PERSPECTIVE

The vascular stem cell niche: roadmap for transplanted neural progenitor cells during environmental enrichment?

One of the challenges of cell transplantation into the brain is poor graft survival. Graft survival may be affected by an immunological response of the host towards transplanted cells, shear injury to cells during transplantation or an unsuitable micro-environment for the transplanted cell type. Neural progenitor cells have an affinity to laminin, which is commonly used to maintain neural progenitors as monolayer cultures *in vitro*. The affinity of neural progenitor cells to laminin is based on the high expression of α6β1 integrin, which is required for binding of neural progenitors to endothelial cells (Hall et al., 2006; Shen et al., 2008). Mice with targeted disruption of β1 integrin have substantial layering deficits in developing neocortex (Loulier et al., 2009). Laminin is an important constituent of the basal membrane of brain capillaries. We also found abundant expression of laminin in the basal cistern along the apical surface of the molecular layer of the dentate gyrus (Jamal et al., 2015). Transplanted dentate progenitor cells did not survive in the transplant core within the dentate gyrus, but initially rather exclusively survived along the laminin scaffold of the basal cistern (**Figure 1A**). The laminin of the basal cistern is connected to the laminin of the capillary network of the dentate gyrus. It appeared as if transplanted dentate progenitor cells initially settled on the dense laminin scaffold of the basal cistern and then migrated into the hippocampal formation using laminin of dentate capillaries as tram tracks (**Figure 1B**, **C**). Even though we expanded neural progenitor cells on laminin-coated tissue culture flasks, which may have selected for neural progenitor cells with a strong affinity to laminin, we observed the same *in vivo* affinity to laminin after growing neural progenitor cells as neurospheres for 5 days before transplantation. Also, previous experiments with neural progenitor cells grown exclusively as neurospheres before transplantation showed the greatest survival of cells along the laminin-rich basal cistern adjacent to the dentate gyrus instead of in the transplant core within the parenchyma of the hippocampal formation (Waldau et al., 2010).

Stereotactic transplantation of neural progenitor cells directly into infarcted area may not produce functional effects due to poor graft survival. Neural progenitor cell transplantation studies have shown poor graft survival in host tissue after stereotactic injection (Waldau, 2010). No neural progenitor cells survived in the transplant core in healthy dentate gyrus in our experiments after 2 weeks. Since laminin seems to be essential for the initial *in vivo* survival of transplanted neural progenitor cells, strategies for better graft survival may involve transplantation of these cells directly on top of blood vessels. In the human brain, the carotid cistern with the supraclinoid carotid artery or the sylvian fissure cistern with its middle cerebral artery branches may provide for the best scaffold for transplanted neural progenitor cells to survive. Neurosurgeons are familiar with the anatomy of these cerebrospinal fluid (CSF) cisterns to directly deliver progenitor cells during a small craniotomy. A small craniotomy may be superior to a stereotactic injection in the vicinity of blood vessels to avoid the risk of vascular injury. Endovascular delivery of neural progenitor cells may also be an option for cell transplantation, but it may not be as efficient since cells have to traverse the capillary intima to reach the laminin-rich basal membrane and since transplanted cells are not stationary due to the blood flow. Once neural progenitor cells have been directly grafted onto the laminin scaffold of intracranial arteries, they may use endogenous arterial branches to reach areas of destination such as a stroke territory. Neural progenitor cells may also profit from the rich nutritional support of the vascular stem cell niche during their migration.

Brain perfusion is regulated by neural network activity. Increased neuronal firing leads to increased regional cerebral blood flow - a phenomenon utilized by arterial perfusion spin labeled functional magnetic resonance imaging to study regional brain activity in relation to various tasks. We examined mice after transplantation in an enriched environment and compared survival of neural progenitor cells to mice

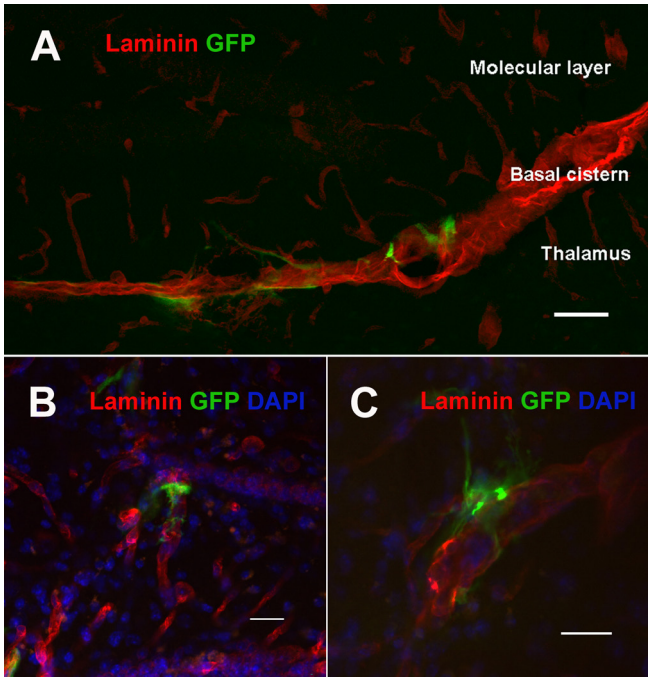

Figure 1 Laminin is important for the initial survival of transplanted neural progenitor cells *in vivo*.
(A) The basal cistern bordering the molecular layer of the dentate gyrus exhibits abundant expression of laminin. Transplanted neural progenitor cells seem to initially survive best on the laminin scaffold of the basal cistern 2 weeks after transplantation. Scale bar: 50 μm. (B) Transplanted neural progenitor cells appear to migrate at the same time along the laminin-rich basal membrane of capillaries into the dentate gyrus. Scale bar: 20 μm. (C) Transplanted neural progenitor cells with strong green fluorescent protein (GFP) expression are tightly associated with capillaries. Scale bar: 20 μm. Laminin: 1:400; polyclonal rabbit; Sigma Aldrich (St. Louis, MO, USA). GFP: 1:2,000; polyclonal chicken; Abcam (Cambridge, MA, USA). 4′,6-diamidino-2-phenylindole (DAPI): 1:500; Hoechst.

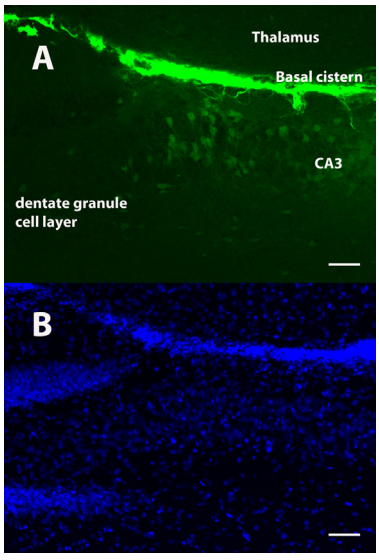

Figure 2 A subpopulation of transplanted cells detaches from the laminin scaffold.
(A) The majority of transplanted cells survive along the laminin scaffold of the basal cistern. A subpopulation of cells with weaker green fluorescent protein (GFP) expression and no apparent capillary association appears to detach from the laminin scaffold of the basal cistern and migrate vertically into the CA3 area of the hippocampus 2 weeks after transplantation. (B) 4′,6-diamidino-2-phenylindole (DAPI). Scale bars: 50 μm. GFP: 1:2,000; polyclonal chicken; Abcam (Cambridge, MA, USA). DAPI: 1:500; Hoechst.

living isolated under standard housing conditions. Blinded counts of progenitor cells showed significantly more transplanted cells in the dentate gyrus after 2 weeks if animals were housed in an enriched environment.

The vascular stem cell niche of the dentate subgranular zone has first been described by Palmer and colleagues (Palmer et al., 2000). Proliferation of endothelial cells and neural progenitor cells is simultaneously stimulated in clusters within the subgranular zone in response to common environmental cues. Potential *in vivo* mitogens are basic fibroblast growth factor and vascular endothelial growth factor. Environmental enrichment also promotes proliferation of endogenous dentate progenitor cells (Kempermann et al., 1997). We observed increased numbers of transplanted dentate progenitor cells in response to environmental enrichment. From our experimental design, it is unclear to tell whether the increased number of progenitor cells with enrichment was due to *in situ* proliferation of transplanted dentate progenitor cells, less cell death after transplantation or increased migration of transplanted progenitor cells from the laminin-rich scaffold of the basal cistern into the dentate gyrus. It is possible that a combination of mechanisms contributed to the increased numbers with enrichment. However, we observed no clusters of transplanted progenitor cells within the dentate gyrus, which argues against the hypothesis of *in situ* proliferation. Transplanted progenitor cells in the dentate gyrus were attached to the laminin-containing basal membrane of capillaries and seemed to use these capillaries as a roadmap to reach their destination. Increased local brain perfusion in response to activated neural networks in the dentate gyrus due to spatial learning and memory retrieval may have triggered the migration of progenitor cells along capillary tracks. Mice with narrower capillaries in the dentate gyrus have been shown to exhibit decreased cell proliferation and survival of newborn neurons (Hara et al., 2010). Therefore, we think that the most likely reason for increased numbers of transplanted progenitor cells in the dentate gyrus with environmental enrichment lies in better nutritional support in the setting of increased regional brain perfusion.

Greater survival of cells in the dentate gyrus with environmental enrichment was region-specific since the total number of surviving cells was higher under standard housing conditions. The non-dentate hippocampal formation showed a non-significant trend towards a higher number of transplanted cells with environmental enrichment. The only other region that showed a significant difference was the cerebral cortex. In the cortex, progenitor cells showed the same tight association with the laminin-scaffold of the endogenous capillary network as in the dentate gyrus. The dentate gyrus and the cerebral cortex have a high density of capillary networks, which may make them most susceptible to the effects of increased perfusion and environmental enrichment (Cavaglia et al., 2001).

We observed a second population of transplanted dentate progenitor cells with weaker green fluorescent protein (GFP) expression, which had detached from the endogenous laminin scaffold and migrated into the interstitial space of the hippocampal formation (**Figure 2A**, **B**). Since we did not observe interstitial cells in the transplant core, it is possible that neural progenitor cells first need to attach to laminin after transplantation in order to survive before they follow an intrinsic program of detachment from laminin, recapitulating the *in situ* behavior of neural progenitor cells in the dentate subgranular vascular stem cell niche.

We used progenitor cells from β-actin-GFP mice to track transplanted cells *in vivo*. Weaker expression of GFP in cells detached from the laminin scaffold may therefore be due to actin/laminin interaction. Clustering of laminin in the extracellular matrix results in laminin receptor clustering and subsequent actin remodeling (Cody and Wicha, 1986). The contact of transplanted progenitor cells to the laminin scaffold may have induced actin and concomitant GFP over-expression. After detachment from the laminin scaffold, GFP expression declined and seemed to be inversely correlated to the distance traveled away from the basal cistern (**Figure 2A**, **B**). Further experiments with laminin-independent neural stem cell labeling techniques are needed to study whether environmental enrichment continues to promote graft survival after 6–8 weeks.

The escape of neural progenitor cells from the laminin scaffold of brain capillaries may also have implications for the brain tumor stem cell theory. Elevated expression of integrin α6 has been found in glioblastoma stem cells, and targeting of integrin α6 attenuates self-renewal and tumor formation (Lathia et al., 2010). However, our results show that neural progenitor cells – which may share some common behavior with glioblastoma stem cells - are able to leave the laminin scaffold and therefore may become immune to integrin targeting. Transplantation of cancer stem cells into the vascular niche may serve as a model to study the early stages of tumor development and the interaction of the vascular stem cell niche with therapeutic agents.

In conclusion, we observed that transplanted neural progenitor cells are closely associated with the vascular stem cell niche and responsive to environmental enrichment. Therefore, survival of neural progenitor cell grafts in humans may be enhanced by physical, occupational or speech therapy to increase regional brain perfusion and help promote survival of transplanted cells in desired target areas. Further research is needed to study whether the association of transplanted dentate progenitor cells with the vascular stem cell niche and the responsiveness to environmental enrichment persists over time.

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