Expansion of rat neural stem and progenitor cells in long-term culture

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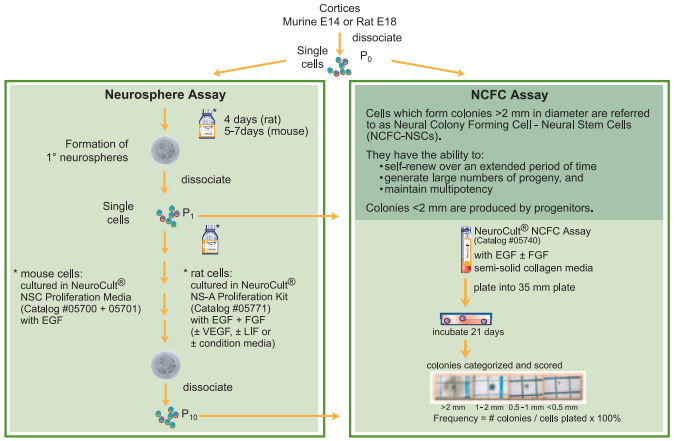
Introduction

A unique population of cells in the mouse and rat CNS proliferate *in vitro* in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) forming cluster of cells called neurospheres. These cells fulfill the functional criteria for neural stem cells (NSCs) including the ability to self-renew, produce large numbers of progeny and retain multipotency. Unlike long term neurosphere cultures derived from embryonic mouse cortical cells which show a consistent fold expansion (~5 fold per passage) in total cells over time, long-term neurosphere cultures derived from embryonic rat cortices exhibit 15 to 40-fold increase in total cell number during early passage (P1 – P2) followed by a lower fold increase of 8 to13 at P3-P4. These cultures then enter a critical stage after P4 when the fold expansion of total cells drops to 2 to 8 fold. Rat cells also tend to adhere to the flask at later passages contributing to difficulties in culturing these cells long term.

To better understand the growth properties of rat NSCs in long-term neurosphere cultures in comparison with the growth properties of mouse NSCs, we cultured embryonic mouse and rat CNS cells in long term neurosphere cultures and at various time points in the cultures we measured stem and progenitor cell content using the Neural Colony Forming Cell (NCFC) assay (Louis et al., SFN 2004). In the NCFC assay, colony size is an indication of proliferative potential and cells forming colonies >2 mm in diameter are referred to as NCFC-NSC and meet all the criteria for a NSC. Cells that form colonies <2 mm, lack self-renewal ability and are likely neural progenitors. We hypothesized that the changes in growth properties of rat cells at later passages in neurosphere cultures are related to changes in the frequency of stem and progenitors cells. We also attempted to examine factors that influence neural stem cell survival in long-term rat neurosphere cultures.

Materials and Methods

Figure 1. Schematic representation of the experimental design showing the neurosphere and Neural Colony Forming Cell (NCFC) assays.



Frequency of NCFC-Neural stem cells (NCFC-NSCs) (%) = (#colonies >2 mm/ cells plated) x 100
Frequency of NCFC-progenitor cells (%) = (#colonies < 2 mm/ cells plated) x 100

Results

Figure 2. Expansion of total rat and mouse cells in neurosphere cultures at each passage from P1 to P10

- A decrease in fold expansion of total rat cells was observed between P5-P9.
- There was a steady fold increase in the total mouse cells numbers from P0 - P10.

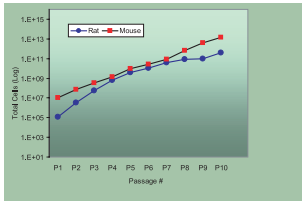


Figure 3. Comparison of the frequency of NCFC-NSC in rat and mouse neurosphere cultures at each passage from P0 – P10

- **Mouse cells**: NCFC-NSC frequency increased from P0 - P1 and remained high from P1 - P7 relative to P0. A decrease in NCFC-NSC frequency was observed from P8-P10.
- **Rat cells**: NCFC-NSC increased from the primary suspension (P0) to P1 however the NCFC-NSC frequency continued to decrease after P1 with a significant decline at P4 to levels below those in the primary suspension (P0).

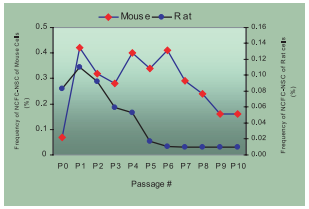
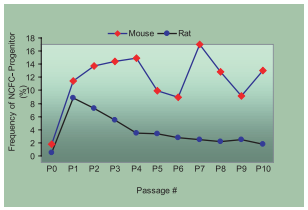


Figure 4. Comparison of the frequency of NCFC-Progenitor in rat and mouse neurosphere cultures at each passage

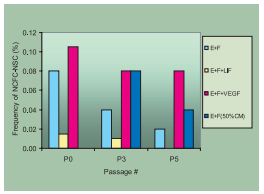
- **Mouse cells**: NCFC-progenitor cell frequency increased after P0 and remained high up to P10.
- **Rat cells**: NCFC-progenitor cell frequency increased from P0-P1 then continued to decrease after P1.



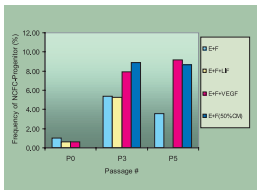
Leukemia Inhibitory Factor (LIF:20 ng/mL), Vasculator Endothelial Growth factor (VEGF:10 ng/mL), or 50% conditioned media (CM obtained from P0-P4 cultures) were added to rat neurosphere cultures containing EGF and FGF. Cells dissociated from neurospheres obtained from P0, P3 and P5 cultures were assayed for NCFC-NSC and NCFC-progenitor content.

Figure 5. The effect of addition of LIF, VEGF or 50% CM on the frequency of rat NCFC-NSC and NCFC-progenitor

A. Frequency of NCFC-NSC

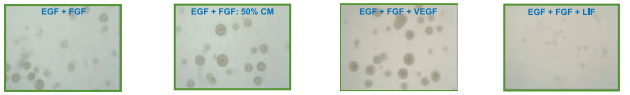


B. Frequency of NCFC-progenitor



- Addition of LIF to rat neurosphere cultures containing EGF + FGF decreased the frequency of NCFC-NSC.
- The addition of VEGF and 50% CM to rat neurosphere cultures containing EGF + FGF increased NCFC-NSC compared to culture containing EGF+FGF alone. An increased in NCFC-Progenitor was observed at P3 and P5.

Figure 6. Morphology of neurospheres in rat neuropshere cultures at P5 containing EGF+FGF ± 50% CM, VEGF or LIF



- Addition of LIF promotes cell attachment, inhibiting neurosphere formation.
- Addition of 50% CM or VEGF decreased cell attachment, increasing neurosphere formation.

Conclusions

- These results indicate that the observed decline in total rat cells beyond P4 in neurosphere cultures is due to an almost immediate loss of NSCs after passage 1.
- This dramatic loss in neural stem cells is not detected in early passages of mouse cells in neurosphere cultures
- Preliminary data indicated that addition of VEGF or conditioned medium to rat neurosphere culture containing EGF + FGF could increase the survival of rat neural stem cells at early passages.

References:
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Schanzer A., Wachs FP., Wilhelm D., Acker T., et al. 2004. Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. Brain Pathol. 14(3): 237-48.

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