REVIEW

Angiogenesis and neuronal remodeling after ischemic stroke

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Abstract

Increased microvessel density in the peri-infarct region has been reported and has been correlated with longer survival times in ischemic stroke patients and has improved outcomes in ischemic animal models. This raises the possibility that enhancement of angiogenesis is one of the strategies to facilitate functional recovery after ischemic stroke. Blood vessels and neuronal cells communicate with each other using various mediators and contribute to the pathophysiology of cerebral ischemia as a unit. In this mini-review, we discuss how angiogenesis might couple with axonal outgrowth/neurogenesis and work for functional recovery after cerebral ischemia. Angiogenesis occurs within 4 to 7 days after cerebral ischemia in the border of the ischemic core and periphery. Post-ischemic angiogenesis may contribute to neuronal remodeling in at least two ways and is thought to contribute to functional recovery. First, new blood vessels that are formed after ischemia are thought to have a role in the guidance of sprouting axons by vascular endothelial growth factor and laminin/β1-integrin signaling. Second, blood vessels are thought to enhance neurogenesis in three stages: 1) Blood vessels enhance proliferation of neural stem/progenitor cells by expression of several extracellular signals, 2) microvessels support the migration of neural stem/progenitor cells toward the peri-infarct region by supplying oxygen, nutrients, and soluble factors as well as serving as a scaffold for migration, and 3) oxygenation induced by angiogenesis in the ischemic core is thought to facilitate the differentiation of migrated neural stem/progenitor cells into mature neurons. Thus, the regions of angiogenesis and surrounding tissue may be coupled, representing novel treatment targets.

Key Words: *angiogenesis; axonal outgrowth; cerebral ischemia; coupling; functional recovery; guidance; neurogenesis; stroke*

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Introduction

Increased microvessel density in the peri-infarct region has been reported and has been correlated with longer survival times in ischemic stroke patients (Krupinski et al., 1994). This raises the possibility that enhancement of angiogenesis is one of the strategies to facilitate functional recovery after ischemic stroke (Arai et al., 2009; Jin et al., 2017; Kanazawa et al., 2019; Marushima et al., 2019).

Effective therapies to promote functional recovery after ischemic stroke could require protection and/or recovery of not only the neurons but also the entire neurovascular unit. The neurovascular unit comprises a conceptual anatomical framework consisting of neurons and their axons, glial cells, and the microvessels that supply oxygen and nutrients to neurons and other cells. Both neurons and microvessels contribute to the pathophysiology of cerebral ischemia (del Zoppo, 2006). Previous observations suggest that focal cerebral ischemia can initiate coordinated responses within the microvasculature and in the neurons nearby where both elements appear to function as a unit.

The most general practical use of the term "ischemic penumbra" is that of a peri-infarct region salvaged by any treatment. Although the ischemic core is defined as a region or regions below perfusion threshold that correlate with characteristic electrophysiological changes, for practical reasons, in animal models, the core has also generally been defined as regions lacking the microtubule-associated protein-2 (microtubule-associated protein-2-negative region), depending upon the setting and model system used. The angiogenesis is observed in the border of the microtubule-associated protein-2-negative ischemic core and peri-infarct region. Although, in the border of the ischemic core, angiogenesis is present after cerebral ischemia. This region might be a novel treatment target after cerebral ischemia (reviewed by Kanazawa et al., 2019). In this mini-review, we discuss how angiogenesis might couple with axonal outgrowth/neurogenesis and work for functional recovery after cerebral ischemia.

Literature Review

We have performed a PubMed literature search of articles published in the period January 1991 to April 2019 on angiogenesis, neovascularization (MeSH Terms), axonal

outgrowth, and/or neurogenesis (MeSH Terms) in ischemic stroke and also cerebral ischemia.

Coupling between Angiogenesis and Neuronal Recovery

Blood vessels and axons run parallel throughout the central nervous system, suggesting a coupling of both components. Post-ischemic angiogenesis may modulate 1) axonal outgrowth and 2) neurogenesis, including proliferation, migration, and maturation of neural stem/progenitor cells (NSCs), and it is thought to contribute to functional recovery. Following ischemic stroke, administration of bone marrow mononuclear cells may be correlated with improved regional cerebral blood flow, regional metabolic rate of oxygen consumption, and improved neurological function (Taguchi et al., 2015). However, direct evidence correlating increased regional cerebral blood flow and functional recovery is hardly proven in humans. Using *ex vivo* coculture systems, possible interactions between angiogenesis and neurogenesis have been examined in, for example, three-dimensional culture models, including neurons, endothelial cells, and extracellular matrices (Uwamori et al., 2017). Coculture of endothelial cells with neural stem cells led to expanded neurogenesis (Shen et al., 2004). Further, conditioned media from endothelial cells protected neuronal cells against oxygen-glucose deprivation via brain-derived neurotrophic factor (BDNF) (Guo et al., 2008). Indeed, several mediators have been shown modulate both angiogenesis and axonal outgrowth and communicate with cells within the affected neurovascular units. These include vascular endothelial growth factor (VEGF) (Kanazawa et al., 2017a, b), transforming growth factor-β (Yi et al., 2010), angiopoetin-1 (Kawamura et al., 2014), platelet-derived growth factor-B (Renner et al., 2003), BDNF (Jiang et al., 2017), and progranulin (Kanazawa et al., 2015) (**Figure 1**). In addition, blood vessels secrete signals (e.g., VEGF, artemin, and neurotrophin) to guide axons and conversely, axons secrete signals to guide blood vessels (Carmeliet and Tessier-Lavigne, 2005). As such, each cell in the neurovascular unit can communicate with other cells using various mediators.

Angiogenesis and Axonal Outgrowth

Angiogenesis is the formation of new microvessels that branch off from pre-existing vessels (Ruan et al, 2015). Hypoxic tissues secrete VEGF; angiogenesis occurs along a concentration gradient of VEGF in neonates (Carmeliet and Tessier-Lavigne, 2005). Angiogenesis also occurs within 4 to 7 days after cerebral ischemia in the border of the ischemic core (Kanazawa et al., 2017a) (**Figure 2**). Meanwhile, axonal outgrowth does not normally appear until 14 days after ischemia, even in the ischemic periphery (Kanazawa et al., 2017a). Cortical circuits can be detected as early as three weeks after ischemia, which enhances functional recovery (Carmichael et al, 2017; Kanazawa et al, 2017a). Angiogenesis may be essential for brain repair following ischemia as it allows increased blood flow and metabolic nutrients to reach the affected brain regions. Furthermore, after ischemia, VEGF from vessel components may promote axonal outgrowth (Jin et al., 2006). Lei et al. (2012) reported that axons bound to laminin express β1-integrin and that laminin/β1-integrin signaling may contribute to axonal development and outgrowth *in vitro*. β1-integrin and laminin are expressed in endothelial cells (Osada et al., 2011). This raises the possibility that angiogenesis and neurogenesis are coupled by VEGF and laminin/β1-integrin signaling, although this has not yet been demonstrated *in vivo*. Taken together, new blood vessels that are formed after ischemia are thought to have a role in the guidance of sprouting axons.

Angiogenesis and Neurogenesis

Neurogenesis is the process of making new functional neurons from endogenous NSCs, including proliferation, migration, and differentiation into mature neurons. Neurogenesis continues throughout the lifespan in two distinct regions: the subventricular zone of the lateral ventricles and the subgranular zone in the dentate gyrus of the hippocampus and increases following cerebral ischemia (Jin et al., 2001). Expression of several extracellular signals (e.g., fibroblast growth factor-2, insulin-like growth factor-1, BDNF, and VEGF) increases after cerebral ischemia, leading to an enhanced proliferation of endogenous NSCs. Among these, VEGF and fibroblast growth factor-2 are released from endothelial cells (Ruan et al., 2015).

Following NSC proliferation, neuroblasts migrate from the subventricular zone to the peri-infarct region, where post-ischemic angiogenesis occurs (Ruan et al., 2015). Microvessels support the migration of NSCs toward the peri-infarct region in at least two ways. First, microvessels supply oxygen, nutrients, and soluble factors, which create a microenvironment suitable for NSC's migration, and BDNF derived from endothelial cells promote vascular-guided NSC migration (Grade et al., 2013). Second, microvessels provide β1 integrin signals which cause NSC to adhere to microvessels and serve as a scaffolding for migration (Fujioka et al., 2017). Integrins are transmembrane receptors that mediate cell adhesion to the extracellular matrix. Extracellular matrix proteins, such as type IV collagen, laminin, and fibronectin, are produced by endothelial cells, pericytes, and astrocytes, and are a component of the vascular basement membrane. Laminin-integrin-dependent adhesion of neuroblasts to vessels facilitates post-ischemic cell migration (Fujioka et al., 2019). Following cerebral ischemia, vascular remodeling occurs around the subventricular zone. VEGF and angiopoetin-1 promote not only angiogenesis but also NSC migration after cerebral ischemia (Ohab et al., 2006; Wang et al., 2007). Stromal-derived factor-1 also contributes to vascular-guided migration (Ohab et al., 2006).

After proliferation and migration of NSCs, how are microvessels involved in their differentiation into mature neurons? Endothelial cells regulate NSC proliferation via BDNF and direct their fate towards neurons *in vitro* (Shen et al., 2004). In the developing brain, neurogenesis is strongly correlated with angiogenesis. Neuronal precursor cells are pres-

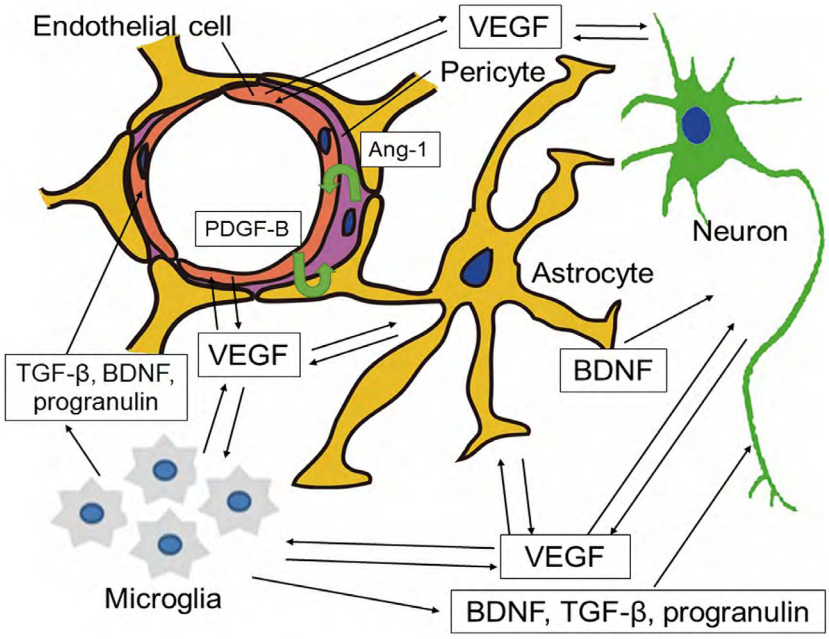

Figure 1 The schema of association between angiogenesis and axonal outgrowth.
After ischemia, several mediators, such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), brain-derived neurotrophic factor (BDNF), platelet-derived growth factor-B (PDGF-B), angiopoietin-1 (Ang-1), and progranulin, may promote both angiogenesis and axonal outgrowth, coupled with cells comprising the neurovascular units.

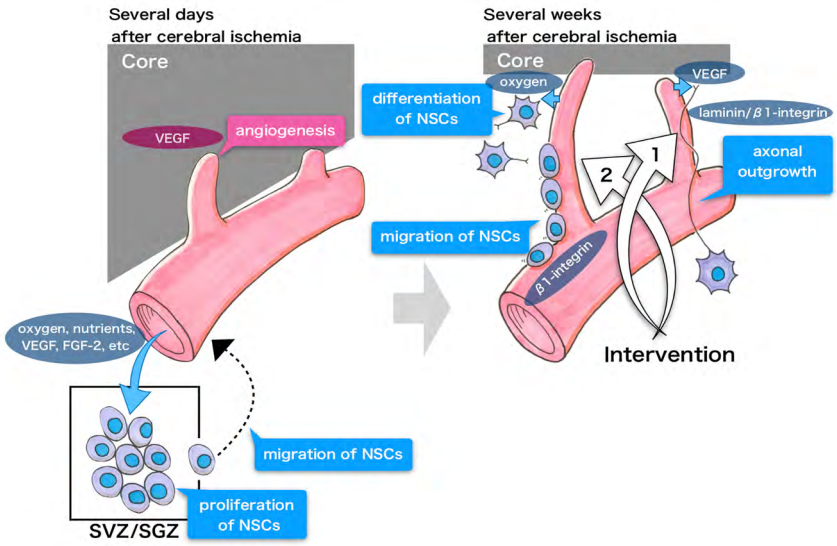

Figure 2 The angiogenesis guides axonal outgrowth and neurogenesis.
Vascular endothelial growth factor (VEGF) and several factors from hypoxic tissues and microvessels promote angiogenesis several days after cerebral ischemia. After intervention, (1) new blood vessels that are formed after ischemia are thought to have a role in the guidance of sprouting axons by VEGF and laminin/β1-integrin signaling. (2) Vascular-guided neural stem/progenitor cells (NSCs) also may migrate peri-infarct regions and migrated NSCs differentiate into mature neurons several weeks after cerebral ischemia. SGZ: Subgranular zone; SVZ: subventricular zone.

ent only in the vascularized cortex. Hypoxia is thought to increase NSC expansion via the hypoxia-inducible factor-1α pathway; the relief of hypoxia induced by angiogenesis in the cortex triggers a switch from NSC expansion to differentiation (Lange et al., 2016). Angiogenesis can be observed in the border of the ischemic core (Kanazawa et al., 2017a, 2019), where severely hypoxic regions are present. Thus, oxygenation induced by angiogenesis in the ischemic core is thought to facilitate the differentiation of migrated NSCs into mature neurons (**Figure 2**). This hypothesis remains to be fully validated.

Interventions and Perspectives

A number of reports using rodent models of focal cerebral ischemia have described the effects of pharmacological and cell-based treatments that appear to increase angiogenesis and axonal outgrowth, as well as lead to functional recovery (reviewed by Kanazawa et al., 2019). The direct causal relationship between angiogenesis and functional recovery has not yet been proven. However, angiogenesis might provide a suitable microenvironment to trigger axonal outgrowth and may induce neurogenesis. The use of agents and/or cell therapies that promote angiogenesis and axonal outgrowth in the ischemic periphery may be therapeutically relevant treatment strategies.

In summary, post-ischemic angiogenesis is involved with the axonal outgrowth and proliferation, migration, and maturation of NSCs, which likely contribute to functional recovery. Direct interactions between angiogenesis in the ischemic core and axonal outgrowth in the ischemic periphery have not been fully elucidated. It is unknown whether angiogenesis could aid in shrinking the ischemic core. The regions of angiogenesis and surrounding tissue may be coupled, representing novel treatment targets.

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