Editorial

LMO2 (LIM Domain Only 2) and Endothelial Cell Migration in Developmental and Postnatal Angiogenesis

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As quoted by the famous astronomer Carl Sagan, "the absence of evidence is not the evidence of absence" (http://wiki.c2.com/?AbsenceOfEvidenceIsNotEvidenceOfAbsence) is the logical corollary that conclusions should not be drawn when a direct test for the presence of evidence cannot be made. This applies to the article by Matrone et al[1] in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, where the authors delineated a novel signaling pathway within the domain of endothelial migration. LMO2 (LIM domain only 2) was first discovered as a recurrent translocation partner of T-cell receptor loci in a subset of patients with T-cell acute lymphoblastic leukemia in 1981.[2,3] Studies on LMO2 have now shown that LMO2 functions as a proto-oncogenic gene not only in T cells but also in hematopoiesis, vascular remodeling, and stem cell biology.[4–9] Matrone et al[1] showed that LMO2 levels in zebrafish embryos regulate intersegmental vessel (ISV) formation. The evidence for a migration defect is the absence of endothelial cells in ISVs in the elegant photomicrographs. Of course direct visualization of the lack of endothelial migration cannot be presented.

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In transgenic zebrafish that express enhanced GFP (green fluorescent protein; under the control of Fli1 [friend leukemia integration 1 transcription factor]) in the vasculature (Tg(fli1:EGFP)^{y1}),[10] the authors show that total proliferating endothelial cells were not different in the LMO2 inhibited embryos versus controls, but GFP$^+$ endothelial-like cells accumulate in the larger conduits with lesser numbers in connecting ISVs allowing the conclusion of impaired migration into the ISVs. Using a stable human umbilical vein endothelial cell (HUVEC) line deficient in LMO2, the authors showed decreased endothelial migration in vitro in a scratch wound assay. This provides evidence that LMO2 deficiency can impair endothelial cell migration, but this assay is not an immediate correlate for developmental cell migration because the migration of cells in this in vitro model is driven by injury to the cells. LMO2 inhibition also decreased neoangiogenesis and tissue regeneration in adult Tg(fli1:egfp)^{y1} zebrafish resected caudal fin.[6]

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(*Arterioscler Thromb Vasc Biol*. 2017;37:1806-1808. DOI: 10.1161/ATVBAHA.117.309953.)

***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
DOI: 10.1161/ATVBAHA.117.309953

Homozygote LMO2-deficient mice are severely anemic and die at E9–10 (embryonic day 9–10) because of failure in yolk sac erythropoiesis.[11] In a study by Yamada et al,[8] transfer of LMO2-deficient embryonic stem cells resulted in normal capillary development until E9; however, post-E9, the vascular system was highly disorganized (in mice that have LMO2-deficient embryonic stem cells integrated into the vasculature) indicating a role of LMO2 in angiogenic remodeling and vascular maturation.[8] Taken together, these studies indicate that LMO2 dysregulation can result in a highly disorganized vascular network. By extension, in diseased tissue with insufficient angiogenesis, we can predict that LMO2 deficiency can not only aggravate tissue injury but also decrease vascular stability and increase vascular thickening (as observed in diabetic retinopathy,[12] hypertension,[13] and coronary atherosclerosis[14]). Clearly, while not simple to detect, the biological process of migration involves complex signaling interactions among multiple cells types for specific cell migration to specific locations. Because the authors performed a global inhibition of LMO2 in the embryos, it is possible that the lack of migratory signals originating from ISVs post-LMO2 inhibition decreased GFP$^+$ endothelial-like cell migration from large conduits into ISVs. Role of LMO2 in regulating paracrine signals between cells from ISVs and large conduits that regulate endothelial migration warrants further study.

The approach taken that linked LMO2 to SPHK1 (sphingosine kinase 1) was interesting. Using a mouse LMO2 CHIP-Seq analysis, the authors show that LMO2 has binding regions in VEGF-A (vascular endothelial growth factor), VEGFR1 (vascular endothelial growth factor receptor 1), VEGFR2, and SPHK1 in zebrafish embryos. SPHK1 and SPHK2 are 2 major isozymes of SPHK; alternate splicing in SPHK1 and SPHK2 produces multiple isoforms.[15,16] Interestingly, whereas LMO2 deficiency decreased VEGF-A in zebrafish embryos, LMO2 deficiency decreased VEGFR2 expression in HUVECs. These results suggest that LMO2 binds to different angiogenic genes during development and in adult tissues. However, whether a species-specific CHIP-Seq (chromatin immunoprecipitation-sequencing) would yield different or additional binding sites for LMO2 is not evident.

To some extent, SPHK1 and SPHK2 seem to have redundant and compensatory functions.[17] Although mice deficient in either isozyme show no obvious phenotypic abnormalities, deficiency in both isozymes results in embryonic lethality.[17–19] SPHK converts sphingosine a ceramide metabolite to S1P (sphingosine-1-phosphate) to decrease ceramide-induced apoptosis.[20] Developmentally, SPHK activation of S1P plays critical roles in embryonic cell migration, cardiac development, neurogenesis, vascular development, and maturation in development, and in disease, SPHK/S1P plays important roles

in protecting from myocardial ischemia and stroke, mitochondrial function, insulin resistance, and inflammation.[21–26] An interesting and novel finding was the discovery that LMO2 has binding sites in SPHK1 but not SPHK2 promoter region. In vivo LMO2 inhibition decreased, and LMO2 overexpression increased SPHK1 levels in zebrafish embryos. LMO2-deficient HUVECs have lower, and LMO2-overexpressing HUVECs have higher SPHK1 expression. On the basis of the bioinformatics analysis, the authors next determined whether LMO2 binds to SPHK1 promoter to regulate SPHK1 expression. In the experimental strategy, the authors used a stable LMO2-deficient HUVEC line to determine the binding interactions between LMO2 and SPHK1 promoter by LMO2 CHIP-PCR (chromatin immunoprecipitation-polymerase chain reaction). Interestingly, despite LMO2 deficiency, PCR analysis of LMO2 CHIP-PCR for SPHK1 promoter showed only a ≈60% reduction SPHK1. Whether the remaining 20% to 40% LMO2 bound to SPHK1 promoter represents any other isoform is not clear. Furthermore, whether LMO2 regulates SPHK1 by direct binding interactions in zebrafish embryos is not clear. The authors next show that whereas SPHK1 inhibition decreased ISV generation similar to LMO2 inhibition, SPHK1 overexpression rescued decreased ISV phenotype induced by LMO2 inhibition in zebrafish embryos. The authors also show that SPHK1 deficiency decreased endothelial migration in HUVECs in a scratch assay. Finally, the authors show that S1P (downstream of LMO2/SHPK1) can rescue impaired angiogenesis induced by LMO2 inhibition in aortic ring angiogenic assays.

Assays seeking to show direct evidence for endothelial migration in vivo simply do not exist. The authors used in vitro (injury) models (scratch/wound healing assays) and aortic ring assays to show evidence of LMO2/SHPK1/S1P in endothelial migration as an in vivo developmental correlate to the findings in zebrafish. Whether LMO2/SHPK1/S1P axis functionally regulates GFP$^+$ endothelial-like cell like migration to ISVs in zebrafish embryos needs to be determined. Even though, LMO2/VEGF-A/VEGFR2 axis was not the focus of the study, VEGF-A,[27] VEGFR1,[28] and VEGFR2[29] deficiencies result in embryonic lethality because of vascular complications, whereas SPHK1-deficient mice were fertile and do not show any developmental abnormalities.[17] To the extent of the limitations inherent in direct assessment, the authors clearly show that LMO2/SPHK1/S1P signaling participates in ISV generation via cell migration.

Studies linking S1P to the VEGF receptor-ligand family are beginning to emerge. A recent study indicated that higher VEGFR2 phosphorylation and VEGF-A levels in SPHK1-deficient mice lungs (http://www.fasebj.org/content/25/1_Supplement/809.1.short). However, increased VEGF-A levels did not induce angiogenesis, but increased tissue permeability and cell proliferation suggesting that a more complex signaling interactions occur between SPHK1, SIP, and VEGF-A pointing that SPHK1 functions as a rheostat for both VEGF-A and S1P (http://www.fasebj.org/content/25/1_Supplement/809.1.short). Whether this signaling component also occurs in developing vasculature is not clear, but points toward more complex signaling between LMO2/SHPK1/SIP and LMO2/VEGF-A/VEGFR2/VEGFR1 axes in angiogenesis and endothelial migration. Furthermore, because S1P activation can also transactivate receptor tyrosine kinases,[26] it is possible that S1P activation by LMO2/SPHK1 in turn can induce VEGFR2 activation, thereby inducing potent signaling networks between cell types in ISVs and larger conduits to regulate endothelial migration.

Finally, even though LMO2 is highly conserved among evolutionary orthologues (http://www.imm.ox.ac.uk/the-lmo-genes-and-proteins), whether LMO2/SPHK1/S1P interactions regulate endothelial migration in vascular development in higher organisms needs to be further examined. More experiments are also needed to determine the molecular mechanisms that regulate LMO2 expression and function in vivo. More importantly, based on LMO2 function in regulating endothelial migration in HUVEC scratch/wound healing assays, it is possible that LMO2 can also play important roles in promoting tissue recovery where modulating endothelial migration is important to achieve therapeutic effects.

Sources of Funding

B.H. Annex was supported by 1R01 HL116455, 1R01 HL121635, and 2R01 HL101200. V.C. Ganta is supported by American Heart Association scientist development grant 16SDG30340002.

Disclosures

None.

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KEY WORDS: Editorials ■ angiogenesis ■ development ■ endothelial migration ■ post-natal ■ signaling ■ vasculature ■ wound healing

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Arterioscler Thromb Vasc Biol. 2017;37:1806-1808
doi: 10.1161/ATVBAHA.117.309953
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636