Cell Cycle 12:24, 3709–3710; December 15, 2013; © 2013 Landes Bioscience

Tie2 (to) Abl

Signaling to endothelial cell survival

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The vasculature plays a crucial role in normal physiology, supplying oxygen and nutrients, as well as maintaining a semi-permeable barrier between tissues and the bloodstream. Endothelial cells (ECs), which form the innermost lining of blood vessels, are critical players in vascular function, by regulating the transport of solutes and immune cells into and out of the bloodstream, regulating vasoreactivity, and maintaining the vasculature in an anti-thrombotic state. Disruption of endothelial homeostasis is a feature of a variety of pathological conditions, including cancer, atherosclerosis, diabetes mellitus, and inflammatory arthritis.[1] Endothelial function is regulated, in part, by a variety of soluble, pro-angiogenic growth factors, including the angiopoietins. Angiopoietin-1 (Angpt1), the primary agonistic angiopoietin ligand, signals through the endothelial receptor tyrosine kinase Tie2 to support functions including EC survival and vascular stability.[2] Angiopoietin-2 (Angpt2) functions as a context-dependent Tie2 antagonist or partial agonist and is highly expressed by ECs in tissues undergoing vascular remodeling or at sites of inflammation.[3]

A recent study from our laboratory has demonstrated a crucial role for the Abelson (Abl) family of non-receptor tyrosine kinases in vascular function in vivo, as well as in the regulation of Angpt1/Tie2 signaling and Angpt1-mediated EC survival.[4] The Abl kinase family includes 2 members, Abl (Abl1) and Arg (Abl2), characterized by the presence of unique C-terminal actin-binding domains. These kinases are activated downstream of both adhesion and growth factor receptors to mediate cellular responses, including cytoskeletal remodeling, adhesion, and migration.[5] Global knockout mice lacking both Abl and Arg kinases in all tissues die during embryonic development and exhibit phenotypes including hemorrhage and pericardial edema,[6] suggesting an important vascular role for these kinases. To examine this possibility, we generated mice lacking Abl kinase expression in ECs, by crossing mice carrying a conditional *Abl* allele ($Abl^{flox/flox}$) on an $Arg^{-/-}$ background to mice expressing Cre recombinase under the control of the endothelial Tie2 promoter. Strikingly, depletion of both Abl and Arg kinases in ECs led to late-stage embryonic and perinatal lethality, with most mutant embryos found dead at birth. While overall vascular structure was unaffected, focal regions of hepatic necrosis were observed in endothelial *Abl*/*Arg* double-null embryos; these areas of tissue death correlated with localized loss of vasculature. Similarly, a subset of adult mice lacking endothelial *Abl* expression (on an $Arg^{+/-}$ background) displayed localized scarring of the left ventricle, which coincided with a complete loss of blood vessels in the affected region. Increased EC apoptosis was observed in endothelial *Abl*/*Arg* double-null embryos, thus suggesting that the enhanced apoptosis promoted localized loss of vascular density with subsequent defects in tissue perfusion, leading to tissue damage, organ dysfunction, and death. Consistent with this scenario, loss of Abl kinase function (either by pharmacological inhibition or knockdown) increased EC apoptosis in response to serum-deprivation stress in vitro.

Unexpectedly, we also found that depletion of the Abl kinases led to decreased expression of the Tie2 receptor both in vitro and in primary liver ECs from endothelial *Abl*/*Arg* double-null embryos. Given the important role of the Angpt1/Tie2 signaling pathway in supporting EC survival, we further examined the effects of Abl/Arg knockdown on Angpt1-mediated signaling and survival responses. Loss of the Abl kinases markedly decreased intracellular signaling responses to Angpt1, particularly inhibiting activation of the pro-survival Akt pathway. Accordingly, Abl/Arg depletion also diminished the anti-apoptotic effects of Angpt1. Interestingly, expression of exogenous Tie2 only partially rescued Angpt1-mediated survival, suggesting that the Abl kinases may modulate Angpt1/Tie2 signaling through additional mechanisms beyond regulating Tie2 receptor levels. Indeed, we also observed activation of the Abl kinases in ECs following Angpt1 stimulation, suggesting a unique dual role for the Abl kinases in the Angpt1/Tie2 pathway, both regulating Tie2 expression and modulating downstream signaling (**Fig. 1**). Further studies will be required to characterize the precise function of the Abl kinases in each of these roles. Interestingly, the activation of distinct Tie2 complexes either at cel–cell or cell–matrix contacts has been linked to preferential activation of the Akt or Erk pathways, respectively.[2] Given the more pronounced inhibition of Angpt1-induced Akt pathway activation by Abl/Arg depletion, an intriguing possibility is that these kinases may modulate Tie2 signaling responses particularly at cell–cell contacts. It will also be of interest to determine whether, in addition to the

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Submitted: 08/13/2013; Accepted: 09/02/2013
http://dx.doi.org/10.4161/cc.26877
Comment on: Chislock EM, et al. Proc Natl Acad Sci U S A 2013; 110:12432-7; PMID:23840065; http://dx.doi.org/10.1073/pnas.1304188110

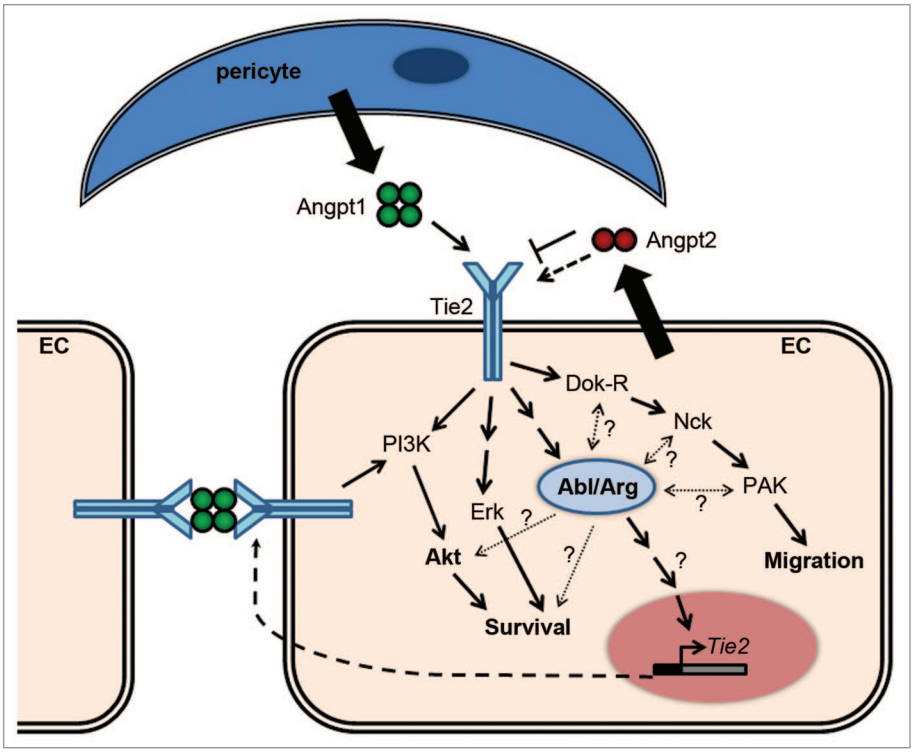

Figure 1. Model for the dual role of the Abl family kinases in angiopoietin/Tie2 signaling. The secreted glycoprotein angiopoietin-1 (Angpt1), which is produced by perivascular cells, including pericytes, binds to and activates the endothelial receptor tyrosine kinase Tie2, activating intracellular signaling pathways to promote responses including endothelial cell survival and migration. In contrast, angiopoietin-2 (Angpt2) is predominantly expressed in endothelial cells and functions as a context-dependent Tie2 antagonist or weak agonist. The Abl kinases (Abl and Arg) positively regulate *Tie2* mRNA expression and are required for maximal Angpt1-mediated pro-survival signaling, primarily through the PI3K/Akt and, to a lesser extent, Erk signaling pathways. In addition, the Abl kinases are activated downstream of the Tie2 receptor. The Abl kinases may also modulate Angpt1-mediated endothelial cell migration through effects on signaling proteins, including Dok-R, Nck, and PAK. The pathway linking the Abl kinases to regulation of *Tie2* mRNA expression remains to be defined. Abbreviations: EC, endothelial cell; Tie2, tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2; Angpt, angiopoietin; PI3K, phosphoinositide 3-kinase; Erk, extracellular signal-regulated kinase; Dok-R, downstream of tyrosine kinase-related protein; PAK, p21-activated kinase.

observed effects on Angpt1-mediated survival, the Abl kinases may modulate other EC responses to Angpt1, including migration as well as anti-permeability and anti-inflammatory effects, which are mediated in part by regulation of cell–cell and cell–matrix adhesion. In this regard, several signaling proteins required for Angpt1/Tie2-dependent EC migration, including Dok-R, Nck, and PAK, are binding partners and/or substrates of the Abl kinases.[3,7]

Alterations in the angiopoietin/Tie2 pathway, including a shift in angiopoietin balance with Angpt2 levels exceeding Angpt1 levels, have been implicated in diverse vascular pathologies.[3] Thus, future studies are required to evaluate the role of the Abl kinases in the modulation of angiopoietin/Tie2 signaling during the progression of these disorders, as well as to fully understand the role of these kinases in both vascular development and adult vascular maintenance.

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