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Coordinated Regulation of Brain Angiogenesis and Barriergenesis

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The genesis of a functional vascular system of the brain requires a complex set of developmental programs: brain angiogenesis and barriergenesis. Brain angiogenesis and barriergenesis are generally characterized by the process of blood formation, branching/sprouting, remodeling and the maturation into blood-brain barrier (BBB). The oxygen tension during the development of vascular system influences vascular vessel formation through regulating angiogenesis and barriergenesis.

First of all, hypoxia is a well-known signal for regulating many physiological and pathological angiogenic processes. We found that the immunoreactivity of pimonidazol, hypoxic marker, was detected in developing brain, and then disappeared in the neonatal brain. The immunoreactivity of vascular endothelial growth factor (VEGF) was spatiotemporally detected in developing brain. It is well known that the expression of VEGF is mainly upregulated by hypoxia inducible factor-1 (HIF-1) under hypoxic condition. HIF-1 is a key transcription factor involved in hypoxia-induced angiogenesis during vascular development and remodeling. Therefore, we suggest that hypoxia may exist widely in developing brain tissues and may act as a signal for brain vessel formation *in vivo*.

Furthermore, we found that hypoxia/reoxygenation is an important regulator of the BBB formation, called barriergenesis. The BBB is an essential barrier for maintaining brain homeostasis and low permeability. Brain capillaries are differentiated and matured into BBB in concert with astrocytes during brain development through oxygen signal. In

order to gain new insights into the processes controlling development and formation of barriergenesis, we identified Src-suppressed C kinase substrate (SSeCKS) by a cDNA RDA. SSeCKS belongs to a family of scaffolding proteins and act as both an anchoring protein and a substrate for protein kinase C. SSeCKS overexpressed in astrocytes during reoxygenation which resulted in a marked reduction of VEGF and angiogenesis. Furthermore, SSeCKS overexpression increased the expression of both angiopoietin-1 (Ang-1), and antipermeability factor, and tight junction proteins, consequently decreased [^{3}H]sucrose permeability. The immunoreactivity of SSeCKS was gradually increased during the vascular maturation period, and SSeCKS-expressing astrocytes closely interacted with zonula occludens-1-expressing blood vessels *in vivo*. We therefore suggest that SSeCKS-expressing astrocytes induce the maturation and stabilization of permeable vessels by inhibiting brain angiogenesis and by enhancing barriergenesis through upregulation of tight junction proteins. Moreover, we suggest that the constitutive expression of SSeCKS in the adult brain may provide a stabilizing signal for barriergenesis under physiological conditions.

Collectively, we suggest that oxygen signal may play an essential role on the cooperative regulation of angiogenesis and barriergenesis of brain vessel development.

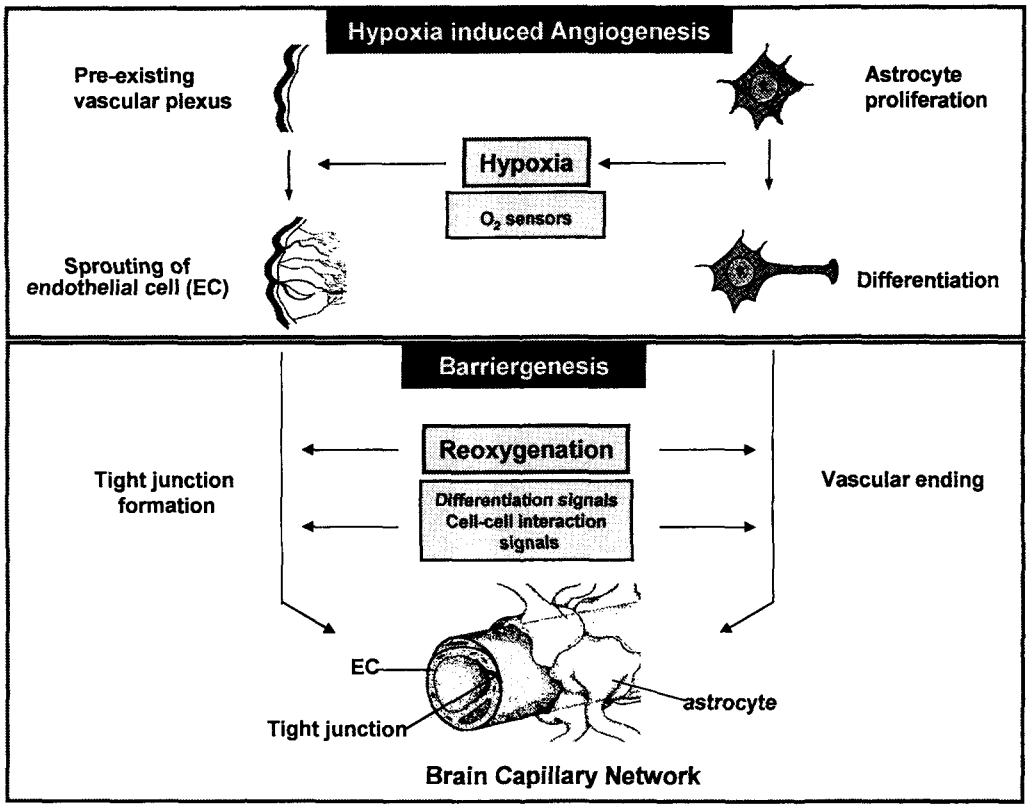

Fig 1. Brain angiogenesis and barriergenesis. Brain angiogenesis and barriergenesis are generally characterized by the process of blood formation, branching/sprouting, remodeling and finally maturation into the BBB in the brain. The oxygen tension during the development of vascular system influences vascular vessel formation through regulating angiogenesis and barriergenesis (Lee SW et al., *Nat. Med.*, 9, 900-906, 2003).

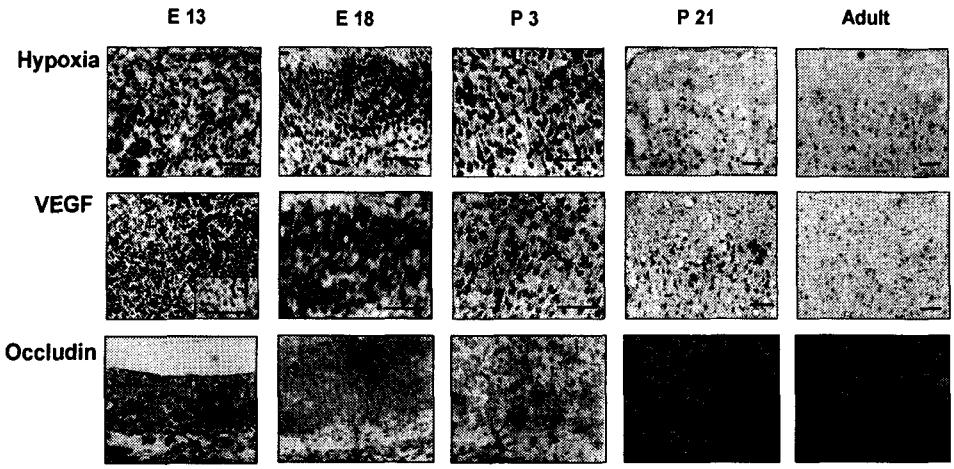

Fig 2. During hypoxia-induced angiogenesis, hypoxic marker and VEGF were expressed in the same place and at the same time in rat brain. In the period of reoxygenation-induced barriergenesis, the expression of tight junction protein, Occludin was increased (Lee YM et al., *Dev. Dynamics*, 220, 175-186, 2001; Song HS et al., *BBRC*, 290, 325-331, 2002; Lee SW et al., *Nat. Med.*, 9, 900-906, 2003).

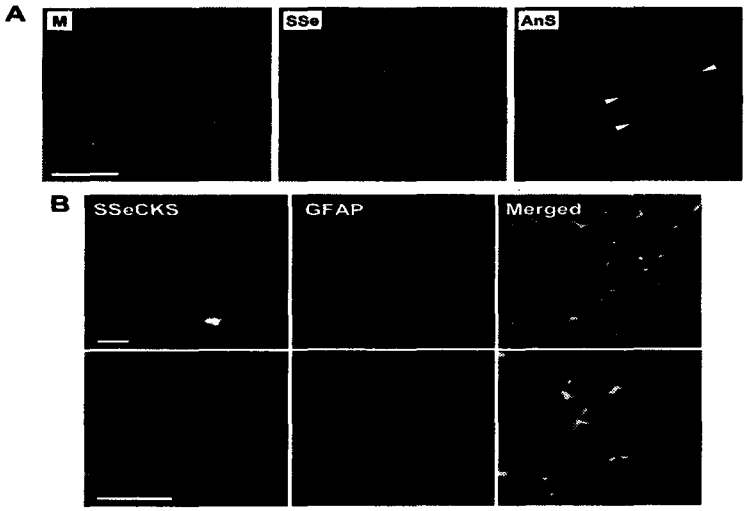

Fig 3. (A) Zonula occludens-1 (ZO-1) immunofluorescence staining. The linear distribution of ZO-1 was detected at HBMEC margins, where cell-cell contact occurs. The linear distribution of ZO-1 after AKAP12-CM treatment was clearer than that in HBMECs treated with mock-conditioned media. (B) Double immunofluorescence staining for AKAP12 (green) and GFAP (red). Immunofluorescence of AKAP12 clearly overlapped with that of GFAP, indicating that AKAP12 is expressed in brain astrocytes (Lee SW et al., *Nat. Med.*, 9, 900-906, 2003).

Building up the blood-brain barrier

Peter Rieckmann[1] & Britta Engelhardt[2]

Astrocyte projections extend to the blood-brain barrier (BBB) and promote its maturation. These astrocytes are now shown to secrete a factor that appears to integrate signaling networks necessary for BBB development and maintenance (pages 900–906).

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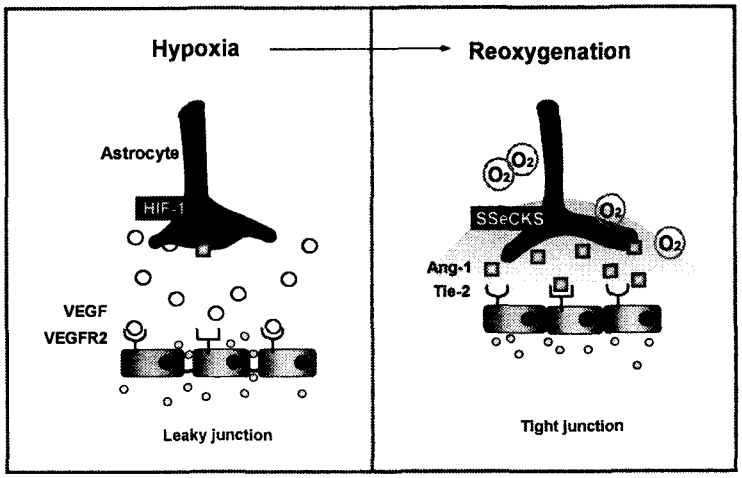

Fig 4. SSeCKS regulates BBB maturation. Expression of SSeCKS in brain astrocytes during reoxygenation results in decreased VEGF and increased Ang-1 secretion. Ang-1 signals through the Tie-2 receptor on cerebral endothelial cells and induces tight junction formation, thereby decreasing paracellular permeability-two events that are crucial to BBB differentiation (Lee SW et al., *Nat. Med.*, 9, 900-906, 2003; Rieckman P & Engelhardt B., *Nat. Med.*, 9-828-829, 2003).