Endothelial Deletion of Phospholipase D2 Reduces Hypoxic Response and Pathological Angiogenesis

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Objective—Aberrant regulation of the proliferation, survival, and migration of endothelial cells (ECs) is closely related to the abnormal angiogenesis that occurs in hypoxia-induced pathological situations, such as cancer and vascular retinopathy. Hypoxic conditions and the subsequent upregulation of hypoxia-inducible factor-1α and target genes are important for the angiogenic functions of ECs. Phospholipase D2 (PLD2) is a crucial signaling mediator that stimulates the production of the second messenger phosphatidic acid. PLD2 is involved in various cellular functions; however, its specific roles in ECs under hypoxia and in vivo angiogenesis remain unclear. In the present study, we investigated the potential roles of PLD2 in ECs under hypoxia and in hypoxia-induced pathological angiogenesis in vivo.

Approach and Results—*Pld2* knockout ECs exhibited decreased hypoxia-induced cellular responses in survival, migration, and thus vessel sprouting. Analysis of hypoxia-induced gene expression revealed that PLD2 deficiency disrupted the upregulation of hypoxia-inducible factor-1α target genes, including *VEGF*, *PFKFB3*, *HMOX-1*, and *NTRK2*. Consistent with this, PLD2 contributed to hypoxia-induced hypoxia-inducible factor-1α expression at the translational level. The roles of PLD2 in hypoxia-induced in vivo pathological angiogenesis were assessed using oxygen-induced retinopathy and tumor implantation models in endothelial-specific *Pld2* knockout mice. *Pld2* endothelial-specific knockout retinae showed decreased neovascular tuft formation, despite a larger avascular region. Tumor growth and tumor blood vessel formation were also reduced in *Pld2* endothelial-specific knockout mice.

Conclusions—Our findings demonstrate a novel role for endothelial PLD2 in the survival and migration of ECs under hypoxia via the expression of hypoxia-inducible factor-1α and in pathological retinal angiogenesis and tumor angiogenesis in vivo. **(*Arterioscler Thromb Vasc Biol*. 2014;34:1697-1703.)**

Key Words: angiogenesis ■ endothelial cell ■ hypoxia-inducible factor-1 ■ phospholipase D2

Angiogenesis involves complex endothelial cell (EC) behaviors, such as proliferation, survival, migration, and tube formation. The most important stimulus promoting angiogenesis is tissue hypoxia. Hypoxia mediates several processes in ECs that are required for each step of angiogenesis.[1] Hypoxia-induced angiogenesis is closely related to pathological situation. The dysregulation of these EC behaviors and thus abnormal angiogenesis are critically associated with hypoxia-induced pathological angiogenesis that occurs during the course of several diseases, such as cancer and vascular retinopathy.[2,3] Therefore, identification of specific molecules involved in hypoxia-induced angiogenesis will facilitate studies to clarify the various molecular mechanisms involved in pathological angiogenesis and may aid the discovery of novel angiogenic drug targets.

See cover image

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Nonstandard Abbreviations and Acronyms	
EC	endothelial cell
eKO	endothelial-specific knockout
HIF-1	hypoxia-inducible factor-1
MLEC	mouse lung endothelial cell
mTOR	mammalian target of rapamycin
OIR	oxygen-induced retinopathy
PLD2	phospholipase D2
VEGF	vascular endothelial growth factor

Cellular responses to hypoxic stress are mediated by multiple mechanisms. Hypoxia-inducible factor-1 (HIF-1) is the most prominent factor that mediates cellular responses to hypoxia by inducing the expression of several target genes.[1] Because HIF-1 activation is preferentially modulated by changes in the amount of the HIF-1α subunit, several factors and mechanisms have been suggested. Among these, the regulation of HIF-1α degradation through post-translational modification by oxygen-sensor proteins and subsequent proteasomal degradation has been well studied. However, although several regulatory mechanisms have been suggested for HIF-1α synthesis, the exact mechanism is yet to be elucidated.[4]

Phospholipase D2 (PLD2) is an enzyme that hydrolyzes phosphatidylcholine to generate phosphatidic acid and choline.[5,6] In response to a variety of upstream stimuli, including growth factors and several stress conditions, PLD2 has been known to function in a variety of biological phenomena by mediating various signaling pathways and cellular functions.[5,7–10] However, the pathophysiological roles of PLD2 in vivo have only recently been reported.[11–13] Furthermore, tissue-specific in vivo functions of PLD2 and its roles in hypoxic response have not yet been studied.

In this study, we determined that PLD2 is involved in survival, migration, and thus sprouting of ECs under hypoxic conditions. Our data suggest that PLD2 contributes to hypoxia-induced expression of HIF-1α through translational regulation and hypoxia-induced gene expression, which are important for the response of ECs to hypoxic stress. Endothelial-specific *Pld2* knockout mice (eKO) showed decreased pathological angiogenesis in oxygen-induced retinopathy (OIR) and tumor implantation models. Our study demonstrated the essential role of PLD2 in the cellular processes of ECs under hypoxic stress and in pathological angiogenesis in vivo.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

PLD2 Is Required for Survival, Migration, and Sprouting of ECs Under Hypoxic Condition

To study the roles of PLD2 in angiogenesis in vivo, we produced *Pld2* floxed mice with 2 LoxP sequence (Figure IA and IB in the online-only Data Supplement). *Pld2* floxed mice were then crossed with *Protamine-Cre* transgenic mice for whole-body deletion of *Pld2*, designated knockout (Figure IA in the online-only Data Supplement). Deletion of *Pld2* was confirmed by verifying protein expression using cells isolated from mice (Figure IC in the online-only Data Supplement). *Pld2* knockout mice were viable and fertile and did not exhibit detectable phenotypes compared with the wild-type littermates. We then isolated ECs from mice to determine whether ablation of *Pld2* affects the cellular function of primary ECs.

ECs modulate their cellular processes, including survival, proliferation, migration, and tube formation, to generate new blood vessels during hypoxic stress.[1] To mimic hypoxia-induced angiogenesis under pathophysiological condition, we investigated whether PLD2 plays roles in the cellular functions of isolated mouse lung ECs (MLECs) under hypoxic conditions. MLECs from *Pld2* knockout mice exhibited decreased survival under hypoxic conditions but no significant differences under normoxia (Figures 1A and IIA in the online-only Data Supplement). Migration was also significantly decreased in *Pld2* knockout MLECs under hypoxia (Figures 1B and IIB in the online-only Data Supplement). Decreased survival and migration under hypoxic condition were also observed in human primary ECs, human umbilical vein ECs, by knockdown of PLD2 (Figure III in the online-only Data Supplement). We then determined whether PLD2 contributes to blood vessel growth in an ex vivo angiogenesis model. The number of endothelial sprouts and branching points from explanted aortic rings under hypoxia were reduced by PLD2 deficiency (Figure 1C). These data suggest that PLD2 is required for EC survival, migration, and vessel formation during hypoxic stress.

Hypoxia Response Gene Expression Is Decreased in *Pld2* Knockout ECs

Cellular responses to hypoxic stress are mediated by multiple mechanisms, including regulation of gene expression.[1,14] We

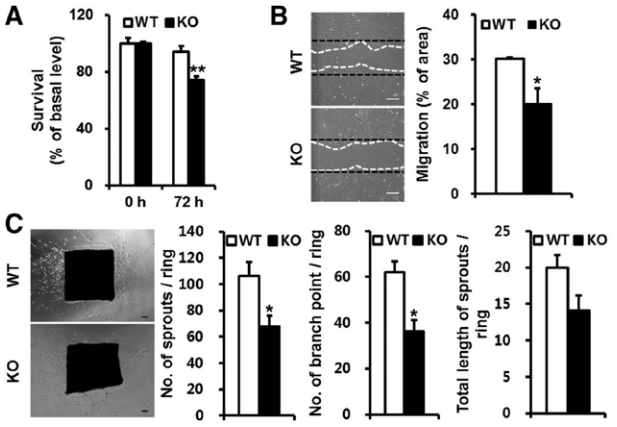

Figure 1. Phospholipase D2 (*Pld2*) deletion reduces survival and migration of endothelial cells and endothelial sprouting from aortic rings under hypoxic conditions. **A**, Survival of mouse lung endothelial cells (MLECs) under 1% O_2 was measured using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. **B**, Confluent MLECs were scratch-wounded and exposed to 1% O_2. The area of migration was quantified at 12 hours after hypoxic exposure (black dotted line: wounded area; white dotted line: leading edge). **C**, Isolated aortic rings from mice were implanted in growth factor–reduced Matrigel and exposed to 1% O_2 for 5 days. The numbers of endothelial sprouts, branch points, and total sprout length were quantified. Scale bar, 100 μm. *P<0.05, **P<0.01. KO indicates knockout; and WT, wild type.

performed cDNA microarray analysis to assess the gene expression induced by hypoxia in MLECs (Figure 2A). Differentially expressed genes were clustered by hypoxia- and PLD2-dependent pattern of gene expression, and cluster 3, corresponding to hypoxia-activated and PLD2-dependent suppressed genes, included the largest number of differentially expressed genes (Figure 2B; Figure IVA and Table I in the online-only Data Supplement). Functional classification showed that many genes in cluster 3 are involved in angiogenesis and related cellular processes (Figure 2C; Table II in the online-only Data Supplement). In the PLD2-dependent clusters (clusters 2–5), HIF-1 target genes were most enriched in cluster 3 (Figure IVB–IVD in the online-only Data Supplement). Quantitative real-time polymerase chain reaction data confirmed that the hypoxia-induced expression of vascular endothelial growth factor A (*Vegf-a*), neurotrophic tyrosine kinase receptor type2 (*Ntrk2*), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (*Pfkfb3*), and heme oxygenase 1 (*Hmox1*) were inhibited in *Pld2* knockout MLECs (Figure 2D). We also verified expression of several genes for nitric oxide synthases, cell adhesion molecules, and oxidative stress that are known to be related to angiogenesis. Among these, NADPH (nicotinamide adenine dinucleotide phosphate) oxidase 2 (*Nox2*) was significantly decreased by *Pld2* knockout (Figure V in the online-only Data Supplement).

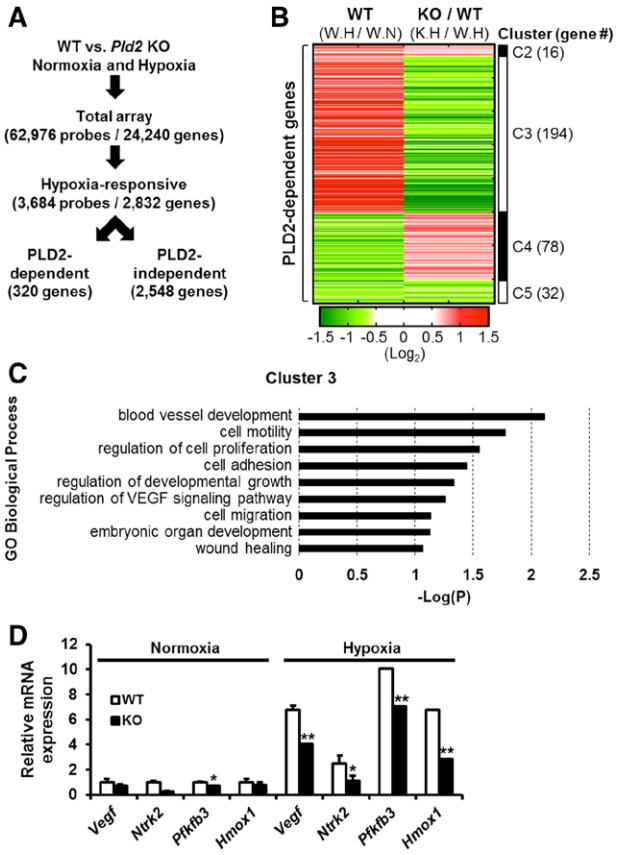

Figure 2. Hypoxia-induced gene expression was decreased in mouse lung endothelial cells from phospholipase D2 (*Pld2*) knockout (KO) mice. **A**, Flow chart showing the strategy for gene expression analysis and the PLD2-dependent gene selection process. **B**, Differentially expressed genes were clustered by hypoxia-responsive and PLD2-dependent genes. Red and green colors represent upregulation and downregulation, respectively. Colored bars represent the gradient of $\log_2$-fold changes (see also Figure II in the online-only Data Supplement). W.H and W.N refer to hypoxia vs normoxia in wild-type (WT) cells, whereas K.H/W.H refers to *Pld2* KO vs WT cells under hypoxic conditions. **C**, Hypoxia-responsive and PLD2-dependent genes in the C3 cluster (194 genes) were functionally classified. **D**, Expression of PLD2-dependent hypoxia-inducible factor-1α target genes was measured by quantitative real-time polymerase chain reaction. **P*<0.05, ***P*<0.01. GO indicates gene ontology; H, hypoxia; K, knockout; N, normoxia; VEGF, vascular endothelial growth factor; and W, wild type.

PLD2 Plays a Positive Role in Translational Control of HIF-1α Expression During Hypoxia

An increase in the amount of HIF-1α subunit is the most important mechanism for HIF-1 activation and target gene expression.[15] Therefore, we measured levels of HIF-1α in hypoxic MLECs. Consistent with the gene expression data, the increased HIF-1α protein levels induced by hypoxia were inhibited in *Pld2* knockout MLECs (Figure 3A). Time-dependent expression of HIF-1α under hypoxia was also reduced in human umbilical vein ECs after knockdown of PLD2 (Figure 3B). Hypoxia-induced HIF-1α expression is regulated by synthesis and degradation. To ascertain whether PLD2 contributes to HIF-1α synthesis or degradation, we measured the time-dependent kinetics of hypoxia-induced HIF-1α expression after treatment with proteasomal or translation

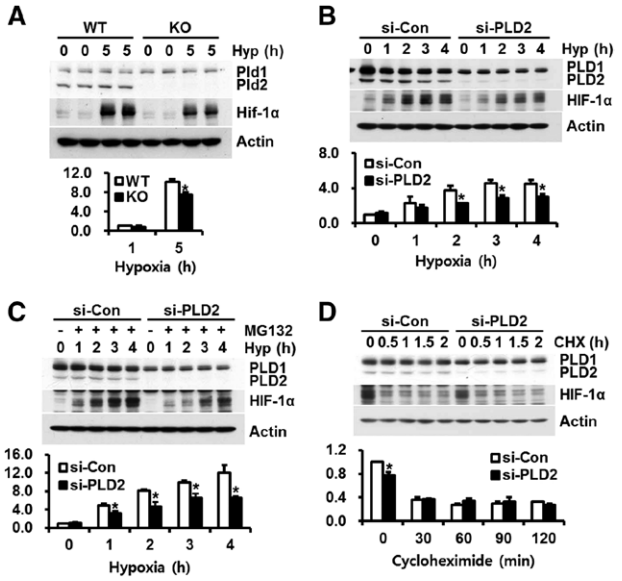

Figure 3. Phospholipase (PLD) D2 is required for hypoxia (Hyp)-induced hypoxia-inducible factor-1α (HIF-1α) expression at the translational level. The expression of HIF-1α was determined by Western blotting. **A**, Mouse lung endothelial cells were exposed to 1% O_2 for 5 hours. **B**, Human umbilical vein endothelial cells (HUVECs) were transfected with small interfering RNA and then exposed to 1% O_2 as indicated. **C**, Transfected HUVECs were treated with 10 nmol/L MG132 to inhibit protein degradation before exposure to hypoxia. Cells were harvested at the indicated times under 1% O_2. **D**, Transfected HUVECs were incubated under 1% O_2 for 4 hours, followed by treatment with 10 μg/mL cycloheximide (CHX) to inhibit protein translation. Cells were harvested at the indicated times after treatment. Quantitative data in each panel represent fold change of HIF-1α/actin. **P*<0.05. KO indicates knockout; si-Con, control siRNA; si-PLD2, PLD2 siRNA; and WT, wild type.

inhibitors (MG132 and cycloheximide, respectively). When proteasomal degradation was blocked, hypoxia-induced accumulation of HIF-1α was still reduced by PLD2 knockdown (Figure 3C). In contrast, when translation was inhibited, silencing PLD2 did not affect HIF-1α levels (Figure 3D). To confirm whether PLD2 regulates the transcription of HIF-1α, we assessed *HIF-1α* mRNA expression, which was not altered by PLD2 knockdown (Figure VI in the online-only Data Supplement). These data suggest that PLD2 is positively involved in the translational regulation of HIF-1α expression.

Ablation of *Pld2* Decreases Retinal Angiogenesis and OIR

Retinal hypoxia plays a crucial role in retinal angiogenesis through the expression of HIF-1α and VEGF-A.[16] To assess the roles of PLD2 during in vivo physiological retinal angiogenesis, we measured retinal vascular development of wild-type and *Pld2* knockout mice at postnatal day 5. Radial growth of blood vessels was delayed in retinae from *Pld2* knockout mice, which showed a decreased number of tip cells and proliferative ECs (Figure VII in the online-only Data Supplement). However, delayed retinal angiogenesis had recovered by postnatal day 14 (data not shown).

Although pathological angiogenesis shares many common processes with physiological angiogenesis, in pathological angiogenesis, formed vessels are not fully functional to resolve oxygen demand, and balance of regulatory mechanisms is disrupted.[2] Persistent angiogenesis aggravates the pathological conditions. To investigate whether endothelial PLD2 is required for angiogenesis under pathological condition, we investigated whether pathological angiogenesis occurs during OIR and tumor growth. To focus on endothelial functions of PLD2, we generated endothelial-specific KO mice, designated *Pld2* eKO, by breeding *Pld2* floxed mice with *Tie2-Cre* transgenic mice (Figure I in the online-only Data Supplement). *Pld2* floxed mice were used as the control mice in each experiment. Endothelial-specific deletion of PLD2 was confirmed by Western blot using isolated ECs and non-ECs from *Pld2* eKO (Figure ID in the online-only Data Supplement). These *Pld2* eKO mice showed normal blood pressure and blood chemistry (Table III in the online-only Data Supplement). There were no significant changes in vasodilation, vasoconstriction, and vasodilator-mediated blood pressure lowering (Figure VIII in the online-only Data Supplement). In the OIR model, vascular regeneration toward the central avascular area was impaired and pathological neovascular tufts were formed by abnormal proliferation of capillary loops. Compared with control mice, *Pld2* eKO retinae presented larger central avascular areas, suggesting decreased angiogenic activity toward the hypoxic avascular area (Figure 4A). Furthermore, *Pld2* eKO mice exhibited less neovascular tuft formation, even in the more hypoxic conditions in the larger avascular areas (Figure 4B). Therefore, PLD2 is involved in hypoxia-mediated angiogenesis during vascular regeneration and pathological angiogenesis.

Tumor Growth and Tumor Angiogenesis Are Decreased in *Pld2* eKO Mice

Angiogenesis occurs during tumor development, and it is essential for tumor growth and metastasis.[17] Furthermore, aspects of angiogenesis are highly correlated with cancer

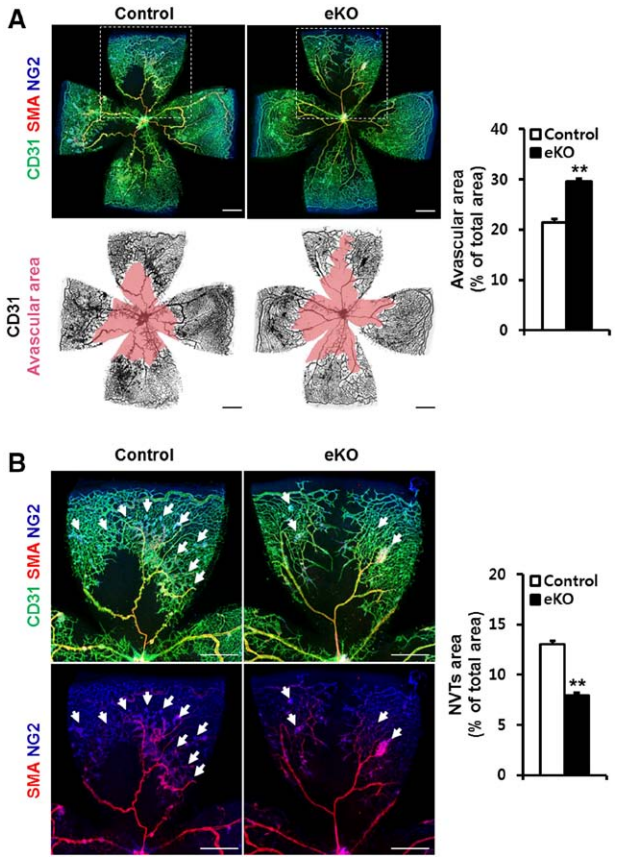

Figure 4. Pathological retinal angiogenesis was decreased in an oxygen-induced retinopathy (OIR) model in endothelial-specific *Pld2* knockout (eKO) mice, despite a larger avascular area. Retinae from OIR model mice were stained by flat-mount staining. **A**, Blood vessels (CD31) and mural cells (SMA and NG2) were visualized by fluorescent staining (**top**). The avascular area is denoted by the pink area (**bottom**) and quantified (**right**). **B**, Arrows indicate neovascular tuft (NVT) formation (arrow) in the retinal quadrant of **A** (square dotted line), and the NVT area of the whole retina was quantified (right). Scale bar, 500 μm. $^{**}P<0.01$. NG2 indicates neural/glial antigen 2; and SMA, smooth muscle cell actin.

patient prognosis. HIF-1α expression driven by hypoxia, which induces the expression of several proangiogenic factors such as VEGF, is critical for tumorigenesis. In particular, endothelial expression of HIF-1α and the VEGF autocrine loop are essential components of tumor angiogenesis.[18] Therefore, we studied the roles of PLD2 in tumor angiogenesis. Lewis lung carcinoma cells were implanted subcutaneously into the mid-back region of mice, and tumor growth was measured. Tumor growth and weight were significantly decreased in *Pld2* eKO mice compared with control mice (Figure 5A and 5B). Tumor angiogenesis was then assessed by CD31 staining of tumor slice. Blood vessel area was decreased in tumors from *Pld2* eKO. The difference between control and eKO was greatest in the intratumoral region, which was more hypoxic than the tumor periphery (Figure 5C). Furthermore, the hypoxic region of tumor sections was larger in *Pld2* eKO mice than in control (Figure 5D). EC proliferation and apoptosis were assessed by staining for CD31 with Ki67 or cleaved caspase-3, respectively. Significantly, fewer proliferative ECs ($CD31^+Ki67^+$) were found in *Pld2* eKO tumor sections, whereas the

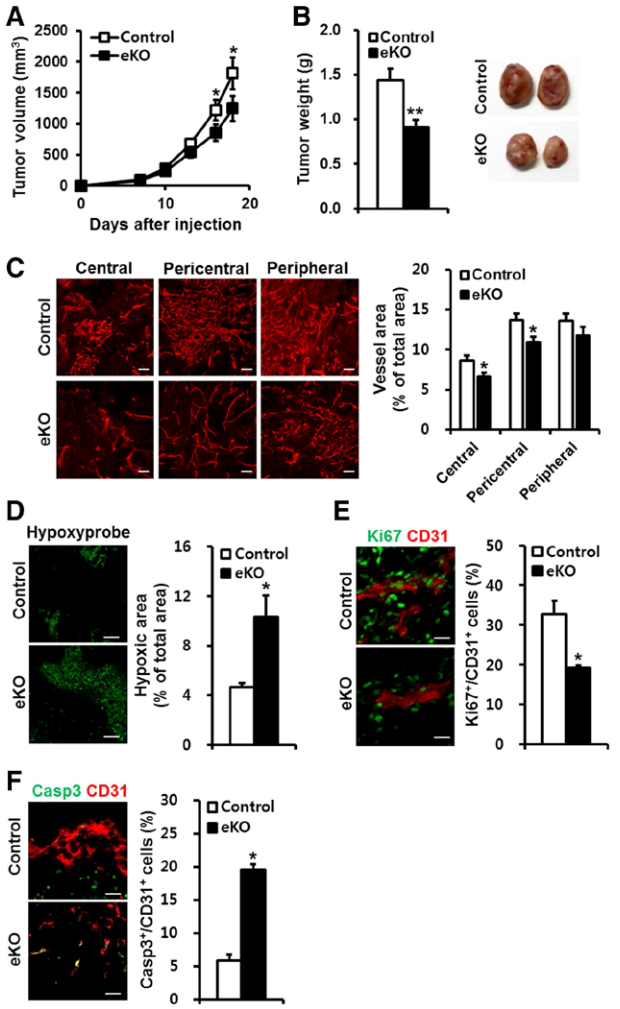

Figure 5. Tumor growth and angiogenesis were reduced in endothelial-specific *Pld2* knockout (eKO) mice. **A**, Implanted tumor volumes were measured every 3 days. **B**, Tumor weight was measured 18 days after implantation. **C**, Blood vessels in tumor slices were stained by CD31, and the vessel area was measured. **D**, Pimonidazole hydrochloride was injected into tumor-bearing mice 1 hour before tumor preparation. Hypoxic regions in tumor sections were stained with fluorescence. **E** and **F**, Proliferative or apoptotic vascular endothelial cells in tumor sections were determined by costaining with CD31 and Ki67 or cleaved caspase-3 (Casp3), respectively. Scale bar, 100 μm. **P*<0.05, ***P*<0.01.

number of apoptotic ECs (CD31⁺cleaved caspase-3⁺) increased (Figure 5F). EC barrier functions and vessel leakage are related in angiogenesis. In particular, tumor blood vessels are leakier than normal vessels because of continuous remodeling; these factors are closely related to tumor prognosis and treatment.[17,19] However, vessel leakage was not affected by deletion of endothelial PLD2 (Figure VIIII in the online-only Data Supplement). Our data suggest that endothelial PLD2 plays a role in pathological angiogenesis during tumor development. Furthermore, PLD2 may be essential for survival and proliferation of ECs in the hypoxic tumor environment.

Discussion

Here, we report that PLD2 is required for the survival and migration of ECs, and thus for EC sprouting under hypoxia, by regulating the expression of HIF-1α at the levels of translation and target gene expression. Endothelial-specific *Pld2* deletion resulted in decreased vascular regeneration and neovascular tuft formation in the OIR model. Furthermore, tumor growth in *Pld2* eKO mice was reduced with decreased angiogenesis in the tumor implantation model. Taken together, these results suggest that endothelial expression of PLD2 is required for cellular response to hypoxia via HIF-1α–dependent pathway and thus is required for angiogenesis in vivo, particularly under pathological conditions (Figure 6).

Cellular responses to hypoxic stress are mediated by multiple mechanisms, including the activation of the DNA-binding transcription factor HIF-1.[15] Under hypoxic conditions, activated HIF-1 induces the expression of several target genes that mediate various adaptive responses to oxygen tension, including angiogenesis. Under hypoxic conditions, the α-subunit of HIF-1 is synthesized, and its degradation is inhibited. Although the post-translational regulation of HIF-1α, which targets it for degradation after hydroxylation by the prolyl hydroxylase-von Hippel–Lindau protein–mediated pathway, has been well studied, HIF-1α is also transcribed and translated during hypoxia.[4] HIF-1α synthesis during hypoxia is regulated predominantly at translational level. Despite the global reduction of translation during hypoxia,[20] stress response proteins, including HIF-1α, continue to be translated. Several mechanisms for the regulation of HIF-1α translation, including internal ribosome entry site–dependent translation,[21] enhanced translation by RNA-binding proteins,[22,23] and calcium concentration,[4,24] have been suggested under different conditions and cell types. In addition, rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), is known to inhibit HIF-1α translation, suggesting that mTOR contributes to HIF-1α translation under hypoxia, although mTOR is inhibited during sustained hypoxia.[25–29] However, the exact mechanisms that regulate HIF-1α translation are unclear. In this study, PLD2 deficiency decreased the expression of a subset of hypoxia response genes, particularly HIF-1α target genes. Hypoxia-induced HIF-1α expression was reduced in MLECs and human umbilical vein ECs after the

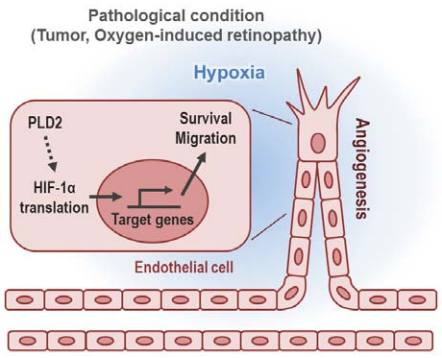

Figure 6. Roles of phospholipase D2 (PLD2) in pathological angiogenesis. Schematic representation of PLD2 functions in hypoxia-induced pathological angiogenesis. Under pathological conditions, PLD2 in endothelial cells exposed to hypoxia upregulates hypoxia-inducible factor-1α (HIF-1α) translation and target gene expression. Thus, PLD2 mediates cellular functions, including survival and migration, and positively contributes to pathological angiogenesis.

deletion or silencing of PLD2. Furthermore, our data showing the effects of MG132 and cycloheximide implicate PLD2 in regulating HIF-1α translation. mTOR is an important regulator of protein translation, and regulation of mTOR activity by phosphatidic acid has been reported to date.[30] Especially, PLD2 is required for mTOR activity after direct interaction and localized generation of phosphatidic acid.[31] Thus, mTOR-dependent pathway could be one of possible mechanism for PLD2-mediated HIF-1α translation. Furthermore, in-depth analysis may reveal the details of molecular mechanism of the PLD2 contribution in translation of HIF-1α.

HIF-1α is expressed ubiquitously, and its elevated expression in cancer cells is important for tumor growth and angiogenesis.[32] In addition, endothelial expression of HIF-1α and hypoxia-driven VEGF expression are essential for EC behavior, vascular homeostasis, and pathological angiogenesis in vivo.[18,33] Thus, endothelial knockout of HIF-1α inhibits solid tumor growth and angiogenesis.[18] Here, we showed that PLD2 is required for the survival, proliferation, migration, and thus sprouting of ECs under hypoxia. Furthermore, endothelial deletion of *Pld2* decreased pathological angiogenesis in OIR and tumor models and modulated tip cell formation and EC viability. There was also a concurrent reduction in the hypoxia-induced expression of Hif-1α, *Vegf-A*, *Pfkfb3*, *Ntrk2*, *Hmox1*, and *Nox2*. Pfkfb3 is a glycolytic activator that regulates proliferation, filopodia/lamellipodia formation, and migration in ECs.[34] Ntrk2 is an EC survival factor that promotes vessel formation and stabilization.[35,36] Hmox-1 protects ECs from oxidative stress and regulates placental angiogenesis.[37] Nox2 is a reactive oxygen species–producing enzyme known to be implicated in pathological angiogenesis.[38–41] Collectively, our findings suggest novel roles for endothelial PLD2 in regulating hypoxia-induced HIF-1α and downstream gene expression, which contribute to the cellular response of ECs to hypoxic stress and angiogenesis. This suggests that PLD2 is a modulator of EC functions during hypoxic stress and angiogenesis under pathological conditions.

Although various roles in different signaling pathways and cellular functions have been suggested, the in vivo functions of PLD2 have only recently been reported.[11–13] In addition, although PLD2 is expressed in several cell types, the tissue-specific functions of PLD2 have not been studied. Therefore, studies using tissue-specific knockout mice are required to clearly define the in vivo functions of PLD2. Angiogenesis occurs as a result of complex processes that occur in various cell types.[2] We generated endothelial-specific knockout mice to assess the endothelial roles of PLD2. Although vessel growth was delayed in *Pld2* knockout retinae at postnatal day 5, the delayed retinal angiogenesis was recovered by postnatal day 14. Therefore, the effects of PLD2 deficiency may be limited in physiological angiogenesis, perhaps by compensatory mechanisms involving the other isoform, PLD1. However, *Pld2* eKO mice showed significantly reduced pathological angiogenesis in an OIR model. Furthermore, tumor growth and angiogenesis were decreased in a tumor implantation model. Interestingly, our data are inconsistent with a previous study using *Pld2* whole-body knockout mice, which reported that only PLD1 plays a role in the tumor microenvironment during tumor angiogenesis and metastasis.[42] Therefore, additional studies are required to clarify isoform specific– or cell type–specific functions of PLDs in angiogenic processes. Taken together, our data suggest that the in vivo functions of PLD2 could be particularly important in pathological states, although it exerts critical roles in general signaling pathways and processes. Therefore, PLD2 could be a target for development of angiogenic drugs with minimal side effects on physiological angiogenesis. Recently, PLD2-specific inhibitors were developed.[43] If effective drug for PLD2 is further developed, specific inhibition of PLD2 could be a potential clinical strategy for several diseases (cancer, diabetic retinopathy, and rheumatoid arthritis) closely related with pathological angiogenesis.

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Disclosures

None.

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Significance

Hypoxia-mediated angiogenesis is important under pathophysiological conditions. Under pathological conditions, dysregulated angiogenesis is closely related to the progression and prognosis of several diseases including vascular retinopathy and cancer. Therefore, identifying specific factors that play roles in pathological angiogenesis is important for developing novel therapeutic strategies. Here, we showed that phospholipase D2 (PLD2) contributes to survival and migration of endothelial cells under hypoxic conditions through regulating translation of hypoxia-inducible factor-1α and expression of its target genes. Furthermore, we demonstrate roles of endothelial PLD2 in pathological angiogenesis with limited effects on physiological angiogenesis, using endothelial-specific *Pld2* knockout mice. Our study suggests PLD2 as a novel regulator of pathological angiogenesis and provides new mechanistic roles of PLD2 in hypoxic response. In addition, to our knowledge, this is the first time that tissue-specific functions of PLD2 have been reported in an animal model.