1016/J.CELL.2013.05.002.

Stoppini, L., Buchs, P.A., Muller, D., 1991. A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods 37 (2), 173–182. https://doi.org/10.1016/0165-0270(91)90128-M.

Sugiyama, T., Osumi, N., Katsuyama, Y., 2014. A novel cell migratory zone in the developing hippocampal formation. J. Comp. Neurol. 522 (15), 3520–3538. https://doi.org/10.1002/CNE.23621.

Taupin, P., 2007. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res. Rev. 53 (1), 198–214. https://doi.org/10.1016/j.brainresrev.2006.08.002.

Urbán, N., Guillemot, F., 2014. Neurogenesis in the embryonic and adult brain: same regulators, different roles (NOV). Front. Cell. Neurosci. 8. https://doi.org/10.3389/FNCEL.2014.00396.

Xu, L., Tang, X., Wang, Y., Xu, H., Fan, X., 2015. Radial glia, the keystone of the development of the hippocampal dentate gyrus. Mol. Neurobiol. 51 (1), 131–141. https://doi.org/10.1007/S12035-014-8692-Y.

Zappaterra, M.W., Lehtinen, M.K., 2012. The cerebrospinal fluid: regulator of neurogenesis, behavior, and beyond. Cell. Mol. Life Sci.: CMLS 69 (17), 2863–2878. https://doi.org/10.1007/S00018-012-0957-X.

Zhao, C., Deng, W., Gage, F.H., 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132 (4), 645–660. https://doi.org/10.1016/J.CELL.2008.01.033.