Can ESC-derived Progenitors Reactivate the Irradiated Neurogenic Niche?

Terry Burns MD PhD; Dominic Rodriguez; James Weimann PhD; Kambiz Ansari; Matthew Li; Theo D. Palmer

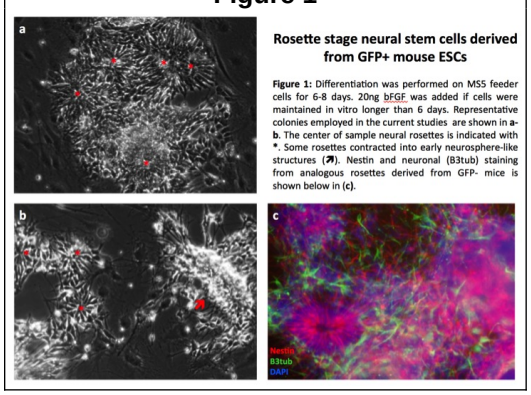
Introduction

Irradiation-induced loss of hippocampal neurogenesis has been implicated in chronic cognitive deficits after irradiation for pediatric and adult brain malignancies. To date no effective therapy exists to restore hippocampal neurogenesis after irradiation. We evaluated the feasibility of using ESC-derived neural progenitors to restore neurogenic capacity to the irradiated neurogenic niche.

Methods

C57Bl/6 mice were lesioned with 10G whole brain or sham irradiation. Three days later, animals received unilateral stereotactic injection of 50,000 GFP-expressing ESC-derived (d7) neural rosette cells into the dentate gyrus and lateral ventricle. Animals were sacrificed at 2 months and evaluated by confocal microscopy.

Figure 1



Rosette stage neural stem cells derived from GFP+ mouse ESCs

Figure 1: Differentiation was performed on MS5 feeder cells for 6-8 days. 20ng bFGF was added if cells were maintained in vitro longer than 6 days. Representative colonies employed in the current studies are shown in **a-b**. The center of sample neural rosettes is indicated with *. Some rosettes contracted into early neurosphere-like structures (↗). Nestin and neuronal (B3tub) staining from analogous rosettes derived from GFP- mice is shown below in (**c**).

Results

Irradiated animals demonstrated near complete (>95%) loss of hippocampal neurogenesis, and partial (<70%) ablation of olfactory bulb neurogenesis. Transplanted animals demonstrated robust GFP+ grafts in or adjacent to the dentate gyrus and in scattered periventricular regions. At the edge of the dentate gyrus graft, GFP+ cells were seen in the subgranular zone and granule cell layer where graft-derived neurons displayed mature dendritic arborization. Rare GFP+DCX+ cells were also present suggesting the potential for ongoing graft-derived neurogenesis. Strikingly, focal re-initiation of endogenous neurogenesis was observed with clusters of host (GFP-negative) DCX+ neurons found immediately adjacent to graft-derived dentate granule neurons.

Conclusions

Our findings provide proof of principle that the irradiated dentate gyrus is capable of both supporting and re-initiating neurogenesis after focal transplantation of ESC-derived cells. The spatially restricted re-initiation of neurogenesis adjacent to graft-derived neurons supports the emerging idea that newly integrated neurons are important positive regulators of ongoing adult neurogenesis.

Figure 2

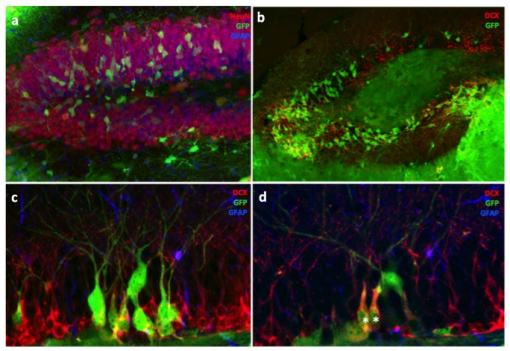


Figure 2: To initially evaluate the ability of ESC-derived NSCs to engraft into neurogenic regions, cells were transplanted into the neurogenic neonatal hippocampus. Here they were seen to generate mature NeuN+ (a) and immature DCX+ (b-d) neurons at 1 month after transplantation.

Figure 3

Loss of Neurogenesis After Whole Brain Irradiation

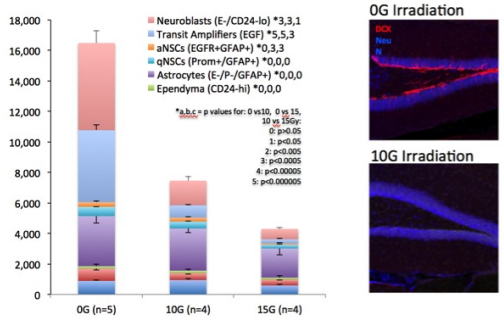


Figure 3: To Evaluate the effects of Irradiation of Neurogenesis, cells from the dissociated subventricular zone were evaluated by FACS, revealing dramatic loss of activated neural stem cells, transit amplifiers and neuroblasts. Staining for new neurons (DCX) in the dentate gyrus similarly revealed dramatic loss of neurogenesis after irradiation.

Learning Objectives

By the conclusion of this session, participants should be able to 1) Describe the potential role of ESC-derived cells in recovery of hippocampal neurogenesis in animal models of brain irradiation. 2) Dscribe the distinction between graft-derived neurogenesis and promotion of endogenous neurogenesis.

Figure 4

Engraftment of Transplanted Cells into the Adult Irradiated Dentate Gyrus

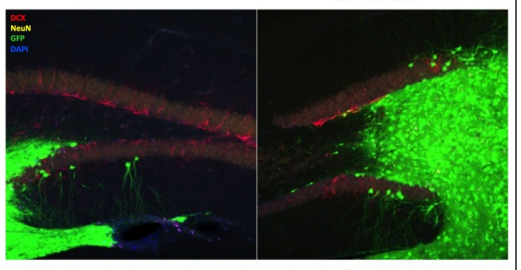


Figure 4: ESC-NSC-derived cells were seen to generate neurons in the granule cell layer of both the irradiated (left) and unirradiated (right) adult brain. Moreover, in the immediate vicinity of the graft, endogenous DCX+ cells are seen suggesting reactivation of endogenous neurogenesis after irradiation. Negligible DCX staining was seen irradiated animals that did not receive cell transplantation.

Figure 5

Neurons, but no Neural Stem Cells at 1 month

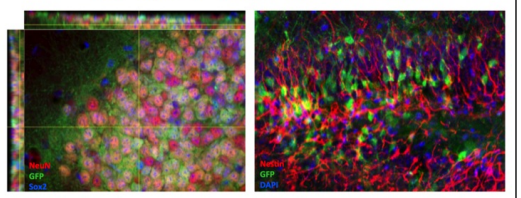


Figure 5: Both within neurogenic regions, and in graft-derived masses, cells differentiated into neural lineages in the irradiated and unirradiated brain (left). However, grafted cells did not express nestin 1 month after transplantation, indicating that no undifferentiated cells remained, and that all grafted cells had committed to either neural on glial lineages.

Figure 6

Nestin-CreER^{T2}/DTR Ablation of Neural Stem Cells

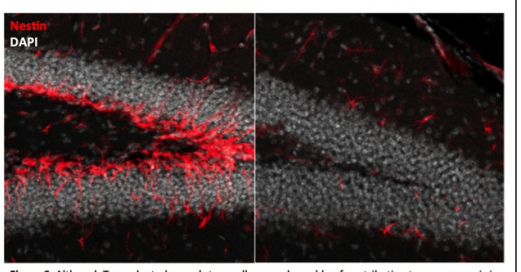


Figure 6: Although Transplanted neural stem cells proved capable of contributing to neurogenesis in the unirradiated and irradiated brain, the fact that grafted cells differentiated into neural or glial cells without engrafting long-term into the neurogenic niche limited their potential to contribute to further ongoing neurogenesis. To test the hypothesis that residual endogenous neural stem cells may be occupying the neural stem cell niche and preventing long-term engraftment, we developed a technique to ablate endogenous neural stem cells. Inducible expression of diphtheria toxin receptor (DTR) on neural stem cells was found to enable ablation of endogenous nestin-expressing cells in the subgranular zone. Ongoing experiments will evaluate the ability of this strategy of "making space" in the endogeneous niche to facilitate repopulation with residual functional endogenous neural stem cells, or transplanted stem cells.