Role of Tissue-Type Plasminogen Activator in Remodeling of the Ductus Arteriosus

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Vascular remodeling (e.g., intimal thickening) is necessary for complete closure of the ductus arteriosus (DA). Smooth muscle cells are reported to contribute to DA remodeling. In contrast, the contribution of endothelial cells remains largely unknown. Recent data showed that tissue-type plasminogen activator (t-PA) was highly expressed in the endothelial cells of rat and human DA. It is well known that t-PA is an activator of the blood fibrinolytic system, but t-PA-induced localized proteolysis has been reported to play an important role in vascular development. We found that t-PA-induced plasminogen-plasmin conversion promoted matrix metalloproteinase-2 activation in endothelial cells of rat DA. Gelatinase activity was noted at the internal elastic laminae (IEL) of rat and human DA on in situ gelatin zymography. The in vivo injection of plasminogen to pre-term rats increased gelatinase activation, IEL disruption, and the subsequent intimal thickening formation in the pre-term rat DA. Human DA results partly supported the rat DA findings, suggesting that t-PA-mediated DA remodeling may also be present in the human DA. Current pharmacotherapy for patent DA (PDA) mainly focuses on increasing vascular constriction. Elucidating the molecular mechanisms of DA remodeling may help to expand the range of therapeutic strategies for PDA.

Key Words: Ductus arteriosus; Endothelial cell; Internal elastic laminae; Intimal thickening; Tissue-type plasminogen activator

The ductus arteriosus (DA) is a fetal blood vessel connecting the pulmonary artery and aorta, and is indispensable for maintaining fetal life. In utero, the DA diverts the right ventricular blood away from the high-resistance pulmonary circulation and into the systemic circulation where gas is exchanged with maternal blood. Closure of the DA is normally completed in the 3 days after birth, but pre-term infants often have a patent DA after birth. Persistent patent DA (PDA) occurs in more than half of pre-term infants, and is related to blood flow imbalance (e.g., pulmonary congestion, and hypo-perfusion of systemic circulation) and subsequent mortality. Although approximately 50% of extremely pre-term infants receive pharmacotherapy with cycloxygenase inhibitors, this involves a risk of renal dysfunction, spontaneous intestinal perforation and necrotizing enterocolitis. Thus, the current treatment strategies for PDA still need improvement.

Complete closure of the DA involves 2 processes: functional and anatomical closure. Functional closure is a vasoconstriction induced by contraction of smooth muscle cells (SMC); anatomical closure is a form of vascular remodeling represented by intimal thickening.

Vasoconstriction of the DA

Postnatal DA constriction is triggered by increased arterial pO₂, decreased blood pressure in the DA lumen, decreased circulating prostaglandin E₂ (PGE₂) level, and a decreased number of PGE₂ receptors.

The postnatal increase of pO₂ inhibits the ductal smooth muscle voltage-dependent potassium channels, such as Kv1.5 and Kv2.1, which causes membrane depolarization and calcium influx via the voltage-dependent calcium channels. This is followed by DA SMC contraction. Voltage-dependent calcium channel and potassium channel genes are upregulated in the mature DA. Calcium influx via voltage-dependent calcium channels, oxygen-induced activation of the Rho/Rho-associated coiled-coil containing protein kinase (Rho/ROCK) pathway is associated with postnatal DA constriction.

Reduced circulating PGE₂ is related to postnatal DA constriction. The fetal DA is dilated by PGE₂, which is derived from the placenta. After birth, the loss of the placenta and the increased flow of the lung, which is the major site of PGE₂ catabolism, cause a decline in circulating PGE₂. In addition, PGE₂ receptors are decreased after birth. These changes promote DA constriction.

Furthermore, we recently reported that serum osmotic change and glutamate are related to postnatal DA constriction. We found that premature infants did not have a physiological decrease in serum osmolarity after birth, and...
that glutamate promoted the DA contraction through glutamate inotropic receptor α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type subunit 1 (GluR1)-mediated noradrenaline production.\textsuperscript{19}

hypo-osmolality contributes to postnatal DA contraction via the transient receptor potential melastatin3 (TRPM3) channel, which is one of the osmotic sensors.\textsuperscript{18} We also found that the glutamate plasma concentration was decreased in human premature infants with PDA, and
Role of t-PA in DA Remodeling

In addition to contraction of the DA, DA remodeling (e.g., intimal thickening) has been suggested to be essential for the complete closure of the DA. In human DA, intimal thickening occurs gradually in mid-gestation and becomes prominent at full term (Figure 1A). Intimal thickening is not complete in pre-term infants and it is weakened in full-term infants with PDA. The formation of intimal thickening in the DA involves some serial steps that occur toward birth (Figure 1B). The disruption of the internal elastic lamina (IEL) and the detachment of endothelial cells from the IEL are the early steps in the formation of intimal thickening in the DA. These processes are followed by the migration of SMC into the subendothelial region, PGE2 receptor ‘EP4’ signaling then promotes the production of hyaluronan in the subendothelial region and the subsequent migration of SMC toward the DA lumen, resulting in intimal thickening. The role of SMC in DA remodeling is therefore important.

Disruption of the IEL

Disruption of the IEL is another hallmark of DA remodeling. The IEL of the DA is constructed similarly to the adjacent aorta in fetal development. Then, IEL disrupts only in the DA in mid-gestation. In humans, the IEL disruption of the DA starts at approximately 17 weeks of gestation and becomes progressively obvious at 22–26 weeks of gestation. The disruption of the IEL is followed by the migration of SMC into the subendothelial region, and intimal thickening becomes visible at the site of the disrupted IEL. Then, the IEL is disrupted more frequently between 27 and 34 weeks’ gestation, and the further migration of SMC promotes narrowing of the DA lumen during late gestation. From these histological observations of human DA, the disruption of the DA IEL is suggested to be incomplete in pre-term infants born at <27 weeks of gestation, who often have PDA. Gittenberger-de Groot reported that human PDA tissue lacked IEL disruption and subsequent intimal thickening, while normal human DA tissue had this remodeling.

The functional role of the IEL as a barrier to the migration of SMC into the subendothelial region was also elucidated by using SMC- and endothelial cell-specific elastin knockout mice. Lin et al demonstrated that IEL was derived from both SMC and endothelial cells, and that areas of neointimal hyperplasia correlated with local IEL disruption in the aorta. In addition to the role of the IEL as a barrier to SMC migration, elastin itself affects the...
phenotype of vascular SMC. Mice with the global knockout of elastin have supravalvular aortic stenosis due to the accumulation of SMC; and tropoelastin, which is a monomer of elastin, inhibits the proliferation and migration of SMC.

In our previous study, PGE₂-EP₄ signaling prevented elastogenesis in the tunica media of the DA via the degradation of lysyl oxidase, which catalyzes elastin cross-links. Unlike the sparse elastic fibers in the DA tunica media, IEL is first constructed and then progressively disrupted toward birth. Therefore, the signaling responsible for the disruption of the IEL is unclear.

Role of Endothelial Cells in the DA
Although we and others have reported that SMC have an important role in the formation of intimal thickening, the role of the endothelial cells adjacent to the IEL is largely unknown. DA endothelial cells contribute to the DA dilatation via the production of endothelial nitric oxide synthetase, and endothelial cells also promote the proliferation of SMC and subsequent intimal thickening via the production of vascular endothelial growth factor. However, many points concerning the function of the DA endothelial cells are unclear. Recently, we found that endothelial cells play an important role in DA remodeling via the marked expression of tissue-type plasminogen activator (t-PA), which mediates matrix metalloproteinase-2 (MMP-2) activation, IEL disruption, and subsequent intimal thickening.

Expression of t-PA in DA Endothelial Cells
We previously reported on the microarray analysis of rat DA and aorta, in which we found that t-PA was dominantly expressed in the endothelial cells of the DA. We also confirmed the expression of t-PA on quantitative polymerase chain reaction using isolated DA endothelial cells and aortic endothelial cells from full-term rat fetuses (embryonic day 21). In accordance with our previous microarray data, t-PA (Plat) mRNA was highly expressed in DA endothelial cells compared with aortic endothelial cells. We collected human DA from neonatal patients with congenital heart disease and examined the expression of t-PA in the intimal thickening region compared with the tunica media. Although these patients had congenital heart disease, they required PGE₁ to maintain patency of the DA, and all DA had remarkable intimal thickening, suggesting that these DA had normally developed until sample collection. We found that the t-PA (Plat) mRNA expression in the intimal thickening region was 3.6-fold higher than that in the tunica media, and on immunohistochemistry for t-PA using rat and human DA, t-PA was markedly expressed in the endothelial cells of both the rat and human DA. We herein introduce our recent findings.

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It is well known that t-PA is an activator of the blood fibrinolytic system, but t-PA-induced localized proteolysis has been reported to play an important role in vascular development. Levin et al investigated the developmental changes of locally expressed t-PA in the rat fetus and found that t-PA expression was dynamically regulated during vascular development. On immunohistochemical staining...
of t-PA using time-series rat fetuses, the t-PA was initially expressed in endothelial cells of the dorsal aorta and extended to the large arteries on embryonic day 13. The expression of t-PA was mostly decreased in the aortic endothelial cells by embryonic day 15. Alternatively, it appeared in the pulmonary, subclavian, vertebral and basilar arteries. After t-PA expression disappeared in the great arteries, t-PA expression was found in the smaller arterial branches. The authors suggested that the primary function of locally expressed t-PA may be localized proteolysis to promote vascular remodeling during fetal development, rather than systemic vascular fibrinolysis.

**Gelatinase Activity in the DA**

T-PA converts plasminogen into plasmin, which activates gelatinase MMP-2 and MMP-9 and elastic laminae are mainly degraded by MMP-2 and MMP-9. Our initial data showed the high expression of t-PA in DA endothelial cells of both rats and humans. Thus, we aimed to elucidate whether DA endothelial cell-induced t-PA plays a role in disruption of the IEL through the activation of MMP. We confirmed the gelatinase activity using isolated DA endothelial cells and aortic endothelial cells from rats (Figure 3A). On gelatin zymography the pro-MMP-2 level in rat DA endothelial cells was higher than in aortic endothelial cells under basal conditions (Figure 3B, control). Plasminogen supplementation increased MMP-2 activation in DA endothelial cells in comparison with aortic endothelial cells (Figure 3B, plasminogen). Neither DA endothelial cells nor aortic endothelial cells had detectable MMP-9, plasminogen. Neither DA endothelial cells nor aortic endothelial cells had detectable MMP-9, plasminogen. Neither DA endothelial cells nor aortic endothelial cells had detectable MMP-9. The authors suggested that the primary function of locally expressed t-PA may be localized proteolysis to promote vascular remodeling during fetal development, rather than systemic vascular fibrinolysis.

**Disruption of Elastic Laminae via MMP Activation**

Although the t-PA-mediated activation of MMP is suggested to be involved in the remodeling of the vascular system, it is difficult to demonstrate the direct effect of t-PA on elastic laminae in vitro because cultured cells on a plastic plate cannot show the disruption of elastic fibers. We previously reported a 3-D vascular model that contains multilayered SMC and elastic laminae. In our recent study, we seeded endothelial cells on the top of the multilayered SMC and confirmed the effect of endothelial cell-derived t-PA on the layered elastic fibers (Figure 4A). In untreated vascular models, layered elastic laminae were confirmed under endothelial cells (Figure 4B, Left). In contrast, supplementation of plasminogen (a substrate of t-PA) increased the gelatinase activation and the disruption of the elastic laminae (Figure 4B, Right). This gelatinase activation was attenuated by the MMP inhibitor EDTA. We also found that t-PA-targeted siRNA in endothelial cells attenuated the gelatinase activation and subsequent disruption of the elastic laminae. These observations suggest that endothelial cell-derived-t-PA involves the activation of MMP and subsequent disruption of elastic laminae.

**In Vivo Plasminogen-Induced IEL Disruption and Intimal Thickening**

A 3-D vascular model showed that plasminogen supplementation facilitated the disruption of elastic laminae via MMP activation. We then investigated whether plasminogen injection increased the IEL disruption and intimal thickening in the pre-term rat DA in vivo. On histology the IEL was almost completely intact on embryonic day 19 and became highly fragmented on embryonic day 21. We also confirmed the high expression of t-PA in DA endothelial cells on embryonic day 19 on immunohistochemistry. We then injected plasminogen on embryonic day 19 and analyzed the fetuses on embryonic day 20 (24h after injection; Figure 4C). We injected the plasminogen i.p. into rat fetuses (Figure 4D). In accordance with the 3-D in vitro vascular model, on in situ gelatin zymography the gelatinase was highly activated in the plasminogen-injected rat DA (Figure 4E, Upper). The IEL disruption and intimal thickening...
ening were moderate and limited in controls (Figure 4E, Lower left). In contrast, the injection of plasminogen increased the elastic laminae disruption and the subsequent intimal thickening (Figure 4E, Lower right).

In our present study, we were unable to measure the serum plasminogen concentration in rat fetuses due to the limited amount of serum, but it has been reported that human infants have lower blood plasminogen concentration than adults. Andrew et al reported that the serum plasminogen concentration in normal human full-term infants is 60% of that in adults, and is lower in pre-term infants due to prematurity of liver function. Furthermore, the activity of fetal plasmin was also lower than that of the adult type. This indicates that infants physiologically have lower blood plasminogen level and plasmin activity; thus, the supplementation of plasminogen may help the t-PA-mediated IEL disruption in the DA.

Although plasminogen is associated with a possible unfavorable effect (bleeding tendency due to activation of fibrinolytic system), in clinical trials of plasminogen injection to premature infants, no apparent adverse effects were reported. Before 1984, when surfactant therapy was started clinically for premature infants with respiratory distress syndrome (RDS), several trials were performed to treat RDS infants, and one of those trials evaluated the effect of plasminogen on RDS. Pathologically, RDS is considered to be a hyaline-membrane disease. The hyaline membrane is composed of fibrin, which is derived from leaked fibrinogen from the pulmonary alveolus. This fibrin inhibits gas exchange and the activity of endogenous surfactant in the alveolus. Plasminogen was used to dissolve the fibrin of the alveolus in premature infants with RDS. Ambru et al reported that the use of plasminogen in premature infants (1,000–2,500 g, at 28–40 weeks of gestation) decreased respiratory distress and mortality without obvious adverse effects in their double-blind, randomized clinical trials. T-PA may promote IEL disruption of the DA without major adverse effects, but we must carefully consider the possibility of plasminogen injection in the treatment of premature infants with PDA.

Conclusions
Rat and human DA endothelial cells had high t-PA expression. Endothelial cell-derived t-PA facilitated IEL disruption through MMP-2 activation, which may aid in the formation of intimal thickening in the DA (Figure 5). The human DA results partly supported the rat DA results, suggesting that t-PA-mediated DA remodeling may also be present in human DA. The elucidation of the molecular mechanisms of DA remodeling may help to expand the range of treatment strategies for pre-term infants with PDA.

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Data Availability
Data sharing is not applicable to this article given that no datasets were generated or analyzed during the current study.

Disclosures
The authors declare no conflicts of interest.

IRB Information
Approval for the human study was obtained from the human subject committees at Yokohama City University (B150305001) and Kanagawa Children’s Medical Center (1502-05). Approval for the animal study was obtained from the Institutional Animal Care and Use Committees of Yokohama City University (F-A-16-010).

References
1. Costello K, Hennessy E, Gibson AT, Marlow N, Wilkinson AR. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. Pediatrics 2000; 106: 659 – 671.
2. Zonnenberg I, de Waal K. The definition of a haemodynamic significant duct in randomized controlled trials: A systematic literature review. Acta Paediatr 2012; 101: 247 – 251.
3. Sellmer A, Bjerre JV, Schmidt MR, McNamara PJ, Hjortdal VE, Host B, et al. Morbidity and mortality in preterm neonates with patent ductus arteriosus on day 3. Arch Dis Child Fetal Neonatal Ed 2013; 98: F505 – F510.
4. Benitz WE. Patent ductus arteriosus: To treat or not to treat? Arch Dis Child Fetal Neonatal Ed 2012; 97: F80 – F82.
5. Hagadorn JI, Brownell EA, Trzaski JM, Johnson KR, Lainwala S, Campbell BT, et al. Trends and variation in management and outcomes of very low-birth-weight infants with patent ductus arteriosus. Pediatr Res 2016; 80: 785 – 792.
6. Clyman RI. Mechanisms regulating the ductus arteriosus. Biol Neonate 2006; 89: 330 – 335.
7. Yokoyama U, Minamisawa S, Ishikawa Y. Regulation of vascular tone and remodeling of the ductus arteriosus. J Smooth Muscle Res 2010; 46: 77 – 87.
8. Yokoyama U, Iwatsubo K, Umemura M, Fujita T, Ishikawa Y. The prostanoid EP4 receptor and its signaling pathway. Pharmacol Rev 2013; 65: 1010 – 1052.
9. Michelakis E, Rebeyka I, Bateson J, Olley P, Puttagunta L, Archer S. Voltage-gated potassium channels in human ductus arteriosus. Lancet 2000; 356: 134 – 137.
10. Reeve HL, Tolarova S, Nelson DP, Archer S, Weir EK. Redox control of oxygen sensing in the rabbit ductus arteriosus. J Physiol 2001; 533: 253 – 261.
11. Nakashiki T, Gu H, Masukawa D, Momma K. Mechanisms of oxygen-induced contraction of ductus arteriosus isolated from the fetal rabbit. Circ Res 1993; 72: 1218 – 1228.
12. Yokoyama U, Minamisawa S, Adachi-Akahane S, Akaite T, Naguro I, Funakoshi K, et al. Multiple transcripts of Ca2+ channel alphal-subunits and a novel spliced variant of the alphalC-subunit in rat ductus arteriosus. Am J Physiol Heart Circ Physiol 2006; 290: H1660 – H1670.
13. Reese J, Walsh N, Poole SD, Brown N, Roman C, Clyman RI. Chronic in utero cyclooxygenase inhibition alters PGE2-regulated ductus arteriosus contractile pathways and prevents postnatal closure. Pediatr Res 2009; 66: 155 – 161.
14. Kajimoto H, Hashimoto K, Bonnet SN, Haromy A, Harry G, Moudgil R, et al. Oxygen activates the Rho/Rho-kinase pathway and induces RhoB and ROCK-1 expression in human and rabbit ductus arteriosus. Am J Physiol Heart Circ Physiol 2006; 290: H1660 – H1670.
15. Clyman RI, Mauray F, Roman C, Rudolph AM, Heymann MA. Circulating prostaglandin E2 concentrations and patent ductus arteriosus in fetal and neonatal lambs. J Pediatr 1980; 97: 455 – 461.
16. Smith GC. The pharmacology of the ductus arteriosus. Pharmacol Rev 1998; 50: 35 – 58.
17. Bouayad A, Kajino H, Waleh N, Fouron JC, Andelfinger G, Varma DR, et al. Characterization of PGE2 receptors in fetal and newborn lamb ductus arteriosus. Am J Physiol Heart Circ Physiol 2001; 280: H2342 – H2349.
18. Aoki R, Yokoyama U, Ichikawa Y, Taguri M, Kumagaya S, Ishiwata R, et al. Decreased serum osmolality promotes ductus arteriosus constriction. Cardiovase Res 2014; 104: 326 – 336.
19. Fujita S, Yokoyama U, Ishiwata R, Aoki R, Nagao K, Masukawa D, et al. Glutamate promotes constriction of the rat
ductus arteriosus. *Circ J* 2016; 80: 2388 –2396.

20. Yokoyama U. Prostaglandin E-mediated molecular mechanisms driving remodeling of the ductus arteriosus. *Pediatr Int* 2015; 57: 820 –827.

21. Yokoyama U, Minamisawa S, Quan H, Ghatak S, Akaïke T, Segi-Nishida E, et al. Chronic activation of the prostaglandin receptor EP4 promotes hyaluronan-mediated neointimal formation in the ductus arteriosus. *J Clin Invest* 2006; 116: 3026 –3034.

22. Yokoyama U, Minamisawa S, Quan H, Akaïke T, Suzuki S, Jin M, et al. Prostaglandin E2-activated Epac promotes neointimal formation of the rat ductus arteriosus by a process distinct from that of cAMP-dependent protein kinase A. *J Biol Chem* 2008; 283: 28702 –28709.

23. Yokoyama U, Minamisawa S, Katayama A, Tang T, Suzuki S, Iwatsubo K, et al. Differential regulation of vascular tone and remodeling via stimulation of type 2 and type 6 adenylyl cyclases in the ductus arteriosus. *Circ Res* 2010; 106: 1882 –1892.

24. Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H, Nishimura T, et al. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem Biophys Res Commun* 1998; 246: 7 –12.

25. Nguyen M, Camenisch T, Snowaert JN, Hicks E, Coffman TM, Anderson PA, et al. The prostaglandin receptor EP4 triggers remodeling of the cardiovascular system at birth. *Nature* 1997; 390: 78 –81.

26. Gittenberger-de Groot AC. Persistent ductus arteriosus: Most probably a primary congenital malformation. *Br Heart J* 1977; 39: 610 –618.

27. Toda T, Tsuda N, Takagi T, Nishimori I, Leszczynski D, Gittenberger-de Groot AC. Persistent ductus arteriosus: Most probably a primary congenital malformation. *Br Heart J* 1977; 39: 610 –618.

28. Tada T, Wakabayashi T, Nakao Y, Ueki R, Ogawa Y, Inagawa M, et al. Persistent ductus arteriosus: The role of CD14+/CD163+ mononuclear cells and VEGF in neointimal mound formation. *Pediatr Res* 2011; 70: 332 –338.

29. De Reeder EG, Girard N, Poelmann RE, Van Munsteren JC, De Reeder EG, van Munsteren CJ, Poelmann RE, Patterson DF, et al. Persistent ductus arteriosus: Most probably a primary congenital malformation. *Br Heart J* 1977; 39: 610 –618.

30. Toda T, Wakabayashi T, Nakao Y, Ueki R, Ogawa Y, Inagawa M, et al. Persistent ductus arteriosus: The role of CD14+/CD163+ mononuclear cells and VEGF in neointimal mound formation. *Pediatr Res* 2011; 70: 332 –338.

31. Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H, Nishimura T, et al. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem Biophys Res Commun* 1998; 246: 7 –12.

32. Toda T, Tsuda N, Takagi T, Nishimori I, Leszczynski D, Gittenberger-de Groot AC. Persistent ductus arteriosus: Most probably a primary congenital malformation. *Br Heart J* 1977; 39: 610 –618.

33. De Reeder EG, Girard N, Poelmann RE, Van Munsteren JC, Patterson DF, Gittenberger-De Groot AC. Hyaluronic acid accumulation and endothelial cell detachment in intimal thickening of the vessel wall: The normal and genetically defective ductus arteriosus. *J Cell Physiol* 1992; 182: 595 –613.

34. Lehti K, Ichikawa Y, et al. Morphological and histological evaluations of the vessel wall: The normal and genetically defective ductus arteriosus. *Am J Pathol* 1998; 153: 139 –157.

35. Liu NM, Yokota T, Maekawa S, Lu P, Zheng YW, Taniguchi H, et al. Transcription profiles of endothelial cells in the rat ductus arteriosus during a perinatal period. *PLoS One* 2013; 8: e73685.

36. Yokoyama U, Sato Y, Akaïke T, Ishida S, Sawada J, Nagao T, et al. Maternal vitamin A alters gene profiles and structural maturation of the rat ductus arteriosus. *Physiol Genomics* 2007; 31: 139 –157.

37. Yarboro MT, Durbin MD, Herington JL, Shelton EL, Zhang T, Ebbey CG, et al. Transcriptional profiling of the ductus arteriosus: Comparison of rodent microarrays and human RNA sequencing. *Semin Perinatol* 2018; 42: 212 –220.

38. Levie EG, Banka CL, Parry GC. Progressive and transient expression of tissue plasminogen activator contributes to remodeling of the rat ductus arteriosus. *PLoS One* 2018; 13: e0190871.

39. Monea S, Lehti K, Kessi-Oja J, Mignatti P. Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism. *J Cell Physiol* 2002; 192: 160 –170.

40. Nijenhuis HR. Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost* 2001; 86: 324 –333.

41. Kruiholf EK, Dunoyer-Geindre S. Human tissue-type plasminogen activator. *Thromb Haemost* 2014; 112: 243 –254.

42. Ishiwata R, Yokoyama U, Matsukasi M, Asano Y, Kadowaki K, Ichikawa Y, et al. Plasmin-induced multilayers of smooth muscle cells as a new experimental model for vascular elastic fiber formation studies. *Atherosclerosis* 2014; 233: 590 –600.

43. Matsukasi M, Kadowaki K, Adachi E, Sakura T, Yokoyama U, Ishiwata R, et al. Morphological and histological evaluations of 3D-layered blood vessel constructs prepared by hierarchical cell manipulation. *J Biomater Polym Ed* 2012; 23: 63 –79.

44. Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. *Am J Pediatr Hematol Oncol* 1990; 12: 95 –104.

45. Clyman RI, Seidner SR, Kajino H, Roman C, Koch CJ, Ferrara SR, et al. Permanent anatomic closure of the ductus arteriosus in newborn baboons: The roles of postnatal constriction, hypoxia, and gestation. *Pediatr Res* 1999; 45: 19 –29.

46. Waleh N, Seidner S, McMurray D, Giavedoni L, Hodara V, Goez S, et al. Anatomic closure of the premature patent ductus arteriosus: The role of CD14+/CD163+ mononuclear cells and VEGF in neonatal mound formation. *Pediatr Res* 2011; 70: 332 –338.

47. Saito J, Yokoyama U, Nicho N, Zheng YW, Ichikawa Y, Ito S, et al. Tissue-type plasminogen activator contributes to remodeling of the rat ductus arteriosus. *PLoS One* 2013; 8: e73685.

48. Clyman RI, Chan CY, Maury C, Chen YQ, Cox W, Seidner SR, et al. Permanent anatomic closure of the ductus arteriosus in newborn baboons: The roles of postnatal constriction, hypoxia, and gestation. *Pediatr Res* 1999; 45: 19 –29.

49. Leppert MF, et al. Persistent ductus arteriosus: Most probably a primary congenital malformation. *Br Heart J* 1977; 39: 610 –618.