Adult Neural Stem Cells Are Irreplaceable After Quiescent Stem Cell Targeted Therapy

Terry C Burns MD PhD; Dominic Rodriguez; Sunny Kumar; Kambiz Ansari; Theo D. Palmer
Stanford University Department of Neurosurgery

Introduction

Quiescent tumor-initiating with neural stem cell-like properties are resistant to therapy and enable tumor recurrence. As such, quiescent tumor stem cells are increasingly targeted by novel anti-tumor therapies. Quiescent neural stem cells (NSCs) are extremely similar to tumor stem cells and may be targeted by these therapies. The capacity of the quiescent NSC niche to recover following ablation remains unknown.

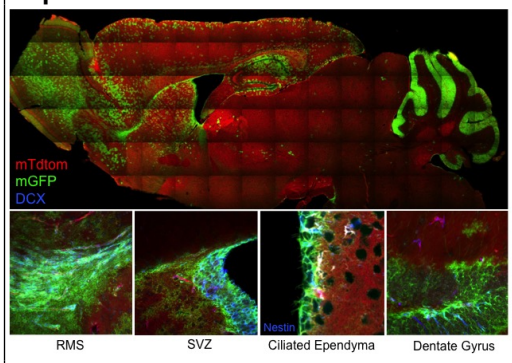
Methods

Nestin-creERT2::diphtheria toxin reporter (DTR) mice were induced to express DTR on NSCs with 3 days 150mg/kg i.p. tamoxifen. Ablation and control mice were treated with 100ug/kg diphtheria toxin DT or vehicle, respectively. Proliferative cells were labeled with BrdU prior to sacrifice. For cell replacement experiments, embryonic stem cell (ESC)-derived NSCs were stereotactically implanted into the dentate gyrus and lateral ventricle.

Results

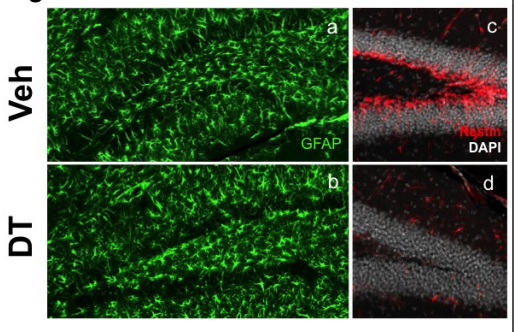
Ablation caused 50-80% reduction of quiescent NSCs and BrdU+ within 10 days. NSC loss persisted for at least 2 months, and was accompanied by 48% loss of neurogenesis at 2 months ($p<0.01$), suggesting inability of the partially vacated neurogenic niche to be repopulated over time by surviving

Figure 1: Persistent Reporter Expression in new neurons at 6 months.



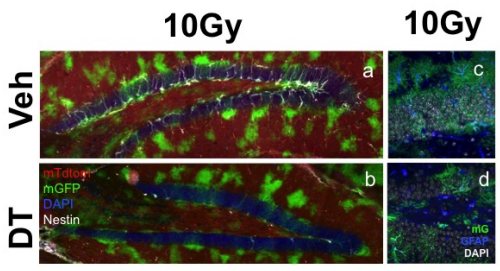
We used specific line of Nestin-creERT2 (Lavado et al, 2010; "Baker mice") crossed to mTmG reporter mice (Muzumdar et al, 2007) and administered 150mg i.p. tamoxin (tam) once daily for 3 days to induce recombination. Thereafter, reporter expression was noted in the vast majority of newly born DCX+ cells in the dentate gyrus (DG) and subventricular zone (SVZ) for up to 6 months.

Figure 2: Ablation of DG NSCs with DT.



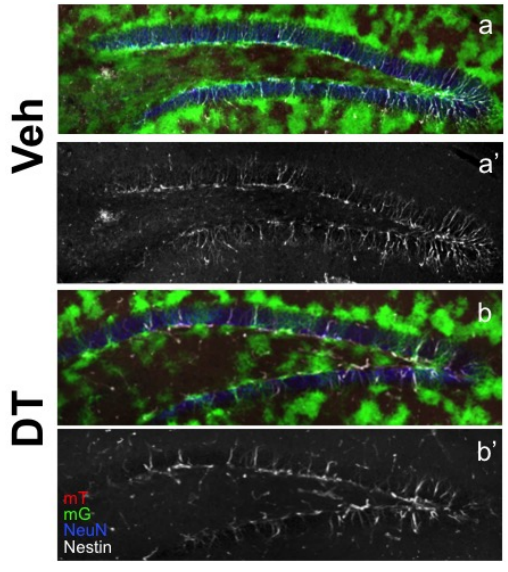
Treatment with tamoxifen and Diphtheria toxin (DT) resulted in loss of radial GFAP+ cells (b) and loss of nestin (d) expression from the subgranular zone (SGZ) by day 10 when compared to vehicle-treated animals (a, c).

Figure 3: Ablation of quiescent DG NSCs with DT.



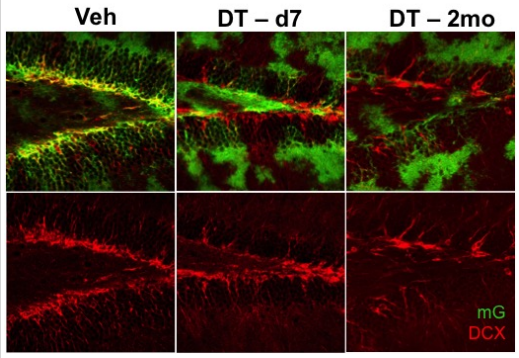
Ablation of irradiated mice via Tam+DT resulted in substantial loss of Nestin+ cells (a, b) and radial GFAP+ cells (c, d) compared to tam+vehicle–treated mice at 1 month.

Figure 4: Limited Repopulation of the SGZ NSC niche.



No significant difference was found in the number of Nestin+ cells at d7 and 2 months; Significantly fewer Nestin+ were present at 2 months after DT than vehicle treatment ($p=0.01$).

Figure 5: Sustained loss of DG neurogenesis after ablation.



Given the sustained loss of Nestin+ cells in the DG after ablation, we asked if this translated to a sustained decrease in newly born neurons, or if compensatory mechanisms could increase the neurogenic yield of this reduced NSC Pool. Similar to nestin, however, DCX cell numbers trended towards being lower at 2 months than 7 days after ablation, and were significantly lower after DT than Vehicle treatment at 2m ($p=0.01$).

Conclusions

We demonstrate for the first time that adult NSCs are unable to repopulate the quiescent NSC niche after partial ablation of quiescent NSCs, although surviving NSCs remain functional and responsive to proliferative stimuli. Primitive ESC-derived NSC also cannot engraft into the vacated niche. These findings suggest that loss of quiescent NSCs irreversibly decreases niche size. Given the importance of neurogenesis for cognition and memory, the potentially irreversible impacts of novel stem cell-targeted antitumor therapies on quiescent NSC pool size should be carefully evaluated.