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Research Report

Bidirectional crosstalk between periventricular endothelial cells and neural progenitor cells promotes the formation of a neurovascular unit

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ABSTRACT

Interactions between neural progenitor cells (NPC) and endothelial cells (EC) from adult vascular beds have been well explored previously. However, the factors and signaling mechanisms that regulate neurogenesis and angiogenesis are most prevalent during embryonic development. This study aimed to determine whether embryonic brain endothelial cells from the periventricular region (PVEC) present an advantage over adult brain EC in supporting NPC growth and differentiation. PVEC were isolated from E15 mouse brains, processed, and sorted with immunomagnetic beads using antibodies against CD31/PECAM. On immunofluorescence (IF) staining, nearly all cells were positive for EC markers CD31 and CD144/VE-Cadherin. In proliferation studies, NPC proliferation was highest in transwell co-culture with PVEC, approximately 2.3 fold increase compared to baseline *versus* 1.4 fold increase when co-cultured with adult brain endothelial cells (ABEC).

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Proliferation
Differentiation

These results correlated with the PVEC mediated delay in NPC differentiation, evidenced by high expression of progenitor marker Nestin evaluated by IF staining. Upon further characterization of PVEC in an angiogenesis assay measuring cord length, PVEC exhibited a high capacity to form cords in basal conditions compared to ABEC. This was enhanced in the presence of NPC, with both cell types displaying a preferential structural alignment resembling neurovascular networks. PVEC also expressed high Vegfa levels at baseline in comparison to NPC and ABEC. Vegfa levels increased when co-cultured with NPC. We demonstrate that PVEC and NPC co-cultures act synergistically to promote the formation of a neurovascular unit through dynamic and reciprocal communication. Our results suggest that PVEC/NPC could provide promising neuro-regenerative therapies for patients suffering brain injuries.

1. Introduction

The complex interplay between neural progenitor cells (NPC) and endothelial cells (EC) is well documented in the central nervous system (CNS) (Ford et al., 2006; Leventhal et al., 1999; Shen et al., 2004; Sun et al., 2010; Teng et al., 2008). Together NPC and EC constitute a "neurovascular niche" that promotes the proliferative and migratory potential of both cell types, ultimately controlling their fate (Ramirez-Castillejo et al., 2006). This complementary relationship is mediated through direct cell–cell contact as well as autocrine and paracrine feedback signals (Flamme et al., 1997; Lambrechts et al., 2003; Shen et al., 2004). Several groups have explored whether interactions between NPC and EC could be exploited in the adult or young–adult brain to promote neurogenesis (Alvarez-Buylla et al., 2002; Chen et al., 2004; Emsley et al., 2005; Golmohammadi et al., 2008). These studies have traditionally used NPC and mature EC (Muffley et al., 2012; Rauch et al., 2008), with scarce data evaluating the interactions of NPC with their developmental and physiological EC partners, *i.e.* embryonic brain EC. This is surprising since angiogenesis and neurogenesis are significantly more robust in embryonic CNS than in adult. Furthermore, there are striking similarities between vascular and neuronal development in the embryonic brain, which suggest that common regulators direct these processes within the growth-promoting milieu of the embryonic CNS (Carmeliet, 2003; Carmeliet and Tessier-Lavigne, 2005).

Blood vessels of the embryonic forebrain (telencephalon) fall into two categories based on anatomical location, growth patterns and developmental regulation, namely pial and periventricular. Pial vessels are a group of capillaries that encircle mouse brain by embryonic day 9 (E9) and form, among other fates, venous sinuses. On the other hand, the periventricular vessel network originates from a vessel deep within the basal ganglia primordium to form the future arterial network (Vasudevan et al., 2008). In mice, this periventricular vascular network develops by E11 in an orderly, ventral-to-dorsal angiogenic gradient, paralleling the neurogenetic gradient. The proliferation, migration and sprouting of new vessels from the embryonic brain endothelial cells (PVEC) arising from this periventricular region coincide with proliferation of neuroepithelial precursor cells, neuronal migration and elaboration of neuronal processes and networks. We thus hypothesized that PVEC would inherently be the ideal partner to promote NPC based neuro-regenerative therapies. In this study, we attempted to characterize PVEC and ascertain the nature of their interactions with NPC. A better understanding of these interactions could simulate conditions similar to the embryonic neurovascular niche to promote neurogenesis and brain repair.

2. Results

2.1. *Isolation and characterization of a novel endothelial cell type*

Approximately 65% of PVEC isolated from E15 mouse brains expressed EC cell surface marker CD31/PECAM on flow cytometric analysis. Subsequently, purification of this population by CD31 immunomagnetic beads resulted in nearly all PVEC expressing EC markers CD31 and CD144/VE-Cadherin on immunofluorescence (IF) staining (Fig. 1B and C respectively). They also expressed the early progenitor/undifferentiated cell marker Nestin (Fig. 1D) (Michalczyk and Ziman, 2005). In contrast, they failed to stain for the neuronal markers β-tubulin III and MAP2+, or the glial cell markers Olig 1, and GFAP, which further confirmed the purity of this population (data not shown).

2.2. *PVEC and NPC crosssignals promote NPC proliferation in vitro via PVEC secreted soluble factors*

After isolating and characterizing PVEC, we examined PVEC's impact on NPC proliferation. Proliferation experiments were performed in a transwell culture system. NPC were cultured in the basolateral chamber, and PVEC were cultured in the apical chamber. 0.4 μm pore membrane inserts were used. This system mimicked the neurovascular niche (adult and embryonic) where EC interact with NPC through a basement membrane. The rate of proliferation of NPC co-cultured with PVEC for 48 h was significantly higher than NPC monocultures or co-cultures with ABEC (Fig. 2). Although we recognize differences between PVEC and ABEC, these cells share many