Stem Cell Research 16 (2016) 579–588

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Embryonic stem cell-derived neural progenitors transplanted to the hippocampus migrate on host vasculature

Chelsea M. Lassiter [a,*,1], Julian S. Gal [a,2], Sandy Becker [a,3], Nathaniel W. Hartman [b,4], Laura Grabel [a,5]

[a] Department of Biology, Wesleyan University, Middletown, CT, 06457, USA
[b] School of Natural Sciences and Mathematics, Stockton University, Galloway, NJ, 08559, USA

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 12 February 2016
Accepted 23 February 2016
Available online 9 March 2016

ABSTRACT

This study describes the migration of transplanted ESNPs either injected directly into the hippocampus of a mouse, seeded onto hippocampal slices, or under *in vitro* culture conditions. We show that transplanted mouse ESNPs associate with, and appear to migrate on the surface of the vasculature, and that human ESNPs also associate with blood vessels when seeded on hippocampal slices, and migrate towards BECs *in vitro* using a Boyden chamber assay. This initial adhesion to vessels is mediated, at least in part, via the integrin α6β1, as observed for SVZ neural progenitor cells. Our data are consistent with CXCL12, expressed by the astroglial-vasculature niche, playing an important role in the migration of transplanted neural progenitors within and outside of the hippocampus.

1. Introduction

There is a functional interdependence between the vascular and nervous systems. The vasculature plays a substantive role in regulating endogenous neural progenitor production (Eichmann and Thomas, 2013), and in determining lineage specification of embryonic neural tissue (Acevedo et al., 2015). During neural tube development, angiogenesis forms the subventricular plexus, vascularizing the embryonic neurogenic zone (Engelhardt, 2014). The neurogenic niches of the adult brain, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus, are well vascularized (Bozoyan et al., 2012; Palmer et al., 2000; Shen et al., 2004; Sun et al., 2015), with neural progenitors in close association with blood vessels (Palmer et al., 2000; Shen et al., 2004; Sun et al., 2015; Tavazoie et al., 2008). A functional role for this relationship is supported by the observation that endothelial cells promote neural stem cell proliferation (Shen et al., 2004), and recent transcriptome analysis reveals spatial and temporal-specific expression of neurogenic factors by brain endothelial cells (Ottone et al., 2014). Interestingly, other endothelial-derived signals such as vascular endothelial growth factor (VEGF), initially identified for its mitogenic role during vasculogenesis (Ferrara and Henzel, 1989), also promote SVZ neural progenitor proliferation and maturation (Wittko et al., 2009), whereas conditional knock-out of the VEGF gene in endothelial cells inhibits neurogenesis (Cao et al., 2004).

In addition to supplying a source of growth factors and signaling molecules, blood vessels also provide a physical framework that can support the migration of neural progenitors during development and in the adult brain (Whitman et al., 2009). In the embryonic and adult rodent SVZ, newly born progenitors migrate along blood vessels toward the olfactory bulb via the rostral migratory stream (RMS) (Bovetti et al., 2007; Kokovay et al., 2010; Snapyan et al., 2009). The vasculature also provides guidance cues for the tangential migration of GABAergic progenitors from the embryonic germinal zones to the mouse cortex (Won et al., 2013). In stroke, there is an increase in angiogenesis around the infarct site (Hayashi et al., 2006), and endogenous neural stem cells and neuroblasts migrate along blood vessels to repopulate damaged regions (Madri, 2009; Thored et al., 2007; Yamashita et al., 2006).

A variety of molecular interactions promote the initial association between blood vessels and SVZ neural progenitors and the subsequent migration of the progenitors. The integrin α6β1 on the neural progenitor surface promotes binding to laminin-coated blood vessels, and this interaction is key to the subsequent exit of neural progenitors from the SVZ to join the RMS (Kokovay et al., 2010). VEGF also acts as a chemoattractant, promoting the repopulation of neural progenitors to ischemic regions damaged by stroke (Marti et al., 2000; Wittko et al., 2009). The chemokine CXCL12 on the blood vessel surface helps direct the migration of SVZ neural progenitors (Kokovay et al., 2010).

* Corresponding author at: Biology Department, Wesleyan University, 52 Lawn Ave, Middletown, CT 06459.
E-mail address: classiter@wesleyan.edu (C.M. Lassiter).

[1] Chelsea Lassiter: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.
[2] Julian Gal: collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.
[3] Sandy Becker: collection and/or assembly of data, data analysis and interpretation, final approval of manuscript.
[4] Nathaniel Hartman: collection and/or assembly of data, final approval of manuscript.
[5] Laura Grabel: conception and design, financial support, manuscript writing, final approval of manuscript.

http://dx.doi.org/10.1016/j.scr.2016.02.043

Astrocytes play a role in promoting vasculature-supported neural progenitor migration. Neural progenitors migrating along blood vessels in the SVZ are in direct contact with the astrocytes that coat these vessels (Bozoyan et al., 2012; Kaneko et al., 2010; Lois and Alvarez-Buylla, 1994). During development, astrocytes help maintain the correct organization of the vasculature in the RMS (Bozoyan et al., 2012). Lesioning the cortex can also activate endogenous SVZ neural progenitor cells and promote their migration to the site of injury, with a role for astrocyte/blood vessel-derived CXCL12 reported (Saha et al., 2013). Taken together, these studies suggest the importance of the blood vessel-astroglial environment in providing cues for directing neural progenitors to sites of injury.

Given the ability of endogenous neural progenitors to migrate on brain vasculature, we investigated the ability of embryonic stem cell-derived neural progenitors (ESNPs) transplanted to the adult mouse brain to use blood vessels as a migratory substrate. This line of investigation is of particular interest given the potential use of ESNPs in cell replacement therapies to treat a variety of neurological disorders.

We now show that grafts of mouse embryonic stem cell (ESC)-derived ESNPs in the hippocampus are well vascularized, with an increase in blood vessel area over time, likely due to an inflammatory response triggered by the injection. Following transplant to the hippocampus, ESNPs are also found in close association with blood vessels at great distances from the injection site, consistent with the vasculature providing a migratory substrate. Both blood vessels and blood vessel-associated astrocytes are coated with CXCL12, and our previous work suggests a role for this chemokine in ESNP translocation (Hartman et al., 2010). Using hippocampal slice culture, we observe a time-dependent association of ESNPs with blood vessels following deposition on the slice surface. We directly examined the interactions of ESNPs with brain endothelial cells (BECs) *in vitro* and observe striking morphological differences between ESNPs co-cultured with BECs and ESNPs grown alone. We demonstrate that BECs produce laminin and ESNPs express the α6β1 integrin and that a function-blocking antibody inhibits the adhesion of ESNPs to a BEC monolayer. In addition, we find that human ESNPs (hESNPs) migrate towards BECs in a Boyden chamber migration assay in response to BEC-secreted factors, including CXCL12. These data suggest that the astroglial-endothelial cell niche promotes the migration of ESNPs away from the initial site of deposition. Understanding the molecular cues that direct ESNP migration can aid investigators in accurately targeting therapeutic cell transplants to regions of damage.

2. Results

2.1. Lesion model and transplant vascularization

Our previous work demonstrated that transplanting mouse ESNPs (mESNPs) into the dentate gyrus (DG) creates a lesion, which leads to degeneration of the suprapyramidal layer of the granule cell layer (Hartman et al., 2010). As previously reported, transplanted YC5/EYFP mESNPs, (see Materials and Methods and Supp. Fig. 1A) repopulate the suprapyramidal layer (Fig. 1A-C) and differentiate into granule neurons (Hartman et al., 2010). To establish whether the disappearance of the suprapyramidal layer of the DG was caused by the presence of the mESNPs or is due to a lesion caused by fluid injection alone, we compared the suprapyramidal layer of the DG following injection of mESNPs versus culture medium containing only rhodamine labeled microspheres. The pressure of fluid injection alone is sufficient to destroy the suprapyramidal layer, based on the virtual absence of NeuN-positive cells at 28 days following injection (Supp. Fig. 2A-D). The layer is not restored under this condition, indicating that endogenous progenitors cannot regenerate this granule cell layer (Supp. Fig. 2A-D).

We next examined the extent of vascularization in the transplant region, or domain marked by microspheres. Three days after YC5/EYFP mESNP injection, CD31-positive blood vessels are readily visible within the transplant (Fig. 1A, A'). Over time, there is an apparent increase in the vascular area within the transplant zone (Fig. 1B, B', C, C'). To determine whether this increase is due to the transplant or is attributable to the lesion alone, we compared the vessel area within a mESNP transplant to the vessel area within the domain marked by microspheres. The increase in vessel area over time in the microsphere-containing domain and the mESNP transplant are comparable (Fig. 1D). These data, combined with our observation that there is an increase in the number of activated microglia within the transplant region (data not shown), suggest that upregulation of angiogenesis is due to an inflammatory response elicited by the fluid injection, and not dependent upon the presence of mESNPs.

2.2. mESNPS migrate upon and exhibit a close association with host vasculature as they move away from the transplant site

At 28 days after injection, a large Sox1:GFP/Ubi:RFP mESNP transplant (red) in the DG interfaces with a laminin-positive vascular plexus (green) (Fig 2A). Upon examination, transplanted mESNPs were also consistently observed outside of the main area of the transplant. Fig. 2A' shows transplanted mESNPs that have migrated to the molecular layer of the hippocampus and are closely associated with blood vessels. Fig. 2A" shows that mESNPs can travel great distances into the cortex, and are again associated with blood vessels. We are able to visualize the needle track of the injection (data not shown) and because it is not adjacent to the cells we observe, conclude that it is unlikely the mESNPs in the molecular layer or cortex were deposited along this trajectory upon injection (Fig. 2 A'A"). These data suggest that transplanted mESNPs migrate extensively using the vasculature as a substrate, consistent with previous work demonstrating that endogenous neural progenitors can use blood vessels as a migratory scaffold (Kokovay et al., 2010; Whitman et al., 2009).

As doublecortin (DCX) expression marks migratory neuroblasts, we examined the proximity of DCX-positive mESNPs to blood vessels (Fig. 2B-D). Fig. 2B and C show a large transplant of YC5/EYFP cells that fills the suprapyramidal layer of the DG 7 days following injection. Vessels within the transplant are visualized via expression of CD31 (Fig. 2B, red; 2B'C', violet). Fig. 2B' and C', corresponding to the boxes shown in Fig. 2B and C, document a transplanted ESNP (GFP-positive), DCX-positive cell closely associated with a CD31-positive blood vessel. This cell is located past the molecular layer of the hippocampus on a blood vessel just ventral to the pyramidal cell layer of CA3, approximately 250 microns away from the dorsal edge of the transplant. The open arrowheads point to the nucleus of the transplanted mESNP and the closed arrowheads point to the nucleus of an endothelial cell. To determine if mESNPs are found closer to blood vessels than expected randomly, we quantified the distance of GFP-positive mESNPs, DCX-positive/GFP-positive mESNPs, and endogenous DCX-positive cells from the nearest blood vessel in comparison to what would be expected for a randomly distributed cell (Fig. 2D). Both populations of mESNPs, as well as endogenous DCX-positive cells, are located closer to blood vessels than expected if cells were distributed randomly (Fig. 2D, D'). Although the average distance between each of the three cell populations and blood vessels does not differ (Fig. 2D'), their relative distribution at distinct distances does, as shown in Fig. 2D. A random distribution of points throughout the hippocampus exhibits a Gaussian curve (blue bars in Fig. 2D). In contrast, mESNPs and their DCX-positive neuroblast derivatives, as well as endogenous neuroblasts, are closer to blood vessels. More than half of the GFP-positive mESNPs are found within 10 microns of a blood vessel (green bars in Fig. 2D). 33% of the DCX-positive transplanted mESNPs are found within 5 microns of a blood vessel (red bar, Fig. 2D), whereas 15% of the endogenous DCX-positive cells are found in this domain (purple bar, Fig. 2D). These data suggest that, amongst these cell types, mESNP-derived neuroblasts are most closely associated with the vasculature, consistent with their migration on the vessel surface.