

Isolation of neurosphere-like bodies from an adult patient with refractory temporal lobe epilepsy

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Epilepsy affects approximately 0.8% of the world population and is most common among those with chronic neurological diseases¹. Some patients are refractory to antiepileptic drugs and they develop refractory epilepsy². This kind of epilepsy presents a higher frequency of sudden death³; surgical treatment is indicated and has shown significantly higher efficacy compared to antiepileptic drug treatment⁴.

The surgical treatment of the patients creates the possibility of isolating neural stem cells (NSCs) *in vivo*. These cells are present in the subventricular zone (SVZ), around the lateral ventricles and in the sub-granular layer in the hippocampus (SGZ), and they give rise to neurons and glial cells during adulthood⁵⁻⁶. The isolation of these cells could lead to a future source of autologous transplants for neurodegenerative diseases, as well as to the storage for future research for basic science studies of NSCs. However, only few studies have described the isolation of NSCs from human brain *in vivo*⁷⁻⁹, and no such study has been performed in Brazil.

Here, we showed for the first time in Brazil the possibility of isolating neural progenitor cells from a woman with refractory epilepsy during interventional surgery.

CASE

A 50-year-old woman with a 15 year history of epilepsy was reported. The surgery was carried out after the concordance of electrophysiological clinical analysis, MRI and ictal single photon emission com-

puted tomography (SPECT) findings that have demonstrated an ictal onset zone in the right temporal lobe, atrophy and loss of volume in the right hippocampus (Fig 1A, B) and increased perfusion in the same side (Fig 1C).

The patient was subjected to an anterior temporal lobectomy and amigdalohippocampectomy, and in approaching the temporal horn of the lateral ventricle, subventricular sampling was done for tissue culture. The protocol was approved by the Ethical Committee from the Hospital Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, and the patient gave written informed consent.

The tissue was immediately transferred to DMEM-F12 at 4°C. The pia mater and associated blood vessels were removed, and the tissue was dissociated with papain following the manufacturer's recommended procedures (Worthington Biochemical Corp). After dissociation, the single cells were cultured in neurosphere medium¹. The cells were cultured in 10-cm non-adherent culture plates. The culture was supplemented with 20 ng/mL epidermal growth factor (EGF), 10ng/mL basic fibroblast growth factor (bFGF) and B27 supplement twice a week. Additional neurosphere medium was administered once every week.

After 14 days *in vitro*, we observed, at least, 26 cellular aggregates similar to neurospheres. These neurosphere-like bodies had different diameters, ranging between 57 µm and 290 µm (Fig 2A). Indi-

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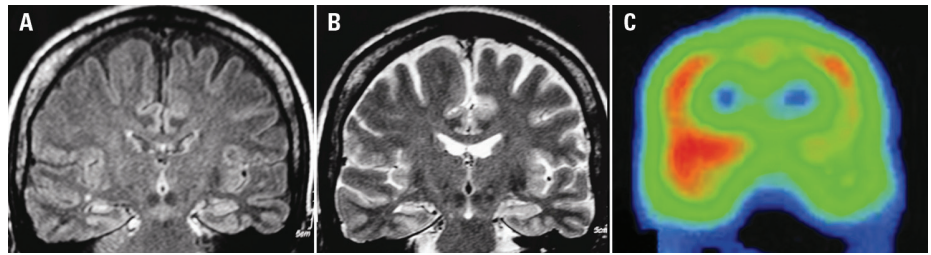
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ISOLAMENTO DE CORPOS NEUROSFERA-LIKE DE PACIENTE ADULTO COM EPILEPSIA DO LOBO TEMPORAL REFRATÁRIA

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Fig 1. Imaging investigation before surgery. [A]: MRI Flair sequence showing the high signal of the atrophic right side of the hippocampus. [B]: MRI T2 sequence depicting loss of volume of the right hippocampus. [C]: The ictal SPECT shows the increase of perfusion in the right temporal lobe, 209×297 mm (300×300 DPI).



vidual neurospheres (n=10) were then transferred to glass coverslips previously incubated with 10 µg/mL poly-L-lysine followed by 20 µg/mL laminin. The growth factors were removed from the culture medium and the neurosphere-like aggregates adhered to the coverslips and showed different patterns of differentiation in the following days. In one pattern, lamellipodia-like projections were observed soon after plating (Fig 2B, 1 day *in vitro*) and in the following days, on top of these, many slender filopodia-like projections were present (Fig 2C, 30 days *in vitro*). The cells were fixed with 4% paraformaldehyde at 37°C. The coverslips were incubated with normal goat serum followed by primary and secondary antibodies for immunocytochemistry. The lamellipodia-like processes were positive for glial fibrillary acidic protein (GFAP), suggesting differentiation into astrocytes and/or radial glial cells from the neurosphere-like bodies. In addition, we showed that on top of these processes, several β-III tubulin positive processes were present, suggesting neuronal differentiation (Fig 2D). A different pattern was observed in other neurospheres, in which only a few projections were present emanating from the neurospheres (Fig 2E, 5 days *in vitro*). One of the neurospheres detached from the substrate, leaving behind 3 attached cells that were used for the recording experiments (Fig 2F, 17 days *in vitro*). After recording, the coverslip with the cells was fixed and immunocytochemistry for GFAP and β-III tubulin was performed. In Figure 2G, it is possible to notice neurites (β-III tubulin positive), some of them closely associated with glial processes (GFAP positive).

To probe the electrical activity of the differentiated cells, depolarizing pulses were applied from -90 to +60 mV in 10 mV steps (holding potential = -70 mV). With this approach, an outward rectifier current was observed typical of glial cells (Fig 2H).

DISCUSSION

Mammalian NSCs have been isolated from the SVZ around the lateral ventricle and the SGZ^{5,6}. These cells are self-renewable as cell aggregates called neurospheres, and they can give rise to all the cells of the nervous system. These neural progenitor cells can generate neurons and astrocytes, but they have lower plasticity than neu-

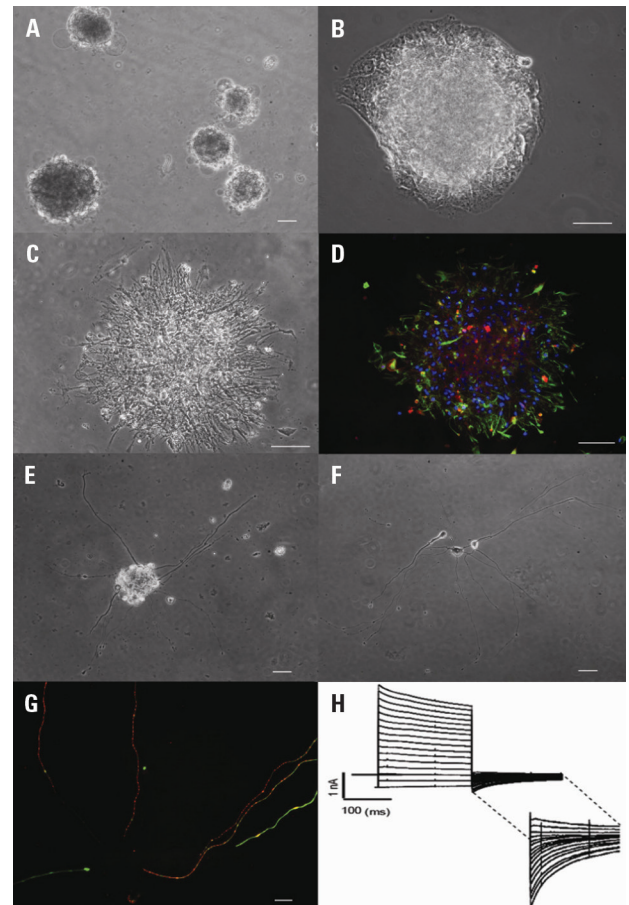


Fig 2. Cells isolated from the SVZ of adult human brain form neurosphere-like bodies and differentiate into neurons and astrocytes. [A] Phase contrast image of neurosphere-like bodies formed 13 days after plating of the dissociated cells in non-adherent culture conditions and in the presence of growth factors. [B-C] Phase contrast images of the same neurosphere-like aggregate 1 day [B] and 30 days [C] after plating in adherent culture conditions and in the absence of growth factors. [D] Photomicrograph of the neurosphere-like aggregate shown in [B-C] immunolabeled for GFAP (green) and β-III tubulin (red). Nuclei are labeled with DAPI (blue). [E] Phase contrast image of a small neurosphere-like body 1 day after plating in adherent culture conditions. [F] Phase contrast image of the cells that remained attached to the plate after detachment of the neurosphere-like aggregate and before electrophysiological recordings. [G] Photomicrograph of cells shown in [F] immunolabeled for GFAP (green) and β-III tubulin (red). [H] Electrophysiological recording from one of the cells shown in [F] showing an outward rectifier current. Scale bars: 504 µm, 209×297 mm (300×300 DPI).

ral stem cells¹⁰. Here, we isolated neural progenitor cells which formed neurosphere-like bodies that gave rise to β -III tubulin-positive neurons and GFAP-positive astrocytes. After differentiation, electrical properties were recorded from these cells showing functionality *in vitro*. In our study, the cells did not differentiate into oligodendrocytes and were not self-renewable (data not shown). Therefore, they are not neural stem cells.

Up to now, few studies have reported the isolation of human neural stem/progenitor cells *in vivo*⁷⁻⁹. Although we did not obtain NSCs, we isolated neural progenitor cells from the SVZ for the first time in Brazil. These cells were isolated after being sampled by means of surgical procedure of an adult patient with refractory epilepsy, and these results can be reproduced by other groups.

Methods of isolation and characterization of progenitor/stem cells have been reported in order to clarify their basic biological mechanisms. Additionally, these cells can be differentiated *in vitro* into all cells of the nervous system, which could be useful in the screening of drugs and, in the future, these cells could be expanded in culture as a source for cellular transplants for neurological diseases.

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