J. Biochem. 2011;149(2):117–119 doi:10.1093/jb/mvq146

JB THE JOURNAL OF BIOCHEMISTRY

JB Commentary

Roles of old players in the suppression of a new player: networks for the transcriptional control of angiogenesis

Received December 5, 2010; accepted December 15, 2010; published online December 20, 2010

Tetsuro Watabe[1,2,*]

[1]Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113-0033; and [2]PRESTO, Japan Science Technology Agency, Kawaguchi, Saitama 332-0012, Japan

*Tetsuro Watabe, Ph.D., Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel: +81-3-5841-3356, Fax: +81-3-5841-3354, email: t-watabe@umin.ac.jp

During the formation of blood vessels, Id1, a member of the helix-loop-helix (HLH) family, and TAL1/SCL, a basic HLH (bHLH) transcription factor, play important roles in the activation of endothelial cells. Recent reports revealed that E2-2, another bHLH transcription factor, inhibits angiogenesis *in vitro* and *in vivo* by suppressing the expression of vascular endothelial growth factor receptor 2 (VEGFR2). Id1 and TAL1/SCL dimerize with E2-2 and relieve the E2-2-mediated down-regulation of VEGFR2 expression, leading to the activation of endothelial cells. These findings reveal a novel interplay between HLH transcription factors that regulate angiogenesis.

Keywords: HLH/E2-2/Id1/SCL/TAL1/VEGFR2.

Abbreviations: bHLH, basic helix-loop-helix; BMP, bone morphogenetic protein; TGF-β, transforming growth factor-β; VEGFR2, vascular endothelial growth factor receptor 2.

Blood vessels play important roles in the maintenance of tissue homeostasis by supplying oxygen and nutrients and by removing waste products. They are also involved in the pathogenesis of multiple diseases, including cancer and diabetic vasculopathy. Embryonic vessels are formed by endothelial cells that arise from angioblasts—endothelial progenitor cells that express vascular endothelial growth factor receptor 2 (VEGFR2). This process of embryonic vessel formation, referred to as vasculogenesis, contrasts with angiogenesis. Angiogenesis is the formation of new blood vessels from pre-existing vessels and requires the activation of endothelial cells by angiogenic factors that transit from resolution to activation phase (*1*). Moreover, bone marrow-derived vascular progenitor cells circulating in the adult peripheral blood contribute to the formation of new blood vessels by differentiating into both endothelial and mural cells.

VEGFR2 is activated by VEGF, and it plays central roles in the differentiation, proliferation and migration of endothelial cells. The expression of VEGFR2 is initiated during the formation of angioblasts and is regulated by multiple cytokines and transcription factors. Kappel and colleagues (*2*) reported that the regulatory sequences for VEGFR2 gene expression are present not only at the 5′-non-coding region but also at the 3′-region of the first intron. Both regulatory sequences are required for proper regulation of VEGFR2 gene expression in endothelial cells. Bone morphogenetic proteins (BMPs), members of the transforming growth factor (TGF)-β family, induce VEGFR2 expression in various stages of vascular development. BMP induces the formation of VEGFR2-expressing ($VEGFR2^{+}$) angioblasts by inducing the expression of Etv2/ER71 transcription factor that induces the expression of VEGFR2 (*3*). In differentiated endothelial cells, BMP signals induce the proliferation and migration of endothelial cells (*4–6*).

These angiogenic effects of BMPs are at least partially mediated by the inhibitors of the DNA-binding (Id) family of proteins, which are direct targets of BMP signals (*7*, *8*). Id proteins (Id1, 2, 3 and 4) belong to the helix-loop-helix (HLH) family of transcription factors. Since basic HLH (bHLH) transcription factors bind DNA as either homodimers or heterodimers to regulate the transcription of their target genes, Id proteins lacking a DNA-binding domain prevent dimer formation and DNA binding by interacting with bHLH proteins (*9*). Id proteins have been implicated in the formation of blood vessels by both *in vitro* and *in vivo* findings. Double-knockout mice deficient for *Id1* and *Id3* exhibit abnormal angiogenesis (*10*). Furthermore, Valdimarsdottir *et al.* (*11*) reported that ectopic Id1 expression activates endothelial cells, and that knock-down of Id1 expression in endothelial cells blocks BMP-induced activation of endothelial cells, suggesting that Id1 is an important target of BMPs in mediating their angiogenic effects.

T-cell acute lymphocytic leukaemia 1/stem cell leukaemia haematopoietic transcription factor (TAL1/SCL), a member of the bHLH family, is required for the formation of blood vessels in the yolk sacs of mouse embryos (*12*). TAL1/SCL is known to form a heterodimer with a group of bHLH family proteins, so-called E-proteins that bind to the E-box consensus sequence. The E-protein family consists of E12/E47, HEB and E2-2 in mammals. TAL1/SCL is reported to induce the expression of VE-cadherin, an endothelial marker, by interacting with E47, leading to the activation of angiogenesis (*13*). However, the molecular mechanisms underlying the HLH factor-mediated regulation of angiogenesis remain to be elucidated.

Tanaka *et al.* (*14*, *15*) identified E2-2 as a novel negative regulator of angiogenesis. E2-2 inhibits

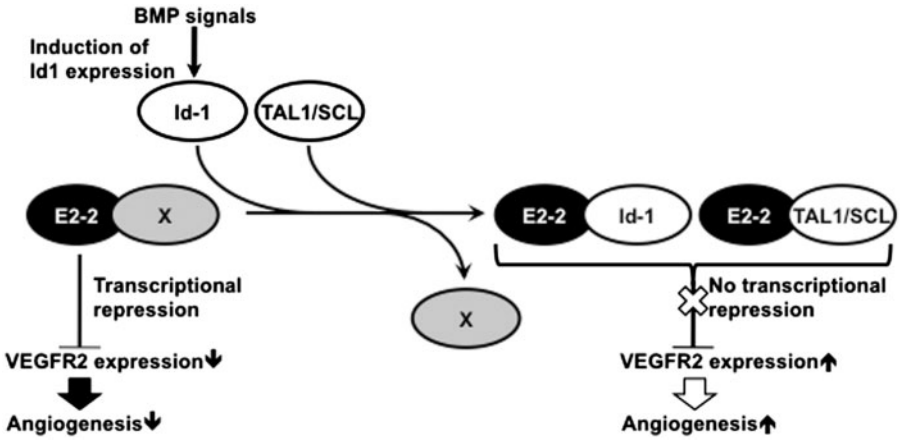

Fig. 1 Hypothetical model for how Id1 and TAL1/SCL suppress the E2-2-mediated suppression of angiogenesis. When endothelial cells are in a resolution phase, E2-2 dimerizes with a hypothetical molecule (X) to repress the transcription of VEGFR2. When angiogenic signals, including BMP, induce Id1 expression, Id1 competes with X for E2-2 binding. TAL1/SCL is also capable of dimerizing with E2-2, leading to the formation of inactive transcriptional repressor complexes. Hence, Id1 and TAL1/SCL relieve the E2-2-mediated suppression of VEGFR2 expression, leading to the activation of the endothelium.

in vitro endothelial migration, network formation and proliferation, and *in vivo* angiogenesis in Matrigel plugs. E2-2 inhibits angiogenesis by down-regulating the expression of VEGFR2. Interestingly, both Id1 and TAL1 can interact with E2-2 and relieve its inhibitory effects on VEGFR2 expression. Furthermore, Id1 inhibits the E2-2-mediated inactivation of angiogenesis both *in vitro* and *in vivo*. These results revealed a novel mechanism by which interaction of E2-2 with HLH transcription factors regulates angiogenesis via the activation of VEGFR2 expression (Fig. 1).

It remains to be elucidated how E2-2 suppresses VEGFR2 transcription. Tanaka *et al.* (*14*) postulated a model in which a hypothetical molecule (X) interacting with E2-2 mediates E2-2-induced transcriptional repression of VEGFR2. Since E2-2-knockout mice do not display vascular abnormalities, other E-proteins such as E2A and HEB may be involved in these transcriptional networks. It would also be interesting to identify other targets of E2-2 that are related to angiogenesis.

Multiple signals are implicated in the regulation of intracellular levels of HLH proteins. Notch and BMP signals induce the expression of Hey1/Herp2/Hesr1, a bHLH transcription factor, which interacts with Id1 (*4*). This interaction leads to Id1 degradation by the activation of a proteasome pathway, which in turn antagonizes the BMP-induced activation of endothelial cells. Protein levels of TAL1/SCL in Jurkat T-cell acute lymphoblastic leukaemia cells are negatively regulated by TGF-β, which activates the AKT-mediated ubiquitin-proteasome pathway (*16*). Because TGF-β negatively regulates the proliferation of endothelial cells (*17*), this interaction between TGF-β and TAL1/SCL may play a role in the regulation of endothelial cell activation. A better understanding of how multiple HLH proteins and related signals regulate the activation of endothelial cells may provide a therapeutic strategy to control pathological angiogenesis.

Conflict of interest
None declared.

References

1. Pardali, E., Goumans, M.J., and ten Dijke, P. (2010) Signaling by members of the TGF-β family in vascular morphogenesis and disease. *Trends Cell Biol.* **20**, 556–567
2. Kappel, A., Rönicke, V., Damert, A., Flamme, I., Risau, W., and Breier, G. (1999) Identification of vascular endothelial growth factor (VEGF) receptor-2 (Flk-1) promoter/enhancer sequences sufficient for angioblast and endothelial cell-specific transcription in transgenic mice. *Blood* **93**, 4284–4292
3. Lee, D., Park, C., Lee, H., Lugus, J.J., Kim, S.H., Arentson, E., Chung, Y.S., Gomez, G., Kyba, M., Lin, S., Janknecht, R., Lim, D.S., and Choi, K. (2008) ER71 acts downstream of BMP, Notch, and Wnt signaling in blood and vessel progenitor specification. *Cell Stem Cell* **2**, 497–507
4. Itoh, F., Itoh, S., Goumans, M.J., Valdimarsdottir, G., Iso, T., Dotto, G.P., Hamamori, Y., Kedes, L., Kato, M., and ten Dijke, P. (2004) Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. *EMBO J.* **23**, 541–551
5. Suzuki, Y., Montagne, K., Nishihara, A., Watabe, T., and Miyazono, K. (2008) BMP-9 induces proliferation of multiple types of endothelial cells in vitro and in vivo. *J. Biochem.* **143**, 199–206
6. Suzuki, Y., Ohga, N., Morishita, Y., Hida, K., Miyazono, K., and Watabe, T. (2010) BMPs promote proliferation and migration of endothelial cells via stimulation of VEGF-A/VEGFR2 and angiopoietin-1/Tie2 signalling. *J. Cell Sci.* **123**, 1684–1692
7. Miyazono, K. and Miyazawa, K. (2002) Id: a target of BMP signaling. *Sci. STKE* **2002**, pe40
8. Miyazono, K., Kamiya, Y., and Morikawa, M. (2010) Bone morphogenetic protein receptors and signal transduction. *J. Biochem.* **147**, 35–51
9. Perk, J., Iavarone, A., and Benezra, R. (2005) Id family of helix-loop-helix proteins in cancer. *Nat. Rev. Cancer* **5**, 603–614

10. Lyden, D., Young, A.Z., Zagzag, D., Yan, W., Gerald, W., O'Reilly, R., Bader, B.L., Hynes, R.O., Zhuang, Y., Manova, K., and Benezra, R. (1999) Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. *Nature* **401**, 670–677
11. Valdimarsdottir, G., Goumans, M.J., Rosendahl, A., Brugman, M., Itoh, S., Lebrin, F., Sideras, P., and ten Dijke, P. (2002) Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. *Circulation* **106**, 2263–2270
12. Visvader, J.E., Fujiwara, Y., and Orkin, S.H. (1998) Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development. *Genes Dev.* **12**, 473–479
13. Deleuze, V., Chalhoub, E., El-Hajj, R., Dohet, C., Le Clech, M., Couraud, P.O., Huber, P., and Mathieu, D. (2007) TAL-1/SCL and its partners E47 and LMO2 up-regulate VE-cadherin expression in endothelial cells. *Mol. Cell. Biol.* **27**, 2687–2697
14. Tanaka, A., Itoh, F., Itoh, S., and Kato, M. (2009) TAL1/SCL relieves the E2-2-mediated repression of VEGFR2 promoter activity. *J. Biochem.* **145**, 129–135
15. Tanaka, A., Itoh, F., Nishiyama, K., Takezawa, T., Kurihara, H., Itoh, S., and Kato, M. (2010) Inhibition of endothelial cell activation by bHLH protein E2-2 and its impairment of angiogenesis. *Blood* **115**, 4138–4147
16. Terme, J.M., Lhermitte, L., Asnafi, V., and Jalinot, P. (2009) TGF-β induces degradation of TAL1/SCL by the ubiquitin-proteasome pathway through AKT-mediated phosphorylation. *Blood* **113**, 6695–6698
17. Watabe, T., Nishihara, A., Mishima, K., Yamashita, J., Shimizu, K., Miyazawa, K., Nishikawa, S., and Miyazono, K. (2003) TGF-β receptor kinase inhibitor enhances growth and integrity of embryonic stem cell-derived endothelial cells. *J. Cell Biol* **163**, 1303–1311