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REVIEW PAPER

EphrinB2/EphB4 pathway in postnatal angiogenesis: a potential therapeutic target for ischemic cardiovascular disease

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Abstract Ischemic cardiovascular disease remains one of the leading causes of morbidity and mortality in the world. Proangiogenic therapy appears to be a promising and feasible strategy for the patients with ischemic cardiovascular disease, but the results of preclinical and clinical trials are limited due to the complicated mechanisms of angiogenesis. Facilitating the formation of functional vessels is important in rescuing the ischemic cardiomyocytes. EphrinB2/EphB4, a novel pathway in angiogenesis, plays a critical role in both microvascular growth and neovascular maturation. Hence, investigating the mechanisms of EphrinB2/EphB4 pathway in angiogenesis may contribute to the development of novel therapeutics for ischemic cardiovascular disease. Previous reviews mainly focused on the role of EphrinB2/EphB4 pathway in embryo vascular development, but their role in postnatal angiogenesis in ischemic heart disease has not been fully illustrated. Here, we summarized the current knowledge of EphrinB2/EphB4 in angiogenesis and their interaction with other angiogenic pathways in ischemic cardiovascular disease.

Keywords Ischemic cardiovascular disease · EphrinB2/EphB4 pathway · Angiogenesis · Vessel maturation · VEGF-Dll4/Notch-EphrinB2 cascade

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Introduction

Ischemic cardiovascular disease such as myocardial infarction (MI) is a major cause of morbidity and mortality world widely. Early reperfusion of the occluded coronary arteries potentially improves cardiac function and outcomes by restoring blood supply to the ischemic areas [1, 2]. However, microvascular rarefaction and/or dysfunction prevents efficient reperfusion to the entire myocardium. In this regard, de novo formation of microvessels, namely angiogenesis and arteriogenesis, has the potential to salvage ischemic myocardium at early stages after MI and is also essential for long-term left ventricular remodeling to prevent heart failure [1, 3, 4].

Angiogenesis, the formation of new capillaries from pre-existing blood vessels (Fig. 1a), is essential for transporting oxygen and nutrients to ischemic region and disposing of waste, which has been most extensively studied. Other blood vessel formations, such as vasculogenesis and arteriogenesis (Fig. 1b, c), are also indispensable in physiologic and pathologic neovascularization [5]. In fact, there are at least two different mechanisms of angiogenesis: true sprouting of capillaries from pre-existing vessels termed sprouting angiogenesis and nonsprouting angiogenesis including bridging and intussusceptions [6]. After birth sprouting angiogenesis participates most extensively in vessel formation. In this review, we will focus on the sprouting angiogenesis.

The Ephrin/Eph system, the largest family of tyrosine kinase receptors in mammals, involves in widespread physiologic and pathologic angiogenesis [7]. Among them, EphrinB2 and its receptor EphB4 play a crucial role in the development of the cardiovascular system and contribute to the function of vasculature [8]. Interference with EphrinB2/EphB4 interactions destabilizes the development of

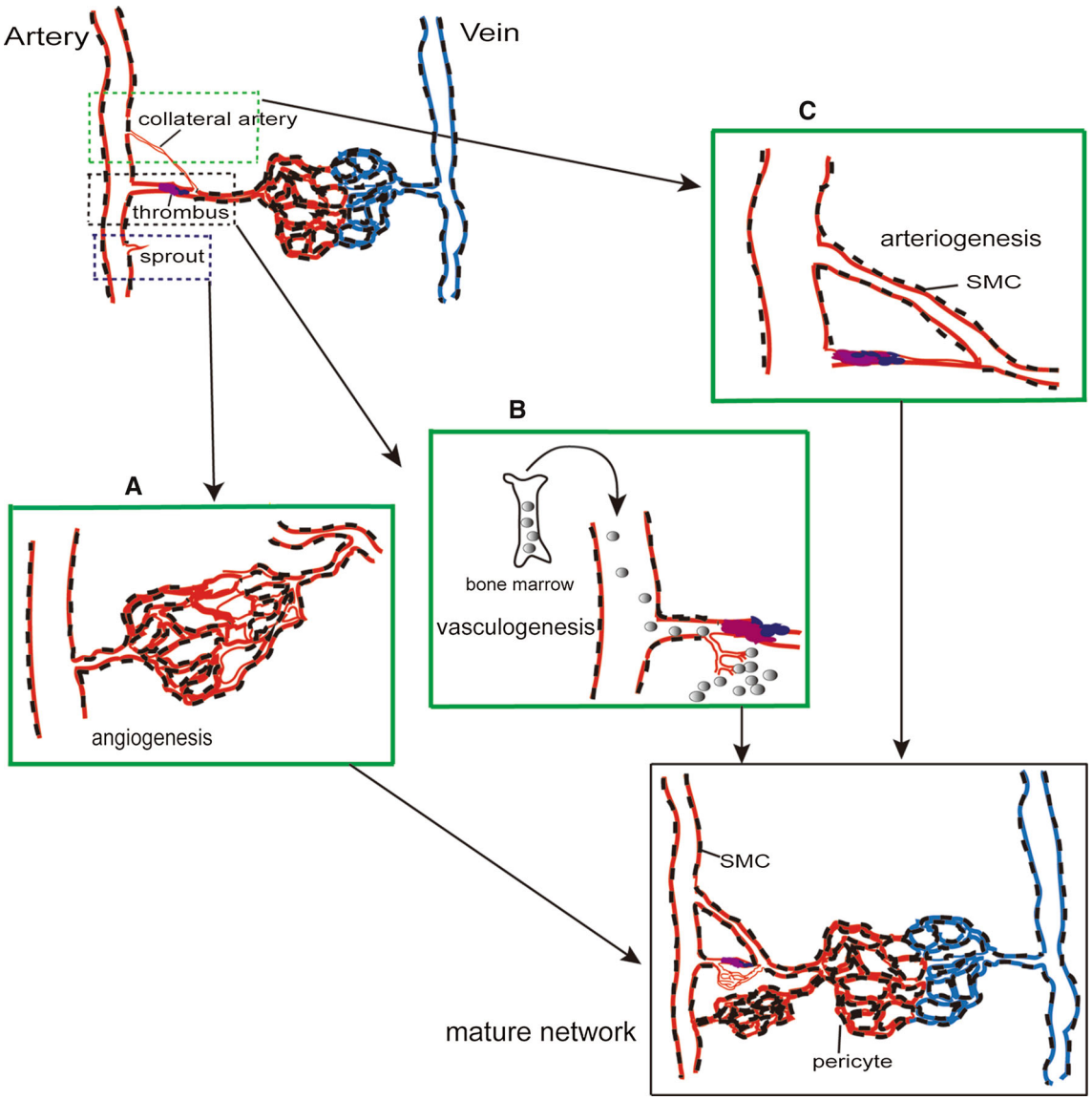

Fig. 1 Schematic overview of the three main ways of neovessel formation. **a** Angiogenesis, new capillaries formation from pre-existing blood vessels by sprouting. **b** Vasculogenesis, new blood vessel formation by endothelial progenitors. **c** Arteriogenesis, the formation of the conduit vessels from small collateral arteries. Ultimately, functional mature vessel networks form to support the ischemic region

capillary network, and deficiency in EphrinB2 or EphB4 displays similar early embryonic lethality due to disorganized vasculature in mice [9–11]. In this review, we will review the role of EphrinB2/EphB4 in postnatal angiogenesis and their potential role in ischemic cardiovascular disease.

Ephrin/Eph family and structural features

The name "Eph" is an acronym from erythropoietin-producing hepatoma where it was found to be highly expressed at first [12], and its ligand Ephrin is short for erythropoietin-producing hepatoma interactor. There are 14 receptors and 8 ligands in mammals. According to their sequence similarity and binding specificities, both the receptors and ligands can be classified into two categories, A and B [13, 14]. In general, A-type receptors only bind A-type ligands, so do B-type receptors and ligands. But there are exceptions, for instance, EphA4 can bind both A-type and most B-type Ephrins. EphB2, besides the EphrinBs, also binds EphrinA5. However, EphB4 has been currently identified as the specific receptor of EphrinB2 [7, 15, 16].

Interaction of the Eph with Ephrin requires cell–cell contact because both the receptor and ligand are

membrane-bound, resulting in bidirectional signaling. Eph-activated signaling is termed "forward," and signaling induced by the Ephrin is named "reverse" [14, 17, 18]. Both EphA and B receptors have similar structures consisting of the extracellular portion, a single transmembrane region and the intracytoplasmic portion [19] (Fig. 2a). The extracellular portion consists of a globular ligand-binding domain; an EGF-like, cysteine-rich region; and two fibronectin-type III repeats. The intracellular portion is composed of a juxtamembrane portion, a tyrosine kinase region, a sterile alpha motif (SAM) domain and a PDZ-binding motif. In contrast to Eph, EphrinA and B have different structures. EphrinA that has an Eph receptor-binding domain in the extracellular portion is tethered to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor, and they have no intracytoplasmic domain to allow signal transmission. EphrinB contains an Eph receptor-binding domain, a transmembrane region, a short cytoplasmic portion with several tyrosine and serine phosphorylation sites and a PDZ-binding motif in the C-terminal [20, 21] (Fig. 2b). While contacting Ephrin on adjacent cells, Eph initiates "forward" signaling by autophosphorylation of several tyrosine residues of intracytoplasmic tyrosine kinase domain. At the same time, the reverse signaling is mediated by the C-terminal region of EphrinB, either through tyrosine phosphorylation by recruitment of other molecules, such as Src family kinases, or a PDZ-dependent way [22].

EphrinB2/EphB4 and angiogenesis

Angiogenesis involves matrix breakdown, endothelial cell (EC) sprouting, branching, pruning, differentiating and recruitment of mural cells, which mainly refers to pericytes and vascular smooth muscle cells (vSMCs), to stabilize the neovasculature, and ultimately establishing a mature circulation system [23, 24]. Postnatal angiogenesis, different from embryonic angiogenesis, participates in numerous pathophysiologic processes, such as ischemic cardiovascular disease, tumorigenesis, wound repair and female reproductive cycle [23, 25].

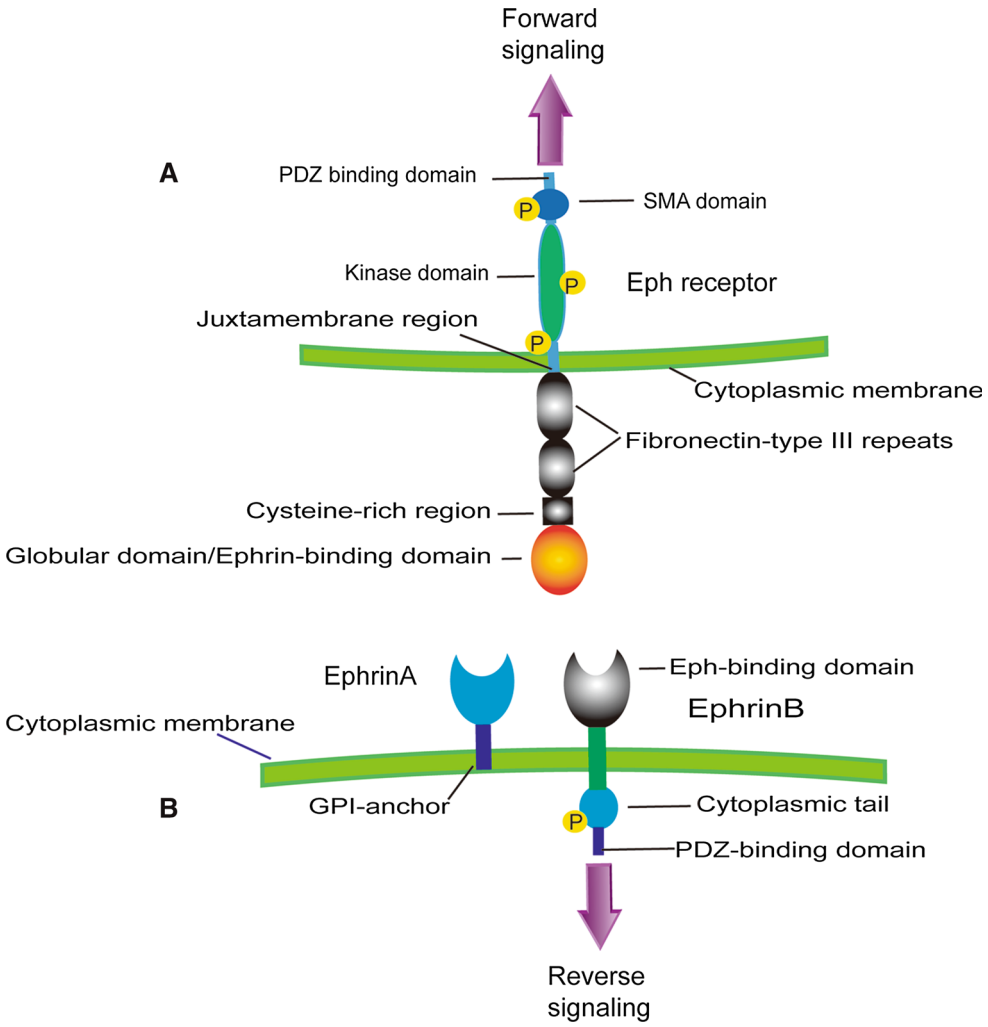

Fig. 2 The structure of Ephrin/Eph family. **a** The Eph receptors include an extracellular portion that consists of a globular, a cysteine-rich EGF-like region, and two fibronectin-type III repeats, and an intracellular portion composed of a juxtamembrane region, a tyrosine kinase domain, a SAM domain and a PDZ-binding motif. **b** EphrinA ligands only have an Eph receptor-binding domain connected to the transmembrane segment via a GPI anchor. EphrinB ligands contain an extracellular Eph receptor-binding domain, a transmembrane region, a short cytoplasmic portion with several tyrosine and serine phosphorylation sites and a PDZ-binding motif in C-terminal. Once engagement with each other, bidirectional signaling is activated with Eph "forward" signaling and EphrinB "reverse" signaling

In the vascular system, both EphrinB2 and EphB4 are expressed on endothelia and mural cells, although each has their own preference, EphrinB2 is mainly on arterial endothelia and mural cells, while EphB4 prefers to venous ECs [26–28]. Compared with classical proangiogenic factors such as VEGF/VEGFR and Ang1/Tie2, EphrinB2/EphB4 appears to have potential advantages: not only promotes sprouting angiogenesis, but also participates in vessel maturation: remodeling and stabilization [29–32].

Sprouting, a coordinating process of endothelial migration and proliferation, involves a large number of molecules, as well as EphrinB2 and EphB4. Stimulation of cultured ECs with soluble dimeric forms of EphrinB and EphB induces forward and reverse signaling to promote sprouting angiogenesis [33]. On one hand, activation of EphB4 forward signaling induces EC migration and proliferation [34, 35]. One study showed EphB4 forward signaling alone could induce sprouting behavior of ECs in vitro [36]. Inhibition of EphB4 forward signaling was sufficient to inhibit VEGF-induced angiogenesis in vivo [37, 38]. On the other hand, stimulation of EC EphrinB2 promotes adhesion, migration, chemotaxis, capillary network formation and sprouting angiogenesis [34, 39, 40]. An important role of EphrinB2 reverse signaling has been shown in sprouting angiogenesis, especially in the regulation of tip cell function [41]. Sawamiphak et al. [42] showed that EphrinB2 clusters localized to tip cell filopodia and their expression was up-regulated by activated VEGFR2, which may be related to the VEGF/VEGFR-Dll4/Notch-EphrinB2 cascade [43, 44]. In a retinal angiogenesis model, EphrinB2 PDZ-signaling-deficient mice (EphrinB2ΔV) exhibited a reduced number of tip cells with fewer filopodia extensions, which indicated that PDZ-dependent reverse signaling of EphrinB2 regulated vessel sprouting by promoting tip cell filopodia extension [45]. Their later work revealed that EphrinB2 at the tip cell filopodia promoted VEGFR2 endocytosis, thereby activating VEGF signaling to direct filopodia extension. The PDZ mutant of EphrinB2 in ECs could not regulate the internalization of VEGFR2, leading to impaired migration or proliferation of ECs due to loss of VEGF responsiveness [42, 45].

EphrinB2 and EphB4 are also important in neovascular remodeling [36]. Prior studies suggested that both EphB and EphrinB may act in a bimodal manner being capable of transmitting both proadhesive and antiadhesive signals [46–49], thus avoiding tanglesome network by restricting intermingling of the vessels [50]. EphB4 can switch the vascularization program from sprouting angiogenesis to circumferential vessel growth, meanwhile reducing the permeability of the vessels [51]. Indeed, forward EphB4 signaling could suppress sprouting angiogenesis by interfering negatively with VEGF and angiopoietin-1 signaling [40, 52, 53]. EphrinB2 reverse signaling was also showed to inhibit endothelial sprouting but promote circumferential growth of vessels. Several studies revealed that reverse EphrinB2 signaling induced low microvascular density but large vessel diameter resulting in tumor progression [51, 54, 55]. Blockade of EphrinB signaling could reduce EC assembly into cordlike structures [31]. In addition, studies also showed that EphrinB2 expression could be up-regulated by shear stress, and this may be related to the differentiation and remodeling of blood vessels induced by shear stress [44, 56, 57]. The bimodal roles of EphB4 and EphrinB2 may be related to the different spatio-temporal conditions of the angiogenesis.

In addition to vessel remodeling, EphrinB2 is also involved in vessel stabilization. EphrinB2 is massively expressed in mural cells that cover arteries and veins during mouse development, playing an important role in pericyte function [58, 59]. In mice with pericyte-specific EphrinB2 deletion, microvasculature is insufficiently covered with pericytes, and capillaries in multiple organs are immature, leading to diffuse tissue edema, hemorrhaging. Furthermore, aberrant collagen was deposited around immature capillaries of skin in these mutant neonates. Interestingly, the EphrinB2-deficient pericytes appeared morphologically normal in many of these mutant mice, but they were only loosely attached, showing a scattered distribution and insufficient contacting with ECs resulting in incomplete vessel coverage. Meanwhile, mutant vSMCs showed attachment defects and discontinuous microvessels covering [58]. A study using EphrinB2ΔV pericytes showed that mutant pericytes had a decreased capacity of stabilizing the capillaries and stimulating synthesis of type IV collagen, a major component of vascular basement membrane, indicating that PDZ-dependent signaling may be associated with the permeability [21]. However, another study showed inconsistent findings, which reported that knock-in mice expressing a PDZ-mutant EphrinB2 were born normally without apparent blood vascular defects, but exhibited marked defects in lymphatic vessel development [60]. The role and mechanism of EphrinB2 in vessel remodeling need to be further studied.

Integrity maintenance among adjacent ECs is also in favor of vascular stabilization. It was shown that the EphrinB2/EphB4 signaling is necessary in the endothelial integrity maintenance through EC/EC interaction [21]. Genetic mouse models have implicated that fine connections were largely absent in cultured EphrinB2 knockout ECs [61].

The downstream signaling of EphB4 and EphrinB2 in angiogenesis

The roles of EphrinB2/EphB4 bidirectional signaling pathways in angiogenesis have been summarized, but little is known about their downstream signals in angiogenesis.

Here we will briefly discuss the downstream cascades of EphB4 receptor and EphrinB2 ligand in angiogenesis, respectively.

Downstream signaling of EphB4 receptor

EphB4-induced EC proliferation is at least in part mediated by PI3K/Akt [35] (Fig. 3a). Inhibition of EphB4 could decrease Akt phosphorylation, thereby inhibiting cell proliferation [62, 63]. Likewise, the blockers of PI3K, Akt, PKG and MEK could inhibit EphB4-induced EC proliferation. However, other main signaling pathways of receptor tyrosine kinases such as Ras, Src and phospholipase Cγ had no effect on the proliferation response [35]. According to the previous studies [64, 65], Akt can specifically phosphorylate endothelial nitric-oxide synthase, which will increase the nitrite production following the stimulation of EphB4, thereby activating cGMP-PKG signaling pathway. Additionally, the signaling downstream of EphB4-PI3K/Akt-eNOs/NO-cGMP/PKG is raf but not Ras-dependent MAPK pathway [35, 66]. Therefore, EphB4-induced proliferation signal may, in part, be mediated through the PI3K/Akt-eNOs/NO-cGMP/PKG-raf/MEK/MAP kinase cascade [35, 67, 68] (Fig. 3a).

Compared with EphB4-induced proliferation, PI3K/Akt-eNOs/NO pathway may also participate in EphB4-mediated EC migration. The signaling downstream of NO in EphB4-mediated migration may be via focal adhesion kinase (FAK) signaling (PI3K/Akt-eNOs/NO-FAK) but not via PKG-MEK pathway [35, 69–73]. Other evidences indicated that PI3K/Akt-NFκB-MMP cascade may also participate in EphB4-mediated EC migration [35, 74, 75] (Fig. 3a). However, in human umbilical vein EC (HUVECs) it has been found that stimulation of EphB4

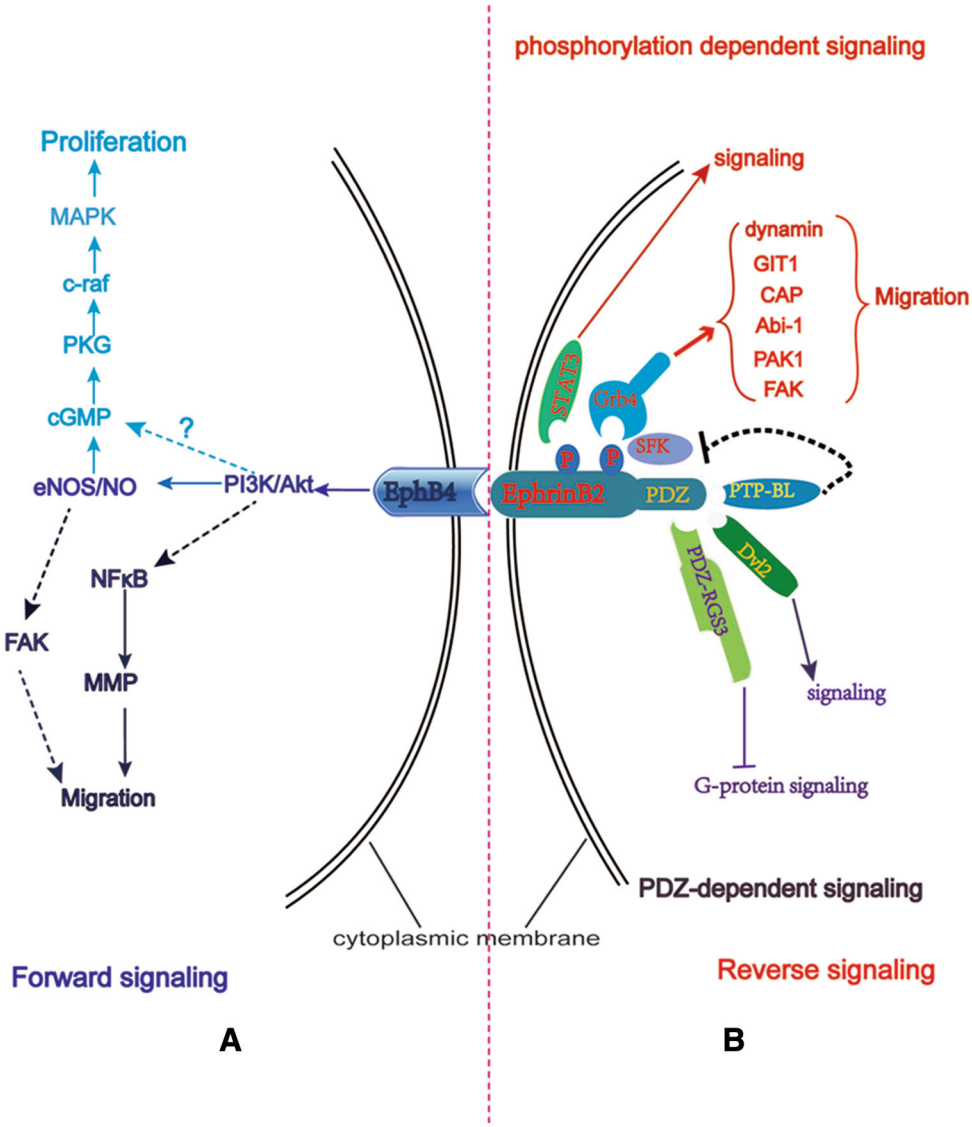

Fig. 3 Schematic representation of the downstream signaling of EphB4 receptor and EphrinB2 ligand. **a** EphB4 forward signaling is mediated mainly by PI3K-Akt pathway, followed by different signaling to induce cell migration and proliferation. **b** EphrinB2 reverse signaling mainly relies on tyrosine phosphorylation and a PDZ-dependent way. On the one hand, with the help of SFK, the phosphorylated-dependent signaling is transduced by binding to Grb4 and STAT3. On the other hand, the PDZ-dependent signaling can be mediated by recruiting PDZ-containing proteins such as PDZ-RGS3, Dvl2 and PTP-BL. PTP-BL may negatively regulate EphrinB phosphorylation via SFK

was unable to stimulate Akt or Erk but acted as a suppressor of Ras/MAPK signaling via the recruitment of p120-RasGAP [52, 71, 76, 77]. The inconsistent results may be context-dependent and related to the critical role of EphrinB2/EphB4 system for proper morphogenesis of capillary endothelium, while neovessels contact with each other.

Downstream signaling of EphrinB2 ligand

Distinct from the forward EphB4 signaling, the EphrinB2-mediated reverse signaling relies on recruiting signaling molecules due to lack of intrinsic catalytic activity. On one hand, the phosphorylation of EphrinB2 is mediated by recruitment of Src family kinases (SFK) [33, 78], followed by binding of the SH2/SH3 domain-containing adaptor proteins such as Grb4 and STAT3 [79–81] (Fig. 3b). Grb4 induces a variety of cytoskeleton regulation signaling such as FAK, G-protein-coupled receptor kinase-interacting protein (GIT) 1, dynamin, Cbl-associated protein (CAP/ponsin), the Abl-interacting protein (Abi-1) and p21-activated kinase (PAK1), thereby mediating migration of ECs [80, 81] (Fig. 3b). STAT3 protein transduces EphrinB signaling from the cell membrane to the nucleus, which was found to be involved in the extracellular matrix-mediated assembly of ECs and pericytes induced by EphrinB reverse signaling [78, 82] (Fig. 3b). On the other hand, the PDZ-containing proteins can be recruited to the PDZ-binding motif of EphrinB2 to mediate the phosphorylation-independent signaling, which is rather important in both angiogenic sprouting and neovascular stabilization as aforementioned [21, 42]. Among PDZ-containing proteins, the regulator of G-protein-signaling (PDZ-RGS3) and disheveled-2 (Dvl2) may be related to PDZ-dependent EphrinB2 reverse signaling [60, 83–85]. But the downstream effector proteins are obscure at present. The PDZ-RGS3 may partly mediate EphrinB2 reverse signaling by regulating the G-protein-signaling pathway [83, 86] (Fig. 3b). The true mechanisms by which EphrinB2 reverse signaling contributes to angiogenesis require further investigation.

There existed evidences of cross-regulation between phosphorylation and PDZ-dependent EphrinB signaling. For example, phosphotyrosine-dependent signaling of EphrinB firstly occurred via binding to EphB receptor, followed by PDZ-dependent signaling. Additionally, the PDZ-containing protein tyrosine phosphatase PTP-BL was recruited to the activated EphrinB and negatively regulated EphrinB phosphorylation via SFK [33] (Fig. 3b).

Conventionally, EphrinB2 reverse signaling requires cell–cell contact. But some studies also showed that EphrinB2 may have some contact-independent functions, suggesting that reverse signaling can also be triggered in a cell-autonomous receptor-independent fashion [21, 58, 87]. This may be related to the existence and the expression levels of cognate receptors on adjacent cells in different spatio-temporal conditions of the angiogenesis [87]. Gene regulation or cross-talks with other angiogenic molecules may be the potential mechanisms involved. Consistent with the notion, several studies showed that EphrinBs can also become phosphorylated without EphB4 engagement, but interacting with some growth factors receptors, such as FGF, PDGF, EGFR, TIE2 receptor [17, 36, 88–90]. However, more studies will be needed to elucidate the precise mechanisms in this field.

VEGF-Dll4/Notch-EphrinB2 cascade

Vascular endothelial growth factor (VEGF) is one of the most powerful angiogenesis activators [91] and promotes EC expression of EphrinB2, but not its phosphorylation [14, 32, 50]. The Dll4/Notch induced by VEGF [92, 93] is another indispensable pathway in neovascularization [94] and can selectively promote the expression of EphrinB2 [43, 44], suggesting that there may exist a cascade among VEGF–Dll4/Notch-EphrinB2 in angiogenesis [43, 44]. VEGF was reported to induce Notch/Delta-directed specific signaling through the PI3K/Akt pathway, which may be related to the Foxc transcription factors [95]. As reported previously, RBPJ protein is the transcriptional mediator of Notch signaling and may be involved in the expression of EphrinB2 [96]. Therefore, VEGF may induce the expression of EphrinB2 in VEGF/VEGFR-PI3K/Akt-Foxc-Dll4/Notch-RBPJ-EphrinB2 cascade (Fig. 4).

Dll4 blockade and soluble EphrinB2 treatment induced nonproductive angiogenesis, characterized by an increase in vascular density but decrease in tissue perfusion, and the effect was additive to that of VEGF [32]. This phenomenon indicates the VEGF-Dll4-EphrinB2 cascade may play a key role in the remodeling of neovessels but not the proliferation of EC [43] (Fig. 4). Activation of EphB kinases suppressed VEGF-induced proliferation and migration through direct inhibition of the Ras/MAPK signaling cascade in ECs, whereas VEGF-induced Flk (VEGFR2) phosphorylation did not alter [52]. Therefore, Dll4 blockade may induce nonproductive angiogenesis, at least in part, by decreasing the negative effect of EphB4 on VEGF-induced EC proliferation [97, 98] (Fig. 4). In line with this notion, knockdown of EphrinB2 with siRNA mimicked the effect of Dll4 blockade [32]. Likewise, the soluble EphrinB2 suppressed VEGF-induced proliferation, sprouting and migration of cultured ECs [99]. Herein, the VEGF-Dll4/Notch-EphrinB2 cascade may inhibit VEGF-induced angiogenesis through EphB4 forward signaling. However, there were some paradoxical results that soluble EphrinB2

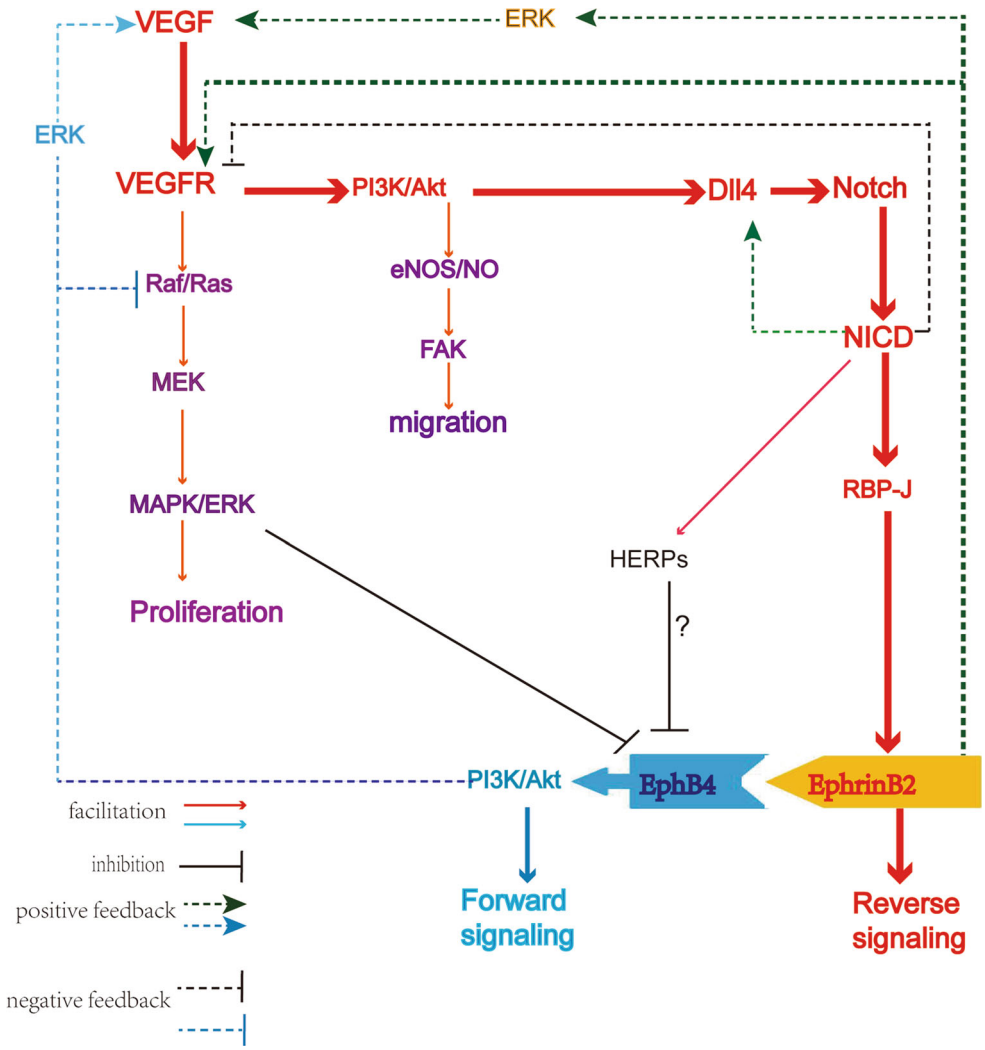

Fig. 4 Proposed pathways relative to VEGF/VEGFR-Dll4/Notch-EphrinB2 cascade. VEGF can not only stimulate EC proliferation and migration directly in different pathways, but also induce the expression of EphrinB2 via VEGF/VEGFR-Dll4/Notch-EphrinB2 cascade, thereby inducing the bidirectional signaling of EphrinB2/EphB4. Also, several feedback loops have been shown in the sketch. The diagram here presented is as simplification of the complex pathways related to the VEGF/VEGFR-Dll4/Notch-EphrinB2 cascade. Moreover, many links between the arrows need to be confirmed

not only could inhibit VEGF-induced angiogenesis, but also induce nonproductive angiogenesis [32], and inhibition of EphB4 forward signaling could inhibit VEGF-induced angiogenesis in vivo sufficiently [37]. Agonists of either EphrinB2 or EphB4 were reported to significantly increase the VEGF mRNA levels in an Erk-dependent way, while respective siRNAs for EphrinB2 and EphB4 inhibiting this increase [100]. This may be the reason for the inconsistent outcomes. The divergence may attribute to the distinct functions of the EphrinB2/EphB4 pathway in specific temporal–spatial conditions, such as different cell types, various microenvironment (in vivo or in vitro), distinct stages (sprouting or remodeling) and divergent systems from different laboratories, or the existence of cross-talk between them such as feedback loop and other relevant pathways.

Different from VEGF-induced proliferation, migration of EC is mediated through PI3K/Akt-eNOS/NO-FAK pathway [69, 93], which may share a common PI3K/Akt pathway with the VEGF-Dll4/Notch-EphrinB2 cascade (Fig. 4). The Ras/MAPK pathway is also involved in VEGF-induced migration [52]. These two pathways of PI3K/Akt and Ras/MAPK/ERK signaling can inhibit each other [101, 102]. This may account for proper remodeling of neovessels through the arrest of EC proliferation and migration.

In addition, there may exist some feedback loops in the cascade (Fig. 4). First, a positive-feedback loop may exist in Dll4/Notch signaling of EC [103]. A negative-feedback loop between Dll4/Notch signaling and VEGF signaling was also discovered [104, 105], indicating that Dll4 may act as a “brake” on VEGF-mediated angiogenic sprouting. This feedback loop may partly participate in Dll4 blockade-induced nonproductive angiogenesis. As an effector downstream of VEGF signaling, EphrinB2 in turn affects the activity or expression of VEGF. EphrinB2 can not only promote the endocytosis of VEGFR [42, 61, 106], but also increase the level of VEGF [100], suggesting a positive-

feedback loop between EphrinB2 and VEGF/VEGFR. Therefore, the up-regulation of VEGFRs induced by inhibition of EphrinB2 signaling may be the result of a compensation for the decrease of VEGF expression or VEGFR endocytosis [107, 108]. Additionally, it was indicated that two evolutionarily conserved binding sites for the RBPJ protein, the transcriptional mediator of Notch signaling, are present in introns 1 and 2 of the *Efnb2* (EphrinB2) gene [96], prompting that the expression of EphrinB2 may in turn affect Notch signaling. Therefore, whether there exist some feedback loops between EphrinB2 and Notch signaling needs further study.

Besides the effect on EphrinB2, VEGF-Dll4/Notch-EphrinB2 cascade also influences the expression of EphB4. VEGF inhibits the expression of EphB4 in adult venous EC directly in a MAPK/ERK-dependent manner [107] (Fig. 4). Notch signaling may exert potent inhibitory effects on EphB4 expression by overexpressing HERPs [103] (Fig. 4). Nevertheless, this effect was not exerted in general [103]. More studies may be required to clarify the mechanisms.

Interestingly, recent studies also showed that Dll4/Notch-EphrinB2 pathway is critically involved in VEGFC/VEGFR3 signaling-induced lymphangiogenesis, which is similar to that involved in VEGF/VEGFR (usually refers to VEGFA/VEGFR2) in angiogenesis. Hence, EphrinB2 may participate in both angiogenesis and lymphangiogenesis, although the structure and function between the blood vessels and lymphatic vasculature are different [60, 61, 109].

EphrinB2/EphB4 and ischemic cardiovascular disease

Angiogenesis is essential for revascularization of ischemic myocardium following infarction. Preliminary clinical study suggests that the therapeutic angiogenesis can provide additional blood flow to the incompletely revascularized areas [110, 111]. EphrinB2/EphB4 pathway may play a pivotal role in modulating the angiogenic process in ischemic cardiovascular disease [19].

Ephrin signaling can be of similar importance as VEGF, and both may function in concert with each other in different cardiac pathologies. EphB4 and EphrinB2 were highly and consistently expressed after 24 h in myocardial infarction, compared with those from the control mice. Furthermore, EC proliferation was increased in the peri-infarcted area post-MI with a tendency at a much greater extent after the EphrinB2-Fc treatment. In noninfarcted mice, treatment with EphrinB2-Fc did not affect the mitotic activity in the myocardium, suggesting that EphrinB2/EphB4 pathway regulates the angiogenesis where new blood vessels are needed, such as the ischemic area, but not affect the normal tissues [112], while the detailed mechanism remains to be uncertain. In cultured human aortic ECs, EphrinB2-Fc induced cell proliferation. In a murine aortic ring angiogenesis model, EphrinB2-Fc was as potent as VEGF in inducing sprout formation [112]. Similarly, in a limb ischemia model, much stronger expression of EphrinB2 was detected in ischemic muscles from growing vessels, compared with that in normal muscles from pre-existing vessels from the contralateral limb [50]. In addition, EphrinB2 and EphB4 levels were higher in the brains of hypoxic-ischemic rats [113], revealing that hypoxia may induce angiogenesis partly by up-regulating EphB4 and EphrinB2 expression, while MI or other ischemic diseases occur [114].

Therefore, specific compounds such as EphrinB2-Fc and EphB4-Fc that can mimic the EphB4 forward and EphrinB2 reverse signaling might be a promising therapy to facilitate angiogenic sprouting in early stage of ischemic cardiovascular disease. At the later stage, sprouting angiogenesis may be turned off to avoid excessive immature vessels when the vessel density is enough. Then, neovascular remodeling begins. The role of EphrinB2/EphB4 signaling pathway should be switched to the negative mode to promote neovascular maturation. The combination with other maturation factors such as Ang1 and PDGF is also beneficial. It is worth noting that overexpression of EphrinB2 prevents appropriate EC assembly [42, 61, 78]. Therefore, optimal EphrinB2 expression level is necessary and the exact dose and time windows require to be further studied.

In addition to promote angiogenesis via interaction between endothelia–endothelia and endothelia–pericytes, Eph/Ephrin system may also participate in inflammatory angiogenesis by inflammatory cells and endothelia interaction [115], which may be partly beneficial to the healing of ischemic cardiovascular disease (Fig. 5). Inflammatory cells, such as neutrophils [116], monocytes/macrophages [117, 118], T lymphocytes [119], express Eph/Ephrin molecules and interact with ECs of the vessels surrounding the ischemic area via the EphrinB2/EphB4 pathway to promote angiogenesis [120] (Fig. 5). As mentioned above, EphrinB2 may also participate in the ischemic cardiovascular disease by inducing lymphangiogenesis, which may be useful for reducing edema and thereby relieving interstitial fibrosis after MI [121–123]. Taken together, EphrinB2/EphB4 pathway may be a potential therapeutic target for ischemic cardiovascular disease by generating functional neovasculature and lymphangiogenesis (Fig. 5). More direct roles of EphrinB2/EphB4 in ischemic cardiovascular disease need to be confirmed.

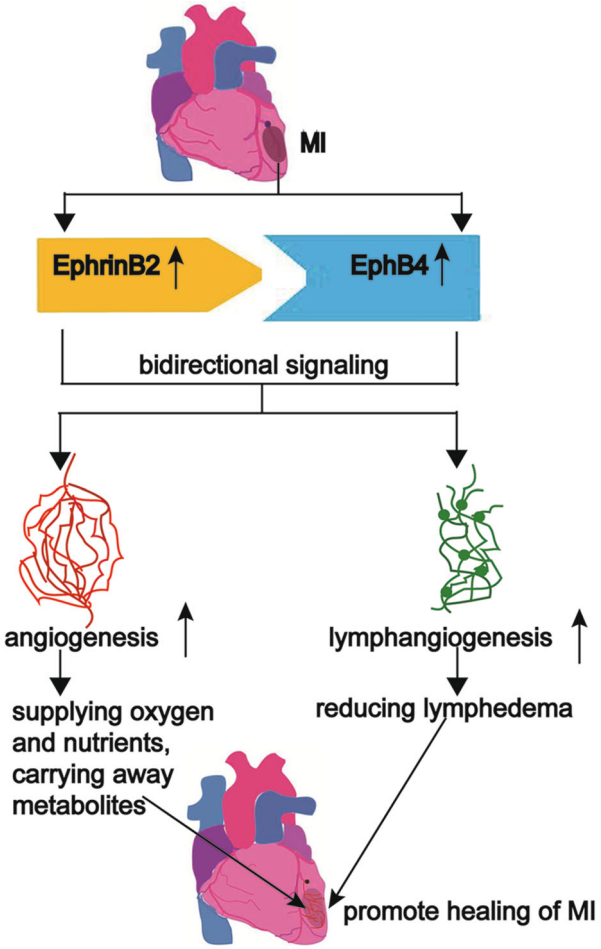

Fig. 5 The role of EphrinB2/EphB4 in ischemic cardiovascular disease. Once myocardial infarction (MI) occurs, ischemia and hypoxia may induce the expression of EprinB2/EphB4 in different cells, such as endothelial cell, pericytes and inflammatory cells, which will induce both angiogenesis and lymphangiogenesis signaling and finally promote the healing of MI

Conclusions and perspectives

In recent years, great strides have been made in studying the signaling network linking EphrinB2/EphB4 to angiogenesis. In this review, we summarized the evidences to support the critical role of the B family of Ephs and Ephrins, especially EphrinB2/EphB4, in postnatal angiogenesis and their potential involvement in ischemic cardiovascular diseases. We outlined the individual roles of EphB4 forward and EphrinB2 reverse signaling and their downstream and upstream cascades in angiogenesis, respectively. Nevertheless, our knowledge remains limited and lots of intriguing questions need to be settled: the role of other subtypes of Ephrin/Eph pairs in angiogenesis; the correlations with other RTK families and other proangiogenic factor; and whether the EphrinB/EphB signaling can modulate the electrical coupling of cardiomyocytes through effects on gap junctions [124].

Further investigation should be focused on the precise mechanisms of temporal–spatial regulation, the potential therapies targeting the genes or the molecules of the Eph family members and the development of specific Eph/Ephrin interfering compounds, such as antibodies, receptor and soluble extracellular domain/Fc chimera, which may provide a novel and promising way to treat angiogenic disorders. And a potential therapeutic medicine should be harmoniously promoting the expression of EphrinB2 and EphB4, not just one of them.

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Compliance with ethical standards

Conflict of interest None declared.

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