Supplementary Methods

Culture and differentiation of neurospheres

Tissues from single animals were dissociated by trituration with a fire-polished Pasteur pipette and used separately to establish neurosphere cultures. For population analyses, cells were plated at 8,000 cells/cm^2 in neurosphere medium (DMEM/F12 containing 2mM L-glutamine, 0.6% glucose, 0.1 mg/ml apo-transferrin, 0.025 mg/ml insulin, 9.6 µg/ml putrescin, 6.3 ng/ml progesterone, 5.2 ng/ml Na selenite, 2 µg/ml heparin) supplemented with EGF (20ng/ml) and FGF2 (10ng/ml), and spheres were collected after three to five days. The total number of viable cells was assessed at each passage by Trypan Blue (Sigma, St. Louis, MO, USA) exclusion. For clonogenic assays, cells derived from primary tissues or primary neurospheres were seeded in 48-well plates at 10 cells/µl, and the number of secondary spheres generated that exceeded the size of 100 µm was assessed after eight to ten days.

To analyze progenitor differentiation, cells dissociated from neurosphere cultures after 2 to 4 passages were seeded at a density of 40 cells/µl on Matrigel-coated coverslips in 48-well plates. Cells were then cultivated sequentially in different media to promote respectively the selection, expansion and differentiation of progenitors, as described in [1, 2]. Briefly, single neurosphere cells were cultivated one day in synthetic medium containing both EGF (20ng/ml) and FGF2 (10ng/ml) to select for undifferentiated progenitors, followed by three days of culture in the same medium containing only bFGF2 (10ng/ml), to promote the expansion of neuronal precursors, followed by 5 days of culture in neurosphere medium containing 1% of FCS to promote progenitor

differentiation. Coverslips were then fixed with 4% paraformaldehyde for 15 minutes, and processed for immunocytochemistry.

Primary antibodies

Mouse monoclonal antibodies to Mash1 (gift from D.J. Anderson), BrdU (1:100, Roche), GFAP (1:1000, Sigma), ßIII-tubulin (1:1000, Babco), PSA-NCAM (1:500, gift from G. Rougon), O4 (1:20, gift from B. Zalc), ß-galactosidase (1:1000, Promega); rabbit polyclonal antibodies to Nestin (1:1000, gift from U. Lendal), Ki67 (1:1000, Novocastra), GFAP (1:1000, Dako), ßIII-tubulin (1:1000, Babco), ß-galactosidase (1:3000, Cappel), Mash1 (1:1000; [3]), pan-Dlx (1:100, gift from G. Panganiban), NG2 (1:500, Chemicon), GFP (1:1000, Molecular Probes), GAD65 (1:500, Chemicon), TH (1:500, Institut Jacques Boy, Reims), PDGFRα (1:1000, gift from C. Heldin), APC (1:200, Calbiochem); NeuN (1:500, Chemicon); rat antibodies against mCD24 (1:300, gift from G Rougon).

Secondary antibodies

Cy3-conjugated goat anti-mouse, -rabbit, and -rat (1:1000, Jackson ImmunoResearch), Alexa 488-conjugated goat anti-mouse and -rabbit (1:600, Molecular Probes), and Cy5-conjugated goat anti-mouse (1:400, Jackson ImmunoResearch).

References

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