Pathophysiologic Role of Molecules Determining Arteriovenous Differentiation in Adult Life

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Keywords

Vascular development · Vascular remodeling · Identity · Eph-B4 · Ephrin-B2

Abstract

The structural differences between arteries and veins are genetically predetermined. Vascular identity markers, the molecular markers specific to veins and arteries, determine the differential development of vessels during embryogenesis and their expression persists in adult vessels. It is revealed that they can be reactivated under various pathophysiologic conditions even after vessel differentiation. Thus, once considered as quiescent in adults, vascular identity markers may actually play significant roles in vascular remodeling. Manipulation of vascular identity and the underlying molecular mechanisms might be a novel strategy to improve vascular remodeling for clinical application.

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Introduction

The structural differences between arteries and veins are genetically predetermined by molecules through controlling the fate of blood vessels during embryogenesis. Some of them are persistently expressed during adulthood as specific identity markers of arteries and veins [1, 2]. New evidence reveals that even after vessel differentiation, vascular identity markers still have the potential to function under pathophysiologic conditions (including surgical procedures) (Fig. 1). This is of particular significance when referring to their roles in potential therapeutic applications. In this review, we will focus on arterial and venous identity markers and their specific roles in some pathophysiologic processes.

Vasculogenesis and Angiogenesis

Formation of a vascular system with proper organizational structure is critical for embryo development and survival. Vasculogenesis and angiogenesis are 2 succes-

sive processes through which blood vessels are formed during embryonic development. Vasculogenesis is a process of in situ differentiation of angioblasts and endothelial cells (ECs) from mesenchyme and their coalescence into tubes of primary vascular plexus. Expansion of this primitive vascular system occurs through angiogenesis, either sprouting angiogenesis or intussusception angio-

genesis. Through these distinct angiogenesis processes, the primary vascular plexus is remodeled into a hierarchical vascular tree with properly branched arteries, capillaries, and veins. Upon initiation of blood flow, the primitive vascular plexus exhibits a remarkable degree of plasticity, indicating that not only the vessel identity but also the global pattern of developing arterial-venous networks is remodeled by blood flow [3].

Fig. 1. Vascular identity markers are involved in vascular remodeling. The dynamic interplay between gene expression, vascular identity markers, vascular structure, epigenetic cues (e.g., hemodynamics), and the biologic response of the vascular wall construct a regulatory network of vascular remodeling.

Arteriovenous Specification

Vascular specification is genetically determined early during the course of development. In this complex developmental program, correct spatial and temporal expression of a diversity of genes is orchestrated by a large set of transcription factors. Endothelial progenitors expressing several endothelial-specific markers can be detected early in embryo development. They are further differentiated to acquire specific arterial and venous properties. The signaling pathways involved in arterial and venous determination are hierarchically organized [4] (Fig. 2).

Arterial Specification

At the top of the arterial specification program, sonic hedgehog triggers the expression of the vascular endothelial growth factor (VEGF), which, at its high concentration, can promote the activation of Notch signaling [5].

Fig. 2. Signaling pathways involved in arteriovenous specification. Shh lies at the top of arterial specification and triggers the expression of VEGF, while at high concentrations, promoting Notch signaling activation. Wnt and other transcription factors, such as Foxc1, Foxc2, and Sox17, cooperate to activate Notch signaling and determine arterial fate. Instead, vein progenitors are exposed to low VEGF concentrations and express COUP-TFII transcription factor, which promotes venous identity by suppressing Notch, Jag1 signaling, and ephrin-B2. BRG1, brahma-related gene 1; COUP-TFII, chicken ovalbumin upstream promoter transcription factor II; Dll, Delta-like; Eph, erythropoietin-producing hepatocellular; Flk, fetal liver kinase; Flt, Fms-related tyrosine kinase; Foxc, Forkhead box C; grl, notch-gridlock; Hey, Hes-related family BHLH transcription factor with YRPW motif; JAG, gap junction alpha; NP, neuropilin; Raf, rapidly accelerated fibrosarcoma; Shh, sonic hedgehog; Sox, sex determining region Y-box; VEGF, vascular endothelial growth factor; Wnt, wingless/integrated.

Besides VEGF, Wnt signaling and other transcription factors such as Foxc1, Foxc2, and Sox17 converge into the Notch pathway and induce arterial differentiation of ECs [6–8], whereas vein progenitors are determined through exposure to low VEGF concentration followed by expression of chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) that inhibits Notch signaling so as to promote venous specification [9]. Several research studies suggested that there is an antagonistic cross talk between VEGF-activated intracellular signaling pathways [10–12]. A VEGF-dependent enhancer of Delta-like 4 (Dll4) has been recently described, which is a minimal region that can drive arterial expression of Dll4 [13].

Venous Specification

Identification of COUP-TFII provides the evidence of a direct transcriptional induction during venous differentiation. COUP-TFII has been found to be expressed only in venous and not in arterial ECs. You et al. [14] elegantly demonstrated that COUP-TFII has a cell-autonomous function in venous specification, and COUP-TFII mutant cells show a partial reduction of venous markers but a strong upregulation of several arterial markers. On the contrary, endothelial-specific overexpression of COUP-TFII leads to a lethal vascular phenotype through hampering the expression of several arterial markers and simultaneously acquiring the venous marker erythropoietin-producing hepatocellular (Eph)-B4 by arteries [14]. The mechanism underlying the action of COUP-TFII exerts an inhibitory effect on Notch signaling and, therefore, promotes venous differentiation by repressing arterial specification [9]. It is revealed that brahma-related gene 1 (BRG1) promotes COUP-TFII expression by binding conserved regulatory elements within the COUP-TFII promoter. BRG1 is a SWI/SNF member ATPase, which serves to remodel chromatin and allows the promoter accessible to transcriptional machinery. In case of BRG1 inactivation, arterial markers will be induced in veins [15].

Notch Signaling Pathway

Components of the Notch signaling pathway are the first group of genes to be implicated in arterial endothelial specification [16]. As large transmembrane receptors, Notch proteins govern fate decisions in numerous cell types, angiogenesis, and vascular homeostasis during both embryonic and adult stages. When bound to the transmembrane ligands Dll or Jagged (JAG), Notch receptors are activated through cleavage and the intracel-

lular domain of the processed Notch receptor translocates into the nucleus. It interacts with the recombination signal binding protein for immunoglobulin Kappa J region to form a transactivation complex and then induces target gene expression [16]. There are 4 Notch receptors (Notch1–4) and 5 ligands (JAG1 and 2 and Dll1, 3, and 4) expressed in mammals. Although early Notch activation can bestow positional determination, this does not irrevocably commit cells to an arterial fate. Lineage-tracing studies reveal that Notch-activated cells initially and positionally committed to the dorsal aorta can later become venous tissue by de- or transdifferentiation [17].

Ephs and Ephrins

As the largest subfamily of growth factor receptors, Eph receptor tyrosine kinases (RTKs) utilize ephrins, similarly with multiple isoforms, as their ligands [18]. Unlike many ligands for other receptor tyrosine kinases, ephrins must be membrane tethered in order to activate their Eph receptors. Ephrins can be divided into 2 subclasses, ephrin-A and ephrin-B, according to their linkage to the cell membrane. Ephrin-B ligands primarily interact with the B subset of Eph receptors, which consist of at least 5 members (Eph-B1-4 and Eph-B6) [19]. Interactions between ephrin-B and Eph-B apparently activate bidirectional signaling, including activation of the intracellular RTK domain of Eph-B, which also involves tyrosine phosphorylation [20].

In view of the vascular defects observed in embryonic mice bearing null mutations in ephrin-B/Eph-B (e.g., inflated or extended pericardium, abnormal cardinal veins or dorsal aorta, and pale yolk sacs with few blood vessels) [21], much attention of researchers has been attracted to the roles of ephrin-B2 and its cognate receptor Eph-B4 in cardiovascular development. Moreover, a distinctively reciprocal pattern of ephrin-B2 and Eph-B4 distribution within the developing vasculature has been revealed, that is, ephrin-B2 distinctively in the endothelium of primordial arterial vessels, while Eph-B4 in the endothelium of primordial venous vessels [22]. It has been confirmed that ephrin-B2 is a marker of arterial endothelium and smooth muscle and its expression persists in adult vessels. Conversely, Eph-B4 is a marker of venous cells and also persistently expressed in adult vessels [1, 2].

Hemodynamic Forces in Vascular Development

During embryonic development, molecular markers that distinguish arteries from veins are expressed before the onset of blood circulation [23]. However, hemodynamic forces imparted by blood flow play important roles

Table 1. Arterial and venous identity markers already identified

Marker	Year	Research	Model
<i>Arterial</i>			
ALK1	2003	Seki et al. [29]	Mouse
CD44	2004	Fischer et al. [30]	Mouse
Connexin-37	2005	Shin et al. [31]	Mouse
Connexin-40	2002	Mukouyama et al. [32]	Mouse
Dll4	2000	Shutter et al. [33]	Mouse
DEPP	2005	Shin et al. [31]	Mouse
Ephrin-B2	1998	Wang et al. [1]	Mouse
IGFB-5P	2005	Shin et al. [31]	Mouse
NP1	2002	Mukouyama et al. [32]	Mouse
Notch1, 3, 4	2000, 2001	Yoshikawa et al. [34]; Villa et al. [35]	Mouse
UNC5B	2004	Lu et al. [36]	Mouse and zebrafish
bHLH transcription factors Hey1 and Hey2	1999	Leimeister et al. [37]	Mouse
JAG1, 2	2001	Villa et al. [35]	Mouse
RGS5	2000	Adams et al. [38]	Tissues from human, macaque, or rat
SOX7, SOX17, and SOX18	2013, 2015	Corada et al. [8]; Zhou et al. [39]	Mouse
aSMA	1998	Benjamin et al. [40]	Rat
GJA5	1982	Spagnoli et al. [41]	Rabbit
<i>Venous</i>			
APJ	1999	Devic et al. [42]	Mouse
COUP-TFII	2005	You et al. [14]	Mouse
Eph-B4	1998	Wang et al. [1]	Mouse
NP2	2001	Herzog et al. [43]	Chick
Endomucin	1999	Morgan et al. [44]	Mouse
VEGFR3/FLT4	1995	Kaipainen et al. [45]	Tissues from mouse and human
TIE2	2001	Moyon et al. [46]	Avian

ALK1, activin receptor-like kinase 1; CD44, classification determinant 44; Dll4, Delta-like 4; DEPP, decidual protein induced by progesterone; IGFB-5P, insulin-like growth factor binding protein-5 protease; NP, neuropilin; UNC5B, unc-5 netrin receptor B; bHLH, basic helix-loop-helix; JAG, Jagged; RGS5, regulator of G protein signaling 5; SOX, sex determining region Y-box; aSMA, a-smooth muscle actin; GJA5, gap junction protein alpha 5; APJ, apelin receptor; COUP-TFII, chicken ovalbumin upstream promoter transcription factor II; ; VEGFR3/FLT4, vascular endothelial growth factor receptor 3; TIE2, tyrosine kinase with immune globulin and EGF homology domains 2.

in embryonic vasculature development. In mature vasculature, different blood flow regimes can induce distinct genetic programs and vessel identity plasticity [3].

Thoma first observed the relationships between mechanical stimuli and vessel form and function in the chick embryo. Thoma's conclusions can be stated in another way; that is, the geometric properties of vessels are optimized to the applied forces and serve to normalize the mechanical stresses [24, 25]. There are multifarious mechanisms of mechanosensation and mechanotransduction involved in transducing hemodynamic signals into biochemical responses, which have been discussed in another review [26]. Embryonic and early postnatal ECs must sense and respond to blood flow to properly shape the developing vasculature. Although arterial and venous specification has been initially hard-wired in the vertebrate em-

bryo, blood flow plays an important role in maintaining these identities and unmasking plasticity when appropriate. In addition, hemodynamic forces also play a critical role in maintenance of dorsal aorta-derived definitive hematopoietic stem cells in zebrafish embryos [27]. In chick embryo yolk sac, alteration of blood flow can cause changes in the global gene expression profile and de novo expression of arterial genes within venous tracts. Moreover, ECs with expression of the arterial-specific genes ephrin-B2 or neuropilin-1 can still be incorporated into the venous system in response to this blood flow change [3]. During cardiac cycles in the early mouse embryo, differences in both the levels of shear stress and the flow rates between arteries and veins may be sufficient to differentially regulate COUP-TFII expression such that COUP-TFII expression would only be present in veins [28].

Arterial and Venous Identity Markers Already Identified

We list vascular identity markers of interest in Table 1, which contains 23 arterial molecular markers [1, 8, 29–41] and 7 venous molecular markers [1, 14, 42–46]. Notably, some membrane proteins functioning in certain membrane structures, such as lipid rafts and caveolae, are also involved in vessel identity determination [47].

Plasticity of Vascular Identity Markers and Potential Therapeutic Applications

Once thought to be quiescent in adults, vascular identity markers may actually play a significant role in vascular remodeling and their expression can also change. Our work and results from other laboratories indicate that vascular identity markers appear to be plastic upon surgical treatment. In addition, they are also involved in other pathologic conditions such as tumors and pulmonary arterial hypertension (PAH) [48–51]. Particularly, activation of Eph-B4 may promote patency of vein grafts and arteriovenous fistula (AVF) due to the prevention of neointimal hyperplasia [52, 53]. In contrast, use of a soluble form of Eph-B4 can reduce tumor angiogenesis and growth [54]. Moreover, pharmacological inhibition of Notch3 reverses hypoxic PAH in mice [51]. Thus, regulation of vascular identity and its underlying molecular mechanisms might be a new path to manipulating vascular remodeling in adults.

Vein Grafts

Human vein graft adaptation in the arterial environment is largely associated with loss of the venous identity marker Eph-B4, without stimulation of the arterial identity marker ephrin-B2. This adaptation is characterized by both sustained downregulation of Eph-B4 expression and transient upregulation of VEGF-A expression followed by its downregulation. The underlying mechanism of these changes was revealed with an *in vitro* adult EC model. Thus, the early phase increase in VEGF-A inhibits the expression of Eph-B4 and upregulates the expression of Dll4 (a Notch ligand), but without stimulating its downstream signaling events such as Notch activation or ephrin-B2 expression in these adult ECs. These results are consistent with the effects of blocking the VEGF/COUP-TFII–Notch signaling–Eph-B4/ephrin-B2 pathway in adult ECs [55]. Therefore, vein grafts lose their venous identity markers without acquiring arterial identity. Activation of Eph-B4 via ligands or expression of constitu-

tively active Eph-B4 inhibits venous wall thickening. Conversely, reduction of Eph-B4 signaling is associated with increased venous wall thickness of vein grafts [56]. It is further confirmed that activation of Eph-B4 signaling also stimulates caveolin-1 phosphorylation during vein graft adaptation, and caveolin-1 subsequently executes its Eph-B4-induced function on vein graft wall thickness via modulation of endothelial nitric oxide synthase function [53]. These results indicate that Eph-B4 is active in adult veins and regulates venous remodeling.

AVF

As the preferred method of hemodialysis access, autogenous AVF presents poor patency and low maturation. The situation in the venous segment of an AVF is dramatically different from that in a vein graft. We have explored the remodeling pattern of the venous segment of the carotid-jugular shunt at a late stage (15 weeks) after operation in adult rats. Our results revealed that the venous identity marker Eph-B4 was lost, but the arterial identity markers ephrin-B2 and regulator of G-protein signaling 5 (RGS5) were gained in the jugular segment of the carotid-jugular shunt. The expression of these 2 arterial identity markers was further strengthened in the pulmonary artery in shunted rats compared with controls. The jugular segment of the carotid-jugular shunt underwent significant intimal hyperplasia with strong expression of smooth muscle cell (SMC) markers. It also presented a distinct transcriptional profile including significant upregulation of 5 arterial markers as compared to the sham-operated jugular vein, among which RGS5 is exactly the gene with the most change (10.14-fold) tested by microarray experiments [48]. In another mouse study, at 3 weeks after surgery, the venous limb of the infrarenal aortocaval fistula gained dual arterial/venous identity markers (i.e., ephrin-B2/Eph-B4). Promoting this AVF to preserve venous identity (Eph-B4) with ephrin-B2 did prevent excessive wall thickening and promote AVF patency [52].

Patch Angioplasty

Studies of rat aortic and inferior vena cava patch models indicate that pericardial patches obtain the identity of the vessel in which they are placed; that is, pericardial patches express the arterial marker ephrin-B2 in arterial environments, whereas pericardial patches express the venous marker Eph-B4 in venous environments [57, 58]. Further study demonstrates that atorvastatin can regulate neointimal growth after pericardial patch angioplasty in the arterial environment by regulating the pathway in-

volved in ephrin-B2 expression [59]. Interestingly, in a rat model of polyester patches implanted into the aorta or inferior vena cava, polyester patches healed by infiltration of host arterial or venous progenitor cells depending on the site of implantation. Moreover, when these synthetic patches were treated with AVF, they would have decreased neointimal thickness with neointimal ECs expressing the arterial identity markers ephrin-B2 and Notch4, in addition to the venous identity markers Eph-B4 and COUP-TFII (dual arterial-venous identity) [60]. These data suggest that synthetic patches heal by acquisition of identity of their environment.

Revascularization of Ischemic Tissues

Some arterial-specific markers are also involved in angiogenesis. It was observed that collateral blood flow was reduced after femoral artery occlusion in 12-week-old transgenic mice with induced EC-specific expression of mutants of the arterial identity marker gap junction alpha 5 (GJA5). These GJA5 mutants had fewer and smaller collateral arterioles, which well accounted for the perfusion deficit. Furthermore, a reduced outward remodeling response was also observed in mesenteric arteries in these GJA5 mutant mice when chronically exposed to increased blood flow, implying that GJA5 might be involved as a positive modulator in adaptation to flow. The reduced number of preexisting collateral arterioles further suggests that GJA5 might play an important role in the formation of native collaterals [61]. Other effective therapies involving clarification of vascular identity for treating peripheral arterial disease have also been developed or are in development [62, 63].

Tumor Blood Vessels

Tumor blood vessels also express Eph-B4 and ephrin-B2, which are indeed associated with mammary gland carcinogenesis and tumor progression. Increasing evidence implicates Eph/ephrin interactions do occur in cancer. It has been demonstrated that tumor angiogenesis and growth were reduced by the use of a neutralizing protein, the soluble form of Eph-B4 [54], and retinal neovascularization was similarly reduced by either soluble Eph-B4 or soluble ephrin-B2 [64]. Moreover, the induction of ephrin-B2 expression is observed during development of Kaposi sarcoma and hepatocarcinoma [50]. Vascular tumors, hemangioma and angiosarcoma, originating from endothelium are closely related to the signaling pathways of involved vascular identity markers. Expression of Tie2, JAG1, Notch4, and Eph-B3 is increased in proliferating hemangioma. The potential role of Eph-B4 in the patho-

genesis of angiosarcoma has been suggested by high level of membranous Eph-B4 [65, 66]. These evidences prove the potential of targeting vascular identity markers for treating cancer and pathologic angiogenesis.

PAH

The involvement of Notch receptor in controlling proliferation of SMCs and maintaining their undifferentiated state has been identified. Research indicates that Notch3 is a marker for PAH disease severity. Overexpression of Notch3 in small pulmonary artery SMCs has been shown in human pulmonary hypertension. Moreover, the abundance of Notch3 protein in the lung is correlated with the severity of PAH both in human and in rodent models of hypoxia-induced or monocrotaline-induced PAH. Results further show that Notch3 is primarily involved in pulmonary vascular remodeling rather than in affecting pulmonary vasoreactivity [51]. As compared to placebo treatment, hypoxic PAH mice with pharmacological Notch3 inhibition presented significant reductions in right ventricular systolic pressures, normal-appearing pulmonary vessels with rarely detected medial thickening or vessel occlusion, less proliferating SMCs but increased apoptotic cells in the remodeled walls of small pulmonary arteries, and regression of right ventricular hypertrophy. Angiograms demonstrated that PAH mice with pharmacological Notch3 inhibition showed a patent distal pulmonary vascular tree, whereas control PAH mice showed blunting of the pulmonary vasculature with the absence of peripheral artery filling. Furthermore, it was shown that mice with homozygous deletion of Notch3 did not develop PAH in response to hypoxic stimulation [51].

Other Vascular Pathophysiologic Conditions

Vascular identity markers have also been found to be involved in other vascular physiopathologic conditions, such as hypertension (RGS) [67], vascular phenotypic remodeling in hyperhomocysteinemia (ephrin-B2 and Eph-B4) [68], brain arteriovenous malformation (VEGF, Notch, ephrin-B2, and Eph-B4) [69, 70], and development and maintenance of the uteroplacental vascular network (ephrin-B2 and Eph-B4) [71].

Summary

Vascular identity markers determine the differential development of arteries and veins. These markers once thought to be quiescent in adults actually are involved in

vascular physiopathologic processes. Manipulation of these potential therapeutic targets might be a novel strategy to improve vascular remodeling in adults.

Acknowledgements

The authors are grateful to Dr. Daisuke Shimura, Dr. Ken Ue-sugi, Dr. Ichige Kajimura, Dr. Eriko Omori, and Dr. Gaku Nakai from the Center for Advanced Biomedical Sciences (TWIns), Waseda University, Tokyo, Japan, and Dr. Yoichiro Kusakari from the Department of Cell Physiology, the Jikei University School of Medicine, Tokyo, Japan, for skillful assistance in experiments.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (S.M.), the Vehicle Racing Commemorative Foundation (S.M.), the Jikei University Graduate Research Fund (S.M.), the Miyata Cardiology Research Promotion Foundation (S.M.), the Natural Science Foundation of China (81670512 to P.L. and 81101042 to F.L.), and the Natural Science Foundation of Hubei Province, China (2016CFB378 to P.L.).

Author Contributions

P.L. prepared the figures; P.L. and F.L. drafted the manuscript; P.L., F.L., Z.Y., Q.-B.J., Q.L., H.-X.W., Y.Y., and S.M. edited the manuscript; P.L., F.L., Z.Y., Q.-B.J., Q.L., H.-X.W., Y.Y., and S.M. approved the final version of the manuscript.

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