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Review

Recent advances of the Ephrin and Eph family in cardiovascular development and pathologies

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SUMMARY

Erythropoietin-producing hepatoma (Eph) receptors, comprising the largest family of receptor tyrosine kinases (RTKs), exert profound influence on diverse biological processes and pathological conditions such as cancer. Interacting with their corresponding ligands, erythropoietin-producing hepatoma receptor interacting proteins (Ephrins), Eph receptors regulate crucial events like embryonic development, tissue boundary formation, and tumor cell survival. In addition to their well-established roles in embryonic development and cancers, emerging evidence highlights the pivotal contribution of the Ephrin/Eph family to cardiovascular physiology and pathology. Studies have elucidated their involvement in cardiovascular development, atherosclerosis, postnatal angiogenesis, and, more recently, cardiac fibrosis and calcification, suggesting a promising avenue for therapeutic interventions in cardiovascular diseases. There remains a need for a comprehensive synthesis of their collective impact in the cardiovascular context. By exploring the intricate interactions between Eph receptors, ephrins, and cardiovascular system, this review aims to provide a holistic understanding of their roles and therapeutic potential in cardiovascular health and diseases.

INTRODUCTION

Erythropoietin-producing hepatoma receptors (Ephs) are the largest known family of receptor tyrosine kinases (RTKs). Their ligands, erythropoietin-producing hepatoma receptor interacting proteins (Ephrins), are classified into two main groups, EphrinA and EphrinB, primarily based on their manner of interaction with the cell membrane. EphrinAs are tethered to the membrane via a glycosyl phosphatidyl inositol (GPI) anchor, whereas EphrinBs are transmembrane (TM) ligands with an intracellular post-synaptic density-95, disks-large and zonula occludens-1 (PDZ)-binding motif. In mammals, Ephs are typically grouped into class A and class B based on their sequence similarity and binding preference for EphrinAs or EphrinBs, with notable exceptions. For instance, EphA4 and EphB2 exhibit the unique ability of binding across subclasses.[1,2]

The ectodomain of Ephs comprises a ligand-binding domain (LBD), two fibronectin (FN) domains, a TM helix, and intracellular regions containing a tyrosine kinase domain, a sterile-α motif (SAM), and a PDZ-binding motif. EphrinAs and EphrinBs have similar extracellular receptor-binding domain (RBD) organization. Additionally, TM EphrinBs possess a TM region and an intracellular PDZ-binding motif.[3–6] It is noteworthy that both Ephrins and Ephs are susceptible for proteolytic cleavage by various enzymes, including matrix metalloproteinases and the blood coagulation complex tissue factor/factor VIIa.[7–10] The activity of cleaved Ephrins and Ephs is predominantly associated with cancer metastasis.[11,12] However, given the presence of proteases responsible for their cleavage on activated endothelial cells (ECs), monocytes, and macrophages, there is emerging evidence suggesting a potential association between cleaved Ephrins/Ephs and atherosclerosis (AS), as well as other cardiovascular diseases (CVDs) (see Figure 1).

Ephs and Ephrins possess dual functionality, acting both as receptors and ligands. This bidirectional signaling enables them to mediate interactions that influence the behavior of both Eph-expressing cells (forward signaling) and Ephrin-expressing cells (reverse signaling).[13] Forward signaling in Eph-expressing cells involves the kinase activity of Ephs, leading to the autophosphorylation of juxtamembrane tyrosine residues and downstream signaling cascades.[14] On the other hand, reverse signaling in Ephrin-expressing cells also relies on the phosphorylation of the cytoplasmic tail of Ephrins,[15] thereby recruiting signaling effectors. Notably, while TM EphrinBs can directly propagate reverse signaling, GPI-anchored EphrinAs rely on other TM proteins to facilitate intracellular signaling.[16,17]

The distribution pattern of Ephs and Ephrins can induce distinct signaling modes. Interactions between Ephs and Ephrins expressed on neighboring cells (in trans) typically activate bidirectional signaling. Conversely, interactions between Ephs and Ephrins coexpressed in the same cell (in cis) often lead to the attenuation of Eph functions and the inhibition of forward signaling.[6,18] Furthermore, interactions

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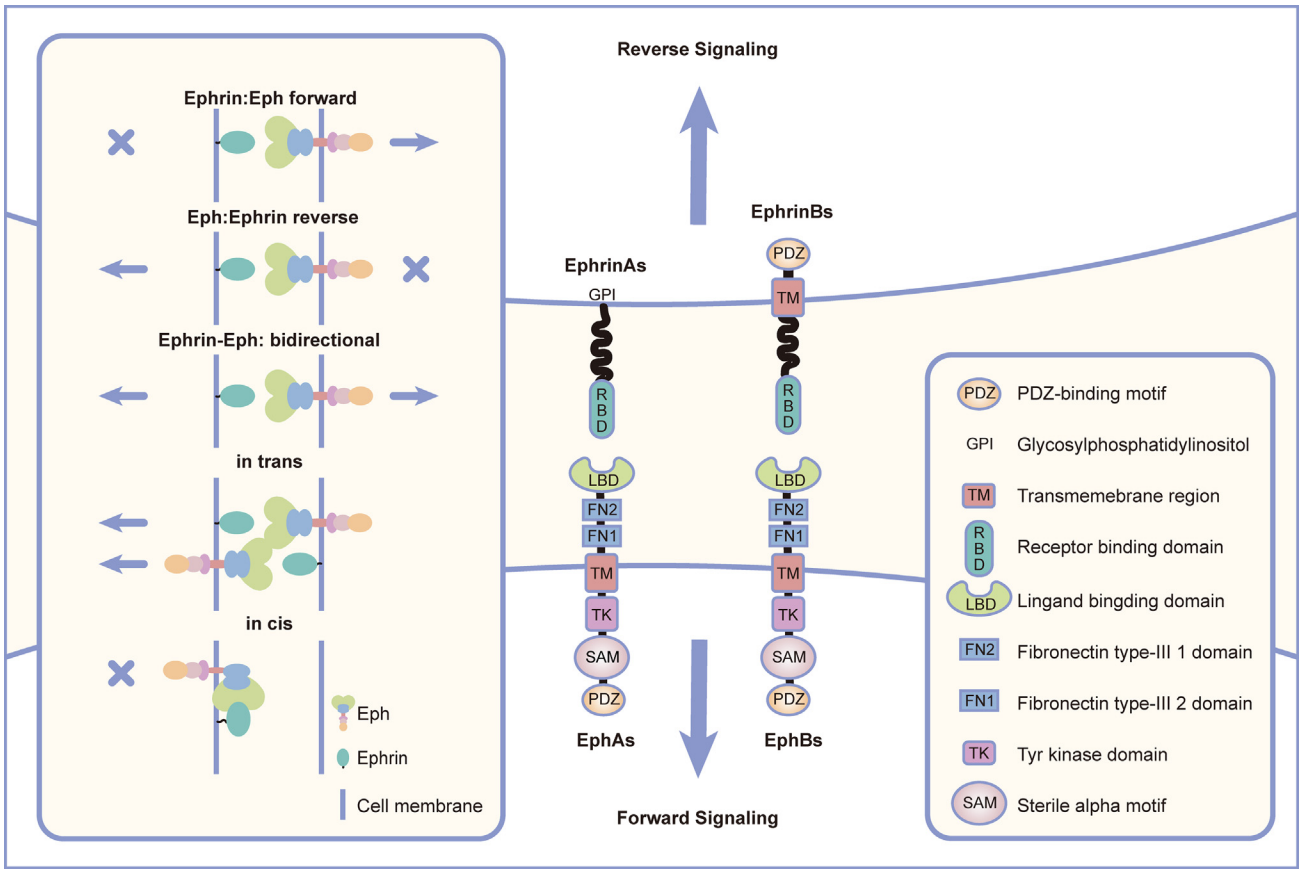

Figure 1. Structures and signal conduction patterns of the Ephrin/Eph family

between neighboring cells can mediate parallel or antiparallel signaling, depending on the relative direction of signaling in each Ephrin-Eph pair.[4]

While bidirectional signaling represents a hallmark feature of Ephs and Ephrins, they also engage in crosstalk with various other pathways. For instance, EphA2 has been identified as a downstream effector of epidermal growth factor (EGF), contributing to cell motility and proliferation.[19,20] In addition to their interactions with each other, Ephs and Ephrins also can interface with cell surface receptors, adhesion molecules, channels, pores, and proteases,[6] forming a complex signaling network and functional system (see Figure 2).

Ephs and Ephrins are involved in a wide range of physiological processes, encompassing embryonic development, neurogenesis, axon guidance, cytoskeletal movement, and tissue boundary formation.[4,18,21–24] Recently, their involvement in CVDs has garnered attention, positioning them as new therapeutic targets for CVDs. In this review, we provide a comprehensive summary of the roles played by Ephs and Ephrins in cardiovascular development and the pathogenesis of CVDs.

EPHRIN/EPH IN CARDIOVASCULAR DEVELOPMENT

Ephs and Ephrins are dispensable for cardiovascular development, orchestrating processes such as embryonic vasculogenesis and angiogenesis. Notably, EphrinB2 and EphB4 are well-known markers of arteries and veins, respectively,[23] and function as key modulators throughout cardiovascular development. During vessel formation, EphrinB2 and EphB4 mediate the repulsion and segregation signals necessary for the organization of ECs.[25,26] For instance, knockdown of *EFNB2* in human umbilical arterial ECs (HUAECs) or *EphB4* in HUVECs resulted in the intermingling of HUAECs and HUVECs, confirming the crucial role of EphrinB2/EphB4 signaling in arteriovenous segregation.[27]

EphrinB2 functions as a proangiogenic effector.[28,29] Research employing EphrinB2 mutants has revealed its pivotal role in angiogenesis, with mutations resulting in defective capillary network formation.[23] In transgenic mice model ectopically expressing EphrinB2, defective recruitment of vascular smooth muscle cells (SMCs) has been observed, leading to sudden neonatal death.[30] Further research has confirmed that EphrinB2 is required for the normal assembly of the blood vessel wall, particularly in the recruitment and organization of SMCs.[31] Moreover, EphrinB2 regulates EC behaviors such as migration and angiogenesis.[26,32] Specific deletion of EphrinB2 in endothelial and endocardial cells phenocopied the embryonic angiogenesis defects observed in conventional EphrinB2 mutants.[33] This highlights the cell-specific role of EphrinB2 in regulating angiogenesis. More specifically, EphrinB2 has emerged as a key regulator of coronary artery development,[34] with the

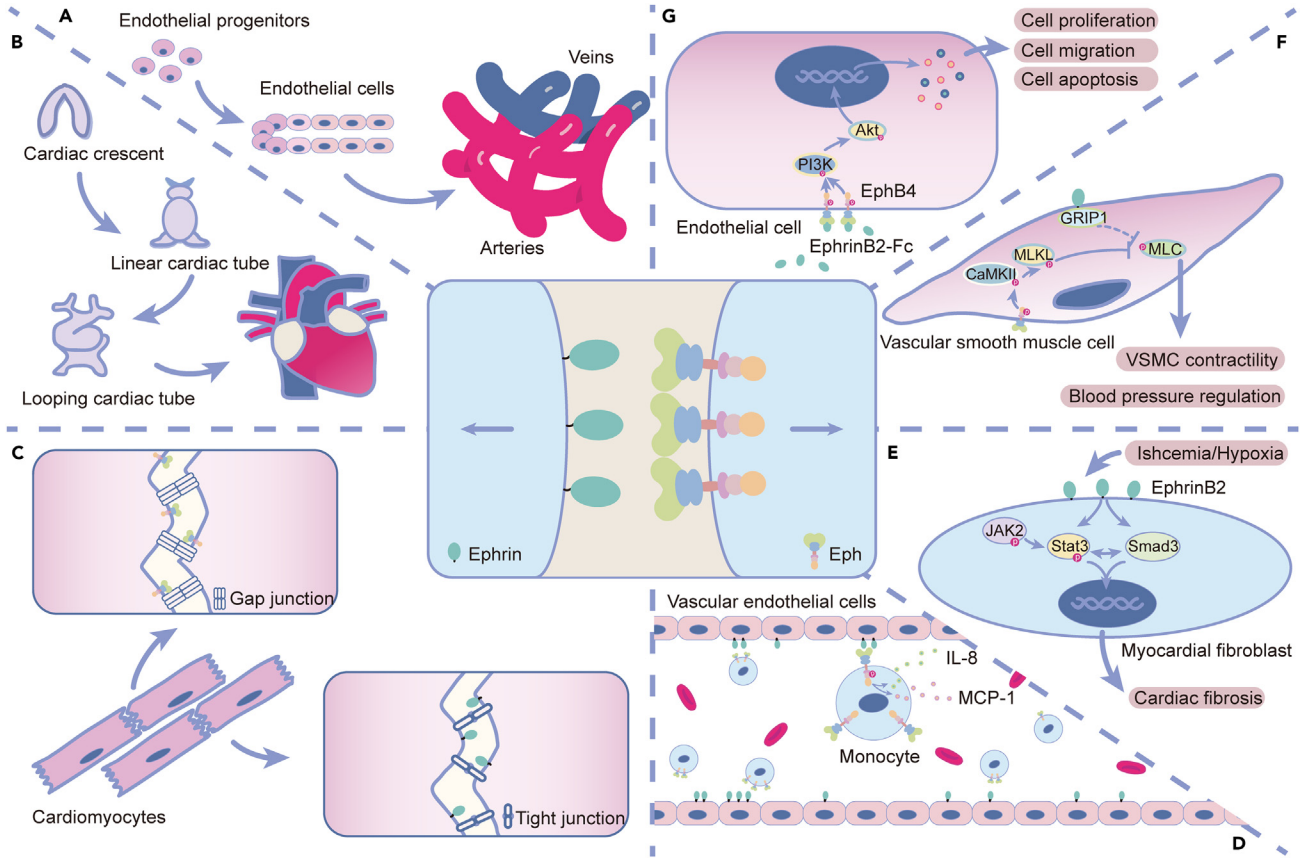

Figure 2. Functions of the Ephrin/Eph family in cardiovascular physiological and pathological status

(A) The involvement of Ephrin/Eph in embryonic vasculogenesis and angiogenesis.

(B) The involvement of Ephrin/Eph in cardiac development.

(C) The distribution of Ephrin/Eph in gap junctions and tight junctions of cardiomyocytes.

(D) The involvement of Ephrin/Eph in monocyte adhesion to vascular endothelial cells.

(E) The involvement of fibroblast EphrinB2 in cardiac fibrosis under ischemic and hypoxic conditions.

(F) The involvement of Ephrin/Eph in blood pressure regulation.

(G) One example of the mechanism by which Ephrin/Eph regulates endothelial cell behaviors.

proangiogenic effect largely relying on its cytoplasmic domain.[35] Recently, EphrinB2 was found downstream of Notch and necessary for proper migration of tip cells to form pre-arterial cells by modulating vascular endothelial growth factor (VEGF)-A signaling, meanwhile early amplification of arterial gene expression and subsequent reduction in the proliferation of HUVECs were observed after knockout (KO) of EphB4.[27]

EphB4 mutants also resulted in defective cardiovascular development and peripheral angiogenesis, mirroring the outcomes observed in EphrinB2 mutants.[24] The EphrinB2-EphB4 interaction was found essential for cardiac lineage development in embryonic stem cells, underscoring the indispensable role of EphB4-forward signaling in cardiomyocyte (CM) development.[36,37] EphB4 expressed on ECs has been verified as a mediator of cardiomyogenesis in embryonic stem cells.[38] EC-specific inactivation of EphB4 was related to capillary rupture, hypertrophic CMs, and pathological cardiac remodeling.[39] Collectively, these studies underscore the crucial role of EphB4 in embryonic cardiac development.

EphrinB2 and EphB4 are involved in venous valve (VV) development and the pathology of VV aplasia.[40] Cardiac valve defects related to early perinatal death were observed in EphrinB2$^{lacZ/lacZ}$ homozygotes, where the cytoplasmic part of EphrinB2 was replaced by βgal,[41] thus verifying the involvement of EphrinB2-reverse signaling in cardiac valve development. Similarly, EphA3-null mice exhibited cardiac abnormalities in atrioventricular valves and septa, accompanied by an increase in perinatal mortality,[42] possibly attributed to the regulation of epithelial-to-mesenchymal transformation (EMT).[42,43] Additionally, EphrinA1 has been implicated in the regulation of EMT and the formation of cardiac valves.[43]

Other members of the Ephrin/Eph family also play crucial roles in cardiovascular development. Double-mutant mice of EphB2 and EphB3 exhibited defects in embryonic vascular development.[44] EphrinB1 and EphrinB2 also showed the ability to induce capillary sprouting.[44] Additionally, EphA2 serves as a regulator of angiogenesis.[45,46] In short, the Ephrin/Eph family contribute significantly to embryonic cardiovascular development.

EPHRIN/EPH IN CARDIOVASCULAR PHYSIOLOGICAL STATUS

EphB receptors are preferentially expressed in CMs, whereas EphrinB ligands are expressed in the vasculature of mouse heart ventricles.[47] This distinct spatial distribution is thought to be related to specific physiological and pathological functions.

Several members of the Ephrin/Eph family have been implicated in the regulation of blood pressure. In humans, *EFNB2* (encoding EphrinB2) is a gene associated with hypertension.[48] Specific deletion of EphrinB2 in vascular SMCs induced hypotension in male mice but not in female mice.[48] Similarly, EphB4 deletion in vascular SMCs led to reduced systolic pressure, mean arterial pressure, and vessel contractility in male mice, but not in female mice.[49] These findings underscore the implication of EphB4-EphrinB2 and sex factors in blood pressure regulation.[48–50] In a chronic hypoxia-induced pulmonary hypertension mice model, either global induction of EphrinB2 KO or pharmacological inhibition of EphB4 exhibited protective effects to a varying extent, whereas SMC-specific EphrinB2 KO did not exert beneficial effects.[51] Specifically, diminished vascular remodeling was only observed in EphrinB2-KO mice, suggesting that this effect was EphrinB2-forward signaling dependent.

Interestingly, EphrinB3-EphB6 exerted opposing effects on maintaining blood pressure by regulating vascular smooth muscle contractility.[50,52] Additionally, EphrinA1-EphA2 signaling is closely related to pulmonary arterial hypertension.[53] According to a genome-wide study, *EPHA6* was identified as one of the sodium-influenced blood pressure loci,[54] indicating its involvement in blood pressure modulation. Although evidence has confirmed the role of Ephrin/Eph family in vasocontraction modulation, the mechanism underlying this modulation of vasocontraction remains incompletely clarified.

EphrinB1 is preferentially expressed on ECs and the lateral membrane of CMs.[55] In adult atrial fibroblasts, EphrinB1 promotes the maturation of human induce pluripotent stem cell-derived atrial CMs,[56] underscoring its significance in normal CM development. General and CM-specific EphrinB1-KO adult mice exhibit disorganization and abnormal morphology of CMs, and the role of EphrinB1 in maintaining postnatal cardiac tissue architecture cohesion is realized, at least in part, by the tight junction component claudin-5.[55] Further research has emphasized the implication of EphrinB1 in postnatal CM crest maturation and cardiac function. Impaired ventricular diastole was observed in young adult CM-specific EphrinB1-KO mice, progressively to systolic heart failure and 100% mortality.[57]

The Ephrin/Eph family plays a crucial role in modulating EC behaviors, including migration and proliferation. EphrinB2 is closely related to the motility and morphology of ECs.[26] Stimulating EphB4 with EphrinB2-Fc promotes the migration and proliferation of ECs via the PI3K pathway.[58–61] Additionally, EphB4 maintains other functions of ECs, encompassing cell integrity, lipid transport, and angiogenesis.[39,62] EphrinA1 mediates the migration and angiogenesis of HUVECs via the activation of ERK-1/2 and its receptor EPHA2,[63,64] while inhibiting the proliferation of ECs.[65] EphA2 plays a critical role in EC migration and assembly and acts as a regulator of postnatal angiogenesis.[45] In normal epithelial cells, EphA2 phosphorylates the tyr-208 residue of claudin-4, a component of tight junctions, thereby mediating paracellular permeability.[66] In a cecal ligation puncture-induced sepsis mice model, KO of EphA4 reduced vascular leak, lung injury, and endothelial dysfunction; administration of a pan-Ephrin inhibitor, EphA4-Fc, also exhibited similar protective effect.[67] This protective effect of EphA4 KO was also observed in maintaining blood-brain barrier integrity after traumatic brain injury.[68] Additionally, alterations in serum levels of EphA2/EphirnA1 were found correlated with endothelial and organ dysfunction in children with fever and infection.[67] In the gastrocnemius muscle after hindlimb ischemia, *EphA4* expression was upregulated.[69] Evidence has demonstrated that inhibiting EphA4 could effectively reduce angiogenesis.[70,71] EphA7 also mediates the migration of HUVECs.[72] Recently, a subset of EphA7^{+} multipotent capillary-resident pericytes was identified, exhibiting high angiogenic and regenerative capacities.[73]

The involvement of Ephrin/Eph family in fibroblast activation and fibrosis has been confirmed in various injuries or diseases affecting the lung, kidney, skin, brain, and spinal cord.[74–79] In ischemic/perfusion-injured kidneys of EphB2$^{-/-}$ mice, multiple fibrosis-related pathways were strikingly downregulated, accompanied by reduced expression of fibrotic markers.[79] Additionally, the soluble ectodomain of EphrinB2 was shed from fibroblasts and mediated lung fibrosis through EphB3 and/or EphB4 signaling.[75] Conversely, evidence suggested that PDZ motif-dependent EphrinB2-reverse signaling in kidney pericytes protects against fibrosis.[76] However, little is known regarding the role of Ephrin/Eph in cardiac fibrosis after myocardial infarction (MI). Our group found that EphrinB2 was highly expressed in advanced failing human hearts and the infarcted/hypertrophic myocardium of mice. Knockdown of EphrinB2 ameliorated cardiac fibrosis and preserved cardiac function in mice after MI. By modulating the interaction between Stat3 and transforming growth factor β (TGF-β)/Smad3 signaling pathways, the profibrotic EphrinB2 regulates myofibroblast activation and modulates cardiac fibrosis after MI.[80] Intramyocardial injection of EphrinA1-Fc decreased cardiac fibrosis in wild-type (WT) mice and EphA2 receptor-null mice four weeks after MI, while poorer cardiac function indices were observed in EphA2-receptor-null mice,[81] indicating that EphrinA1-Fc ameliorated cardiac fibrosis after MI through other EphA receptors or potentially EphA-independent signaling. Interestingly, in another EphA2-mutant mouse (EphA2-R-M) model of MI with hyperglycemia, diminished cardiac fibrosis was observed compared to that in WT mice after MI, while this antifibrotic effect was not observed in normoglycemic EphA2-R-M mice.[82] Recently, EphrinB2 was demonstrated to drive the osteogenic differentiation of adult cardiac fibroblasts in a calcium influx-dependent manner, indicating an unrecognized role of EphrinB2 in regulating cardiac calcification through Ca^{2+}-related signaling.[83]

The Ephrin/Eph family also modulates inflammation. In mouse models of MI, intramyocardial injection of EphrinA1-Fc exerted a cardioprotective effect, reducing the recruitment of neutrophils and leukocytes through decreased chemoattractants.[84] Endothelial EphrinA1 binding to Ephs on CD4^{+} T lymphocytes activates chemotaxis of T lymphocytes.[85] Additionally, EphrinA1 mediates tumor necrosis factor alpha (TNF-α)-induced monocyte adhesion to ECs by regulating the surface presentation of adhesion receptors of VCAM-1 and ICAM-1, without influencing their expression levels.[86] In human atherosclerotic plaques, EphrinB1 and EphB2 were found markedly upregulated on macrophages and T lymphocytes, with their extracellular domains exhibiting inhibitory effects on monocyte migration.[87] However, stimulating

monocytes with EphrinB2 promoted the release of MCP-1 and interleukin-8, indicating that the interaction between endothelial EphrinB2 and monocyte EphB receptors promoted the proinflammatory phenotype of monocytes.[88] EphA2 was found to be phosphorylated and required for thrombin-induced ICAM-1 expression via nuclear factor κB (NF-κB) in HUVECs.[89] Similarly, EphA2 was also required for the expression of another adhesion receptor, VCAM-1, via nuclear factor of activated T cells (NFAT) activation in human aortic ECs.[90] Deletion of EphA2 in macrophage attenuated macrophage infiltration, accompanied by reduced expression of proinflammatory cytokines and chemokines in AS.[91–93] EphA4 acts as a key regulator of monocyte phenotype transition in brain injury. The absence of bone marrow-derived EphA4 exerted neuroprotection and significantly reduction in monocyte/macrophage infiltration, accompanied by a shift in monocytes from a proinflammatory to anti-inflammatory phenotype.[94]

EPHRIN/EPH IN CVDs

Previous studies have addressed the roles of the Ephrin/Eph family in CVDs, including AS, ischemic cardiac disease, cardiac hypertrophy, arteriovenous malformation, and hindlimb ischemia (see Table 1).

Coronary artery disease

AS

EphA2, EphA8, and EphB2 are located on murine chromosome 4 and the human chromosomal region 1p34-36. Through genome scan, Athsq1 (chromosomes 4) was identified as a candidate susceptibility locus for AS in mice, with high homology with chromosomal 1p32-36 in human.[113] Similarly, the human chromosomal region 1p34-36 was confirmed as a susceptibility locus for MI.[114] These pieces of evidence suggest a potential association between Ephs and AS and MI. In human atherosclerotic plaques, EphrinB and EphB2 were significantly upregulated, with localization on plaque macrophages, T cells, and ECs.[87,115] Moreover, EphrinB1 and EphB2 showed ability to inhibit spontaneous and cytokine-dependent migration of monocytes,[87] which is the basic and initial pathological process in AS. Additionally, EphrinB-EphB interactions were involved in monocyte adhesion to ECs.[112,115] *Efnb2* expression was found upregulated in human coronary artery ECs under athero-prone conditions,[116] consistent with high spatial synchronization at the AS-prone site of the aortic arch in mice.[88] EphrinB2 expressed on ECs functions as a chemoattractant and regulates the expression of MCP-1 and IL-8, as well as monocyte adhesion and activation.[88]

The interaction between EphrinA and EphA also promotes the adhesion of monocytes to ECs. EphrinA1 and EphA2 were markedly upregulated in the endothelial layer in human and mouse atherosclerotic plaques and human aortic ECs stimulated with oxidized low-density lipoprotein.[93] Additionally, EphrinA and EphA were found upregulated during monocyte maturation and differentiation; and the adhesion ability of HL60 monocytes was promoted after stimulation by EphA and EphrinA1.[117] EphrinA1 regulates the adhesion of monocytes to ECs, showing its strong relationship with the pathology of AS.[86,101] EphA2 is consistently upregulated in the aortic arches of two mouse models of AS.[118] Knockdown of EphA2 in ApoE$^{-/-}$ mice reduced the formation of atherosclerotic lesions and inflammation,[92] potentially related to the attenuation of endothelial EphA2-mediated activation of monocyte adhesion and proinflammatory genes expression.[93] As a widely used drug for plaque stabilization, statins showed the effect on modulating inflammation on the vascular wall. However, evidence indicates that atorvastatin exacerbates macrophage inflammation by promoting proinflammation genes expression through EphA2.[97] EphA2 expressed on ECs and SMCs mediates atherosclerotic inflammation and the progression toward advanced AS, while macrophage EphA2 showed no effect on this process.[91] The p38 mitogen-activated protein kinase/VEGF pathway might be involved in this EphA2-modulated pathological processes.[119] High circulating levels of EphA2 were detected in patients with acute coronary syndrome and were negatively correlated with the left ventricular ejection fraction, suggesting that EphA2 could be used as a predictor and therapeutic target for CVDs.[96,120,121]

MI

Previous research has shown that EphrinA1 protects against MI. The administration of EphrinA1-Fc significantly exacerbated cardiac dysfunction and remodeling after MI in EphA2 receptor-null mice.[81] Conversely, EphrinA1-Fc injection exerted cardioprotection in WT mouse hearts after MI and ischemic/reperfusion (I/R) injury.[81,84,122] These findings strongly demonstrated the pivotal role of EphrinA1-EphA2 signaling, especially the EphA2-forward signaling, in heart injury and repair. This cardioprotective effect of EphrinA1-Fc is, at least in part, associated with the preservation of CM structure and mitochondrial bioenergetics.[123] Consistently, EphA2-mutant mice exhibited poorer cardiac function and aggravated cardiac injury, irrespective of whether the infarcted heart was hyperglycemic or not.[82,95] Evidence suggests that the cardioprotective effect of EphA2 might be mediated through the PI3K/Akt pathway.[98] However, the effect of EphA2 on the CVDs remains somewhat ambiguous. As mentioned previously, while EphA2 on ECs and SMCs promotes the pathological process of AS, it seems that EphA2 confers protection in MI. In AS, EphA2 on ECs and SMCs enhances inflammation infiltration and SMC phenotype transition, while the protective effect of EphA2 on MI is still unclear concerning its functional cells (potentially CMs). This inconsistency may stem from the diversity of functional cell types and signal transduction patterns (forward or reverse signaling) as well as downstream effectors. Thus, more work is warranted to elucidate its role.

EphrinB2 was markedly elevated in ischemic hindlimbs and infarct zones of MI, suggesting its involvement after ischemic injury.[28,80] Previous research has revealed that EphrinB2 regulates angiogenesis and limits fibrosis after kidney injury.[76] However, our group found that EphrinB2 promotes cardiac fibrosis after MI, and this profibrotic effect is conducted by modulating the interaction of Stats and

Table 1. Research on the role of Ephrin/Eph in cardiovascular diseases

Ephrins/Ephs	Functions	Mechanisms	Models	Reference
EphrinA1/EphA2	Angiogenesis	EphA2 deletion decreased capillary density after MI.	EphA2-mutant mice	DuSablon et al.,[82]; O'Neal et al.[95]
		EphA2 deletion decreased angiogenesis and migration of ECs.	HUVECs and EphA2-deficient mice	Brantley-Sieders et al.[45]; Tian et al.[96]
	Inflammation	EphA2 deletion enhanced inflammation infiltration after MI.	EphA2-R-mutant mice	DuSablon et al.,[82]; O'Neal et al.[95]
		EphA2 activation promoted proinflammation gene expression in ECs stimulated with EphrinA1-Fc.	HAECs	Funk et al.[93]
		EphA2 promoted ICAM-1 expression in ECs induced by thrombin.	HUVECs	Chan et al.[89]
		EphA2 deletion reduced inflammation and proinflammatory genes expression in macrophages after AS.	$EphA2^{-/-}Apoe^{-/-}$ mice	Finney et al.,[91]; Jiang et al.,[92]; Zeng et al.[97]
		Deletion of EphA2 or EphA4-Fc reduced vascular leak and proinflammatory cytokines in sepsis	EphA2-KO mice	Khan et al.[67]
	SMC phenotype	EphA2 deletion reduced SMC proliferation in AS.	$EphA2^{-/-}Apoe^{-/-}$ mice	Finney et al.[91]
	Fibrosis	EphrinA1-FC significantly reduced fibrosis in mouse hearts after MI.	Mice	Whitehurst et al.[81]
		EphA2 deletion reduced interstitial fibrosis after hyperglycemic MI.	EphA2-receptor-mutant mice	DuSablon et al.[82]; O'Neal et al.[95]
		EphA2 deletion increased interstitial fibrosis after MI.		
	CM survival	The EphA2 agonist, Doxazosin, protected against CM injury after MI, with its antagonist LCA acting oppositely.	Langendroff perfused isolated rat hearts	Kaur et al.[98]
		Reduced EphA2 phosphorylation by LCA protected against apoptosis of CMs.	HL-1 cells	Jehle et al.[99]
EphA3	Heart development	Deletion of EphA3 caused defects in atrioventricular valves and septa.	EphA3-knockout mice	Stephen et al.[42]
	Angiogenesis	miR-210 modulates the proangiogenic cell functions in hindlimb ischemia through EphA3.	hindlimb ischemia mice model	Besnier et al.[100]

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Table 1. *Continued*

Ephrins/Ephs	Functions	Mechanisms	Models	Reference
EphrinA1-EphA4	MC adhesion to ECs	EphrinA1 and EphA4 promoted TNF-α-mediated monocyte adhesion to endothelium.	HUVECs	Ende et al.[86]
		Activation EphA4 with EphrinA1-Fc in multiple ECs promoted MC adhesion to ECs	HUVECs, HUAECs, HAECs, and HCAECs	Jellinghaus et al.[101]
EphrinB1-EphA4/EphB1	Thrombus growth and stability	Platelets EphrinB1-EphA4/EphB1 promoted platelets aggregation and clot retraction.	*In vitro*	Prévost et al.[102]
EphA4	Heart development	Knockout of EphA4 leads to obvious atrial hypertrophy.	EphA4-KO rats	Li et al.[103]
	Vascular integrity	ECs deletion of EphA4 reduced blood-brain barrier permeability after traumatic brain injury	EC-specific EphA4-KO mice	Cash et al.[68]
EphA7	ECs migration	As the target of miR-137, EphA3 potentially regulates miR-137-modulated cell viability and migration of ECs.	HUVECs	Lu et al.[72]
	Angiogenesis potency of pericytes	$EphA7^+$ pericytes improved blood flow recovery after hindlimb ischemic injury.	Transplantation of $EphA7^+$ pericytes in hindlimb ischemia mice	Yoshida et al.[73]
EphB2-EphB4	ECs functions	EphB2 and EphB4 signaling is necessary for EC formation of cordlike structures.	*In vitro*	Salvucci et al.[104]
EphB4	Vascular formation and integrity	Deletion of EphB4 in ECs leads to cardiac capillary rupture in mouse.	EC-specific EphB4-KO mice	Luxán et al.[39]
		EphB4 regulates migration and proliferation of ECs via the PI3K pathway.	Human microvascular endothelial cells	Steinle et al.[59]
		Deregulation of EphB4/RASA1/mTORC1 leads to defective vascular phenotypes of ECs.	Zebrafish	Kawasaki et al.[105]
		Mutations in EphB4 caused CM-AVM.	genome-wide linkage study	Amyere et al.[106]
		Activated EphB4 promoted proangiogenic potency of endothelial progenitor cells after hindlimb ischemia.	Hindlimb ischemia mice model	Foubert et al.[62]
	CM differentiation	EphB4 is required for CM differentiated from embryonic stem cells.	*In vitro*	Chen et al.,[36] Wang et al.,[37] Chen et al.[38]
EphrinB2–EphB4	Arteriovenous identity	EphrinB2-EphB4 signal modulates cell segregation and arteriovenous specification.	*In vivo* and *in vitro*	Füller et al.,[25] Stewen et al.,[27] Groppa et al.[32]
	SMC contractility and vascular remodeling	Deletion of EphB4 exhibited hypotension; EphrinB2-reverse signaling also modulates SMC contractility.	EphB4-KO male mice	Wang et al.[49]

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Table 1. *Continued*

Ephrins/Ephs	Functions	Mechanisms	Models	Reference
		The EphrinB2-EphB4-forward signaling and EphBs-EphrinB2-reverse signaling both regulated blood pressure through SMC contractility modulation.	SMC EphrinB2-KO mouse and vascular SMCs from SMC-specific EphB4-KO mouse	Wang et al.[48]
		EphrinB2 deletion and EphB4 inhibition protected against chronic hypoxia-induced pulmonary hypertension.	Global EphrinB2-KO mice	Crnkovic et al.[51]
	Inflammation	Blockage of EphrinB2-EphB4 signaling reduced immune cells infiltration in transplanted graft.	Rat infrarenal aortic transplant model	Langford et al.[107]
EphB6	Blood pressure regulation	EphB6 depressed contractility of vascular SMCs through EphrinBs-forward signaling.	EphB6-KO mice	Luo et al.[50]
EphrinA1-EphA	ECs functions	EphrinA1-EphA2 signaling promoted migration and angiogenic potency of ECs.	HUVECs	Tang et al.,[63]; Saik et al.[64]
		EphrinA1-EphA2 signaling repressed proliferation of ECs.	HUVECs	Wiedemann et al.[65]
	Vascular permeability	Activation EphAs with EphrinA1-Fc increased vascular permeability.	*In vivo* and *in vitro*	Larson et al.[108]
EphrinA3	ECs functions	EphrinA3 regulated tubulogenesis and chemotaxis of ECs under hypoxia	HUVECs	Fasanaro et al.[109]
		EphrinA3 repressed angiogenesis in ECs under hypoxia	HUVECs	Song et al.[110]
EphrinB1	Heart development	Deletion of EphrinB1 caused cardiac tissue organization and increased mortality under pressure overload.	EphrinB1-KO mice	Genet et al.[55]
		EphrinB1 regulated maturation of CM crest and diastolic function.	CM-specific EphrinB1-KO mice	Karsenty et al.[57]
		Deletion of EphirnB1 on ACF reduced contraction of co-cultured iPSC-aCMs.	*In vitro*	Brown et al.[56]
EphrinB-EphB	Synchronized contraction	EphB activation with EphrinB-Fc inhibited synchronized contraction of cultured CMs through jap junction.	In primary isolated rat CMs	Ishii et al.[47]
EphrinB2	Vasculogenesis and angiogenesis	EphrinB2 promoted neovascularization of venous angiogenesis.	*In vivo* cornea micropocket assay	Hayashi et al.[28]
		EphrinB2-expressing natural killer cells improved angiogenic potency of ECs through endothelial EphB4	*In vitro*	Wolf et al.[29]

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Table 1. *Continued*

Ephrins/Ephs	Functions	Mechanisms	Models	Reference
		EphirnB2 modulates SMC adhesion and migration. Deletion of SMC EphrinB2 led to defects in microvessel assembly.	*In vivo* and *in vitro*	Foo et al.[31]
		Carboxy-terminally truncated EphrinB2 exhibited defects in vasculogenesis and angiogenesis.	Mouse	Adams et al.[35]
	Cardiac remodeling after MI	Endocardial deletion of EphrinB2 led to defects in coronary artery development.	Mouse	Travisano et al.[34]
		EphrinB2-Fc promoted capillary density in the border zone after MI	Mouse	Månsson-Broberg et al.[61]
		Overexpression of EphrinB2 reduced sympathetic hyperinnervation and ventricular arrhythmia through RhoA after MI	Mouse	Wang et al.[111]
	ECs functions	EphrinB2 modulated cell shape and migration of ECs by its PDZ-binding domain.	HUVECs and HUAECs	Bochenek et al.[26]
		EphrinB2-Fc promoted migration and angiogenesis of HUVECs *in vivo* and *in vitro* through the PI3K pathway.	Mouse corneal neovascularization and Matrigel plug assays	Maekawa et al.[58]
		EphrinB2-Fc promoted cell migration and proliferation but suppressed cell apoptosis in HUVECs	*In vitro*	Zheng et al.[60]
EphrinB2-EphB	Vasculogenesis	EphrinB2-OE mice exhibited abnormal vasculogenesis and fetal thoracic aortic dissection.	CAGp-ephrinB2 Tg mice Tie-2p-ephrin-B2 Tg mice	Oike et al.[30]
	MC adhesion to ECs	Endothelial EphrinB2 promoted MCs adhesion to ECs through EphB on MC	*In vitro*	Braun et al.,[88]; Pfaff et al.[112]

ACF, atrial cardiac fibroblast; AS, atherosclerosis; AVM, arteriovenous malformation; CM, cardiomyocyte; ECs, endothelial cells; HAECs, human aortic endothelial cells; HCAECs, human coronary endothelial cells; HUAECs/HUVECs, human umbilical artery/vein endothelial cells; iPSC-aCMs, induced pluripotent stem cell-derived atrial cardiomyocytes; KO, knockout; LCA, lithocholic acid; MC, monocyte; MI, myocardial infarction; SMC, smooth muscle cell.

TGF-β/Smad3 signaling.[80] In murine hearts, EphrinB2-Fc promoted capillary density in periinfarct zones,[61] and this pro-angiogenesis effect is conducted by extracellular domain of EphrinB2, in other words, not the EphrinB2-reverse signaling. As mentioned earlier, EphrinB2-Fc significantly promoted angiogenesis after MI in mouse,[61] possibly through EphB4. The receptor of EphrinB2, EphB4, could potentially serve as a therapeutic target for MI. According to Zhang et al.,[124] intramyocardial injection of EphB4 promoted lymphatic vessel regeneration in mice after MI, accompanied by a markedly increase in VEGFR3 expression in the border zone 3 weeks after MI. Lymphangiogenesis is another crucial factor in cardiac repair after ischemic injury. Due to insufficient lymphangiogenesis, myocardial edema could last for several months after MI. With intramyocardial administration of VEGFR3-selective designer protein, Henri et al.[125] observed improved myocardial fluid balance, reduced inflammation, and fibrosis, consequently leading to improved cardiac function in a murine MI model. Thus, EphB4 holds the potential to facilitate cardiac repair and remodeling after MI by mediating both angiogenesis and lymphangiogenesis, while available evidence points that fibroblast EphrinB2 primarily serves as a profibrotic effector in MI. EphB4 and EphrinB2 might work together at the early stage after MI to protect from cardiac dysfunction and rupture, since moderate fibrosis is needed during this time. After that, overwhelmed EphrinB2 signaling may cause excessive fibrosis leading to heart failing. Double-gene-edited mice could be helpful to figure out the overall effect of the EphrinB2/EphB4 signaling after MI.

The downregulation of EphrinA3 has been associated with the angiogenic capacity of ECs. By downregulating EphrinA3, miR-210 modulates tubulogenesis and chemotaxis in ECs.[100,109] Additionally, extracellular vesicles secreted from miR-210-infected adipose-derived stem cells demonstrate proangiogenic effects on hypoxic ECs by inhibiting EphrinA3.[110] Also, exercise-induced miR-210 has been found protective against cardiac I/R injury. In this context, EphrinA3 emerges as a potential target of miR-210, playing a crucial role in modulating the proliferation and apoptosis of CMs during this process.[126] EphrinA3, emerging as a potential therapeutic target of cardiac ischemic injury, should be examined in further research.

CM structural abnormality

Based on bulk and single-cell transcriptomic analysis, a significant increase in dermatopontin-positive (DPT^+) fibroblasts was confirmed in patients with dilated cardiomyopathy.[127] DPT^+ fibroblasts had strong interactions with ECs through several signaling pathways, including Ephrins-Ephs such as EphrinA5-EphA3, EphrinA1-EphA3/4, and EphrinB2-EphA4. With high expression in the atria of the heart, EphA4 is related to atrial hypertrophy and electrocardiography abnormalities.[103]

As mentioned previously, the interaction between EphrinB1 and claudin-5 has been identified as pivotal in maintaining postnatal cardiac tissue architecture.[55] EphrinB1-KO mice exhibited disorganized cardiac tissue and delayed atrioventricular conduction.[55] In failing human hearts, changes in EphrinB1 localization, but not in protein levels, were observed in parallel with reduced claudin-5 on CMs and ECs.[128] However, the impact of the change in the localization of claudin-5 and EphrinB1 on cardiac function remains unclear.

EphB4 is essential for maintaining normal cardiac structure. EC-specific inactivation of EphB4 in adult mice resulted in an increase in heart weight and CM hypertrophy, accompanied by reduced ejection fraction.[39] Conversely, inactivation of EphrinB2 in ECs, the ligand of EphB4, only induced CM hypertrophy, resulting in a significant increase in the relative CM area.[39] In Acp1-KO mouse, striking resistance to pressure overload-induced cardiac hypertrophy and heart failure was observed, which may be potentially related to Ephrins.[129] Circulating levels of EphB4, together with other biomarkers of cell adhesion, were useful in predicting the clinical outcome of chronic heart failure.[130]

Though indirectly, all these works suggested that the Ephrin-Eph family may have a pivotal role in CM structure maintenance, such as cardiomyopathy or hypertrophy.

Congenital heart defects

Apart from its known roles in cardiac fibrosis and angiogenesis, EphrinB2 has been suspected to be highly associated with congenital heart defects, such as ventricular septal defect. The *EFNB2* gene, located at the terminal region of human chromosome13q, is associated with various syndromes, including abnormalities in cardiac function and morphology.[131,132] Similarly, EphrinA1 and EphA3 have been related to congenital heart defects, especially defects in cardiac valve formation. Frieden and colleagues found that EphrinA1 KO caused congenital heart defects such as significantly thicker aortic and mitral valves in mice, by inhibiting EMT during heart valve morphogenesis, ultimately leading to cardiac function deterioration in adult mice.[43] A new *de novo* heterozygous deletion mutation in the *EFNB1* has been associated with rare arterial septal defect in patients with craniofrontonasal syndrome.[133,134] Among the members of the Eph family, EphB2 has been linked to Noonan syndrome, which frequently presents with cardiac defects.[135] Knockdown of EphA2b (but not EphA2a) attenuated cardiac defects in a zebrafish model of LEOPARD syndrome, which manifested as multiple Lentigines and café-au-lait spots, Electrocardiographic conduction abnormalities, Ocular hypertelorism/Obstructive cardiomyopathy, Pulmonary stenosis, Abnormal genitalia, Retardation of growth, and Deafness.[136,137]

Cardiac arrhythmia

Recent research revealed a significant association between *EPHA3* and mitral valve prolapses with atrial fibrillation.[138] In newborn EphA3-null mice, which died quickly within the first 2 days after birth, bradycardia and first-degree atrioventricular block were observed.[42] Yet, the precise role of EphA3 in the cardiac conducting system remains incompletely clarified. Therefore, researchers should notice the emerging role of EphA3 in cardiac electrophysiological conduction.

The EphrinB/EphB signaling is related to electrical coupling and CM contraction, partly through effects on gap junctions.[47] Data from whole-exome sequencing indicate a potential association between EPHB4 and atrioventricular nodal reentry tachycardia.[139] Stimulating

EphB receptors with EphrinB1-Fc induced a significant time-dependent loss of synchronization contraction between two clusters of CMs, accompanied by reduced intercellular communication via gap junctions.[47] This unsynchronized contraction between neighboring CMs may induce arrhythmia, a common but often lethal complication of MI.

Cardiac sympathetic hyperinnervation is associated with arrhythmia and high mortality after MI. Evidence has shown that overexpression of EphrinB2 inhibits sympathetic sprouting and thus further reduces the incidence of ventricular arrhythmia after MI in mice, in part by activating RhoA.[111] High-throughput sequencing has revealed the upregulation of *EPHA4* and *EPHB2* in the cardiac stromal cells of patients with arrhythmogenic cardiomyopathy, while *EFNB2* was downregulated.[140] This research identified a special cluster of the Ephrin-Eph family through functional network analysis,[140] indicating the involvement of the Ephrin-Eph family in arrhythmogenic cardiomyopathy and cell-to-cell junction cardiomyopathy, characterized by the detachment of myocytes and alterations in intracellular signal transduction.[141]

Arteriovenous malformations

The distribution of the Ephrin/Eph family on ECs exhibited cell type specificity. EphB6 is uniquely expressed on aortic ECs, while EphrinA4 and EphB1 are expressed on valve ECs,[142] alongside two well-known markers of arteries and veins, EphrinB2 and EphB4, respectively.[23] This cell-specific distribution indicates the involvement of Ephrin/Eph in valve formation and a switch between valve and aortic lineages.

It was reported that pathogenic variants in *EPHB4* could lead to capillary malformation-arteriovenous malformation (CM-AVM) syndrome,[143,144] and new variants in *EPHB4* are associated with CM-AVM.[106,145] This vascular malformation could be boiled down to EPHB4/RASA1/mTORC1 signaling in ECs.[105] Recently, in human-derived primary cerebral cavernous malformation (CCM) ECs, an increased ratio of EphirnB2/EphB4 and mutations in both EphrinB2 and EphB4 were identified,[146] showing a possible but indirect link between aberrant EphrinB2/EphB4 signaling and the pathophysiology of CCM.

Typically, EphrinB2 and EphB2 are expressed on arterial ECs, while monocytes express EphB4. Monocytes adhere to ECs and activate into macrophages, which could be the main cause of rejection after transplant. By using EphB4 monomer to block the EphirnB2-EphB4 binding, less inflammation and neointima formation were observed in an infrarenal aortic transplant rat model.[107] Additionally, ectopic expression of EphrinB2 and EphB2 was observed on ECs in venous malformations.[147] This phenomenon might reflect a non-physiologic alteration in malformed veins. The formation of arteriovenous fistula (AVF) is associated with the acquisition of dual arteriovenous identity of EphrinB2 and EphB4. EphrinB2 is associated with the development of dural AVF.[148] Endocardial EphrinB2 deletion induces AVF, manifested with malformations and transmural communications.[34] Notably, EphB4 promotes AVF patency and venous remodeling in a manner dependent on Akt1 and caveolin-1.[149,150] Altogether, EphrinB2-EphB4 signaling might be an important mechanism underlying both congenital and acquired AVF.

Ephrin-Eph interactions are also involved in post-aggregation events in thrombosis,[102,151] suggesting a potential role in common thrombotic diseases such as pulmonary thromboembolism and deep venous thrombosis. EphA4, EphB1, and EphrinB1 are expressed on the surface of platelets. Blockade of EphA4 decreased platelet P_2Y_{12}-induced granule secretion and thrombus formation.[151] Additionally, Blocking the interaction between EphrinB1 and EphA4/B1 attenuated thrombus formation and clot retraction,[102] demonstrating the involvement of Ephrin-Eph interactions in promoting the growth and stability of thrombi. Thrombin can lead to a wide range of cellular and molecular changes in ECs and trigger endothelial inflammation. During this process, phosphorylated EphA2 was proven necessary for thrombin-induced ICAM-1 via NF-κB in HUVECs,[89] thus potentially related with sequential inflammation induced by thrombin.

Pulmonary hypertension

RNA sequencing data provided evidence that *EFNA1* (encoding EphrinA1) functions in idiopathic pulmonary arterial hypertension (IPAH), and further study confirmed that the protective effect of EphrinA1 against IPAH was mediated by the receptor, EphA2.[53] In EphA2 receptor-null mice, VEGF blockade followed by hypoxia caused more severe pulmonary hypertension than in control mice.[53]

In angiotensin II-induced hypertrophic mouse heart, EphrinB2 was markedly upregulated.[80] In a chronic hypoxia-induced pulmonary hypertension model, global inducible EphrinB2-KO mice exhibited reductions in pulmonary vascular remodeling and right ventricular hypertrophy, while SMC-specific EphrinB2 KO did not exert beneficial effects; conversely, pharmacological inhibition of EphB4 reduced right ventricular systolic pressure in mice upon chronic hypoxia exposure.[51] This study suggests that the protective effect of EphrinB2 against chronic hypoxia-induced pulmonary hypertrophy is partially dependent on EphB4. However, the specific cell type, or in other words, the functional site of EphrinB2-EphB4 signaling, remains unclear.

PROMISING INTERVENTIONS TARGETING EPHRIN/EPH FOR CVDs

Promising interventions targeting Ephrin/Eph have been thoroughly elucidated in several reviews.[152–154] Herein, the following discussion addresses the interventions targeting Ephrin/Eph in research on CVDs (see Table 2).

Fusion proteins, comprising the extracellular domain of the protein and the Fc region of human IgG, are the most common interventions used in CVD research. The cardioprotection effect of EphrinA1-Fc has been confirmed in a mouse model of MI.[81,84,122,123] EphrinA1-Fc activates EphA2 on ECs, inducing the expression of VCAM-1 and E-selectin and subsequent monocyte adhesion,[93] thereby confirming the involvement of EphrinA1/EphA2 in AS. EphrinB2-Fc activates multiple signaling pathways in HUVECs, modulating cell proliferation, survival, and migration.[60,61] Additionally, EphrinB1-Fc-induced high levels of EphB desynchronize the contraction of cultured CMs.[47]

Table 2. Promising interventions targeting Ephrin/Eph for CVDs

Type	Drugs	Target	Effects	Reference
Fusion proteins	EphrinA1-Fc	Activation of all EphA receptors	Wildly used as an agonist for EphAs. Exhibited pro-angiogenesis and cardioprotection effects after MI.	Whitehurst et al.,[81]; DuSablon et al.,[84]; Funk et al.,[93]; Dries et al.,[122]; Torres et al.[123]
	EphrinB1-Fc	Activation of EphB receptors	EphrinB1-Fc induced desynchronized contraction of cultured CMs by upregulating EphBs.	Ishii et al.[47]
	EphrinB2-Fc	Activation of EphB receptors	Wildly used as an agonist for EphBs. Exhibited pro-angiogenesis on ECs.	Maekawa et al.,[58]; Zheng et al.,[60]; Protack et al.[149]
	Soluble EphA2-Fc	Inhibiting the activation of multiple EphAs	EphA2-Fc dose-dependently reduced tubule formation of ECs *in vitro*.	Saik et al.[64]
Small-molecule drugs	Lithocholic acid (LCA)	interfering with the interaction of Eph-Ephrin	Pre-treatment of LCA diminished the cardioprotection of post-conditioning against ischemia by inhibiting EphA2.	Kaur et al.[98]
			LCA decreased CM apoptosis by repressing EphA2 phosphorylation.	Jehle et al.[99]
	Doxazosin	EphA2 and EphA4 agonist	Post-conditioning by doxazosin protected against ischemic injury in rat hearts.	Kaur et al.[98]
	ALW-II-41-27	EphA2 tyrosine kinase inhibitor	ALW-II-41-27 reduced macrophages proinflammatory genes expression induced by atorvastatin via inhibiting EphA2.	Zeng et al.[97]
	green tea catechin EGCG	Tyrosine kinase inhibitor	EGCG inhibited EphrinA1-mediated cell migration and angiogenesis *in vitro*.	Tang et al.[63]
Peptides	TYY	Inhibiting the binding of EphA4-Ephrins	TYY binding to EphA4 inhibited EphA4-Ephrins signaling and blocked angiogenesis *in vitro*.	Han et al.[70]
	TNYL-RAW	Inhibiting EphB4 binding with EphrinBs	By inhibiting EphB4, TNYL-RAW impaired CM development in ES cells.	Chen et al.[36]
	SNEW	Inhibiting the binding of EphB2-EphrinBs	SNEW inhibited ECs forming into cordlike structures *in vitro*.	Salvucci et al.[104]
Synthetic biomimetic materials	Polyethylene glycol-immobilized EphrinA1	EphrinA1-Fc conjugated with PEG, agonist for EphAs	PEG-EphrinA1 promoted angiogenic capacity of ECs *in vitro* and *in vivo*.	Saik et al.[64]
	Hydrogel-immobilized EphrinA1	EphrinA1-Fc immobilized on hydrogel, agonist for EphAs	Hydrogel-EphrinA1 dose-dependently stimulated adhesion and tubule formation of ECs.	Moon et al.[155]

CMs, cardiomyocytes; ECs, endothelial cells; MI, myocardial infarction; PEG, polyethylene glycol.

Typical small molecular interventions include lithocholic acid (LCA) and doxazosin. The former is a competitive and reversible ligand for Ephs, which can interfere with Eph-kinase activation.[156] The latter functions as a specific agonist of EphA2 and EphA4.[157] Peptides such as TYY, SNEW, and TNYL-RAW inhibit the binding between Ephs and Ephrin ligands through their higher affinity for certain Ephs.[70,158] New synthetic biomimetic materials, such as hydrogel-immobilized EphrinA1 and polyethylene glycol-conjugated immobilized EphrinA1, have also been used in recent research.[64,155] Cardiovascular diseases imposed a huge burden on our society and economy[159]. Despite many interventions that target Ephrin/Eph, there is a considerable amount of work to be done before real clinical application becomes feasible. Besides, due to the abroad effects and targets of the Ephrin/Eph family, how to make the interventions more precisely is a big challenge.

Conclusion

In this review, we summarized recent advancements in understanding the involvement of the Ephrin/Eph family in cardiovascular development, as well as in physiological function and pathological progression. The Ephrin/Eph family plays essential roles in cardiovascular embryogenesis and postnatal angiogenesis, from vessel formatting and CM development to the maintenance of cell structure and function. Mutations in the Ephrin/Eph family often result in congenital heart diseases. Moreover, available evidence strongly supports the extensive involvement of the Ephrin/Eph family in leading CVDs such as AS and MI, through modulating angiogenesis, inflammation, fibrosis, and cell proliferation and survival via multiple downstream effectors. Despite that, controversies exist. For instance, EphA2 has actually opposite effects on inflammation regulation in MI and AS. Besides, due to the wild range of targets and effects of the Ephrin/Eph family, the application of relevant interventions in CVDs is limited. It would be important to precisely target at the Ephrin/Eph family, for instance, with combination of bioengineering technologies. Yet, there are still many unknowns concerning the function of the Ephrin/Eph family in CVDs. It is a far more intricate network than has been uncovered thus far; thus, improving the understanding of this family and identifying potential therapeutic targets are imperative.

Limitation of the study

In this review, we summarized recent advances in the roles of the Ephrin/Eph family in cardiovascular development and pathologies. While our review may not encompass all relevant studies due to limitations in literature search, it provides a general overview. Some findings may be inadequately introduced or discussed, particularly the ambiguous ones. We recommend more in-depth research and discussions to better clarify the roles of the Ephrin/Eph in CVDs.

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AUTHOR CONTRIBUTIONS

M.X. and Y.X. had the idea for the review. Y.Z. and S.-a.S. performed the literature search and drafted the original manuscript. J.S., H.M., and J.L. critically revised the work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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