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From Death to Recovery Following Hypoxia Ischemia: If TGFβ Is a Central Regulator, Is Integrin β8 the Switch?

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Neonatal hypoxic ischemic brain injury is a significant cause of mortality and lifelong neurological morbidity. Despite its public health significance, little is know about the underlying molecular pathways linking hypoxia to neuronal ischemia and cell death. In this issue of Neurotoxicity Research, Li et al. show that the integrin β8 is upregulated in cultured astrocytes in response to hypoxia, which subsequently activates a TGFβ-dependant neuroprotective pathway. These findings support a novel function for astrocyte-derived integrin β8 and sheds light on the molecular mechanisms underlying hypoxic ischemic brain injury.

TGFβ-mediated Cytoprotection

TGFβ is a pleotropic cytokine with well-characterized cytoprotective and apoptotic effects (Annes et al. 2003; Flanders et al. 1998; Unsicker and Krieglstein 2002). Whether TGFβ promotes cell survival or induces apoptosis depends largely on the cellular and environmental context, as well as the specificity of TGFβ activation and signaling. This specificity is achieved through various mechanisms including pericellular extracellular matrix localization, regulated liberation of active TGFβ from its latent form, and a vast array of intracellular signaling molecules integrating TGFβ effects on target tissues. The integrin αvβ8 is a critical activator of TGFβ in vitro and in vivo. Using cell culture assays, Cambier et al. (2005) showed that astrocyte-derived αvβ8 binds to and activates TGFβ, which then transactivates vascular endothelial cell TGFβ dependent signaling. Conditional deletion of β8 from dendritic cells abrogates TGFβ-mediated activation of regulatory T cells and results in an immunophenotype identical to that found in TGFβ knockout mice (Travis et al. 2007). Mice with a mutated form of TGFβ1 blocking integrin-mediated activation develop a phenotype identical to that of TGFβ1 null mice (Yang et al. 2007) and similar to that of αv or β8 deficient mice (Bader et al. 1998; Zhu et al. 2002a, b). Finally, genetic loss of TGFβ in mice results in apoptotic neuronal loss accompanied by diffuse astrogliosis, and increased neuronal susceptibility to kainic acid-induced excitotoxic injury (Brionne et al. 2003). Moreover, in vivo TGF-β1 administration in mice protects against ischemic brain injury (McNeill et al. 1994; Zhu et al. 2002a, b), and overexpression of TGFβ1 from astrocytes protects against excitotoxic neuronal injury (Brionne et al. 2003). While these experiments support β8's role in the activation of TGFβ, and TGFβ's role in neuroprotection, there has been little direct evidence demonstrating transcellular activation of neuroprotective signaling pathways by β8. Li et al. (2009) provide such evidence. They show that in the presence of β8 expressing astrocytes, TGFβ protects against hypoxia-induced apoptotic cell death, in part by upregulating canonical antiapoptotic proteins BCL2 and BCLxl. Importantly, BCLxl is induced by TGFβ1 via TGFβ receptor, ALK1 activation of NF-kappaB, promoting neuronal survival after injury (König et al. 2005). It will be interesting to see if this same β8–TGFβ–ALK1 pathway is important for neuronal maintenance and for protection against hypoxic ischemic injury in vivo.

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TGFβ Activation

As was previously shown by other groups (Cambier et al. 2005; Mobley et al. 2009), Li et al. (2009) demonstrate that astrocyte-derived β8 activates TGFβ. Different from other studies (Cambier et al. 2005), however, they found that neither matrix metaloprotease (MMP) inhibition nor β8 knockdown could completely abrogate TGFβ activation. While the authors note this may be due to ineffective MMP inhibition, or due to incomplete β8 knockdown, it is also possible that alternative activators of TGFβ are upregulated in response to hypoxia, such as other integrins or receptors. For example, the VEGF co-receptor, neuropilin 1 (Nrp1) is highly expressed on neurons, regulates neuroprotection in response to hypoxia (Oosthuyse et al. 2001), and was recently found to bind to and activate TGFβ (Glinka and Prud'homme 2008). Interestingly, there are numerous parallels between Nrp1 and β8. For instance, Nrp1 and β8 knockouts have similar cerebrovascular phenotypes. Also, the adult neurological phenotype of αv or β8 deficient mice (McCarty et al. 2005; Proctor et al. 2005; Mobley et al. 2009) is strikingly similar to that of mice with deletion of the hypoxia-response element in the VEGF promoter (Oosthuyse et al. 2001), where hypoxia-induced VEGF was found to have a neuroprotective role mediated in part through neuronal Nrp1. Considering these parallels, it will be interesting to determine whether neuro-glial Nrp1 can specifically activate TGFβ in response to hypoxia, and whether this occurs in the context of the β8–TGFβ interaction.

Hypoxia-Induced β8 Expression

Li et al. (2009) show that astocytic β8 is upregulated in response to hypoxemia and that the timing of hypoxia-induced TGFβ activation mirrors peak expression levels of β8. Why might β8 expression be responsive to hypoxia/ischemia? During development, β8 plays an essential role in vascular ingression and remodeling in the brain (Zhu et al. 2002a, b; Proctor et al. 2005). Here, it is plausible that glial-derived β8 regulates neovascularization, coupling the metabolic needs of developing neuroepithelial cells to the vasculature that supplies oxygen and nutrients. It is tempting to speculate that β8 plays a similar dual role in regulating neo-vascularization and neuronal survival in response to hypoxic damage. How does hypoxia signal astrocytes to upregulate β8? Based on the observations of Li et al. (2009), β8 may have direct or indirect autocrine signaling effects through TGFβ. TGFβ and hypoxia cooperatively signal through hypoxia inducible factor (HIF)-1α to regulate transcription of endothelial-derived VEGF, and control angiogenesis and endothelial apoptosis (Ferrari et al. 2006; Sánchez-Elsner et al. 2001). HIF1α and its major target gene, VEGF, are upregulated in response to hypoxia/ischemia, and may protect against neuronal cell death in this setting (Sheldon et al. 2009). Considering these recent reports, it will be important to determine whether β8 is involved in regulation of HIF1α and VEGF, and alternatively how hypoxia, HIF1α and VEGF may regulate expression of β8. This line of study may more fully elucidate the pathophysiology of hypoxic ischemic brain injury and could help identify novel treatment targets. Taken together the findings of Li et al. (2009), one may speculate that β8 is critically important for neuronal maintenance and protection against hypoxic insult in vivo. Testing of this hypothesis will require astrocyte specific deletion of β8, and in vivo characterization of these mice in various injury paradigms including hypoxia/ischemia brain injury.

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