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REVIEW ARTICLE

The biological function of ELABELA and APJ signaling in the cardiovascular system and pre-eclampsia

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Abstract

Pre-eclampsia (PE) is a pregnancy-specific syndrome that is characterized by hypertension and proteinuria. The etiology of PE is not completely understood but is believed to involve placental insufficiency and maternal vascular damage. Growing evidence supports an important role for the apelin receptor (APJ) system in regulating cardiovascular physiology. There are two vertebrate APJ ligands, APELIN and ELABELA, both of which mediate vasodilatory functions. A recent study linked deficient ELABELA signaling and the development of PE, though the molecular mechanism remains largely unknown. In this review, we summarize the biological function of the ELABELA and APJ system in cardiovascular homeostasis and discuss the potential mechanisms by which ELABELA and APJ regulate placenta trophoblast invasion and vascular functions and participate in the development of PE.

Keywords Pre-eclampsia · APELIN · ELABELA · APJ signaling

Introduction

Pre-eclampsia (PE) is a pregnancy-specific syndrome of gestational hypertension that complicates 3–5% of pregnancies worldwide [1]. PE is associated with multi-organ damage and is lethal to both gravidas and newborns if improperly addressed. The etiology of PE remains incompletely understood and is probably heterogeneous. Placental dysfunction, including inadequate cytotrophoblast migration and invasion, poor vascular remodeling of the uterine spiral arteries, and placental hypoperfusion, are believed to be central to the development of PE [2]. Placenta hypoperfusion and the associated ischemia–reperfusion (I/R) injury stimulate the placenta to release soluble anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), causing widespread maternal vascular endothelial dysfunction and vasoconstriction through the nitric oxide (NO)- and endothelin-1 dependent pathways [1, 3]. In addition, early abnormal immune activity in the placenta may impact cytotrophoblast migration and invasion, and subsequent chronic systemic inflammation in the mother may also contribute to the genesis of PE [4, 5]. Therefore, PE is likely a multifactorial syndrome caused by alterations in multiple regulatory pathways at different anatomical sites. Understanding the molecular events underlying the pathology is essential for developing preventative and therapeutic approaches for PE.

The Apelin receptor APJ (also known as APLNR or AGTRL1), is a Class A G protein-coupled receptor (GPCR) [6]. APJ receptor signaling is an important regulator of blood pressure, angiogenesis, cardiovascular function, fluid homeostasis, energy metabolism, cell proliferation/migration, apoptosis, oxidative stress, and inflammation [7–10]. A recent study revealed that the APJ system also plays a critical role in the development of PE. Deletion of *Elabela* (also known as *Apela* or *Toddler*), a ligand for the APJ receptor, triggers PE-like symptoms such as hypertension and proteinuria in mouse, and ELABELA administration could relieve these symptoms [11]. However, the specific mechanism through which the APJ system leads to PE is unknown. In this review, we summarize the latest studies on the functions of the APJ system in cardiovascular system

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and discuss the potential mechanism through which the APJ system contributes to the development of PE.

Function of APELIN-APJ signaling in cardiovascular system

Activation of APELIN receptor can inhibit forskolin-stimulated cAMP production, and its activation is sensitive to pertussis toxin, indicating that APJ is coupled to inhibitory G proteins (Gi) [12]. The human APJ protein consists of 380 amino acid residues and is highly conserved among vertebrates [6]. To date, two endogenous ligands, APELIN and ELABELA have been identified; of these ligands, APELIN was the first identified and is the better studied APJ ligand [13]. APELIN is initially produced as a 77-amino-acid preproprotein. After removal of the signal peptide, the 55-residue proapelin may be further cleaved by endopeptidases at several paired basic residues (Arg-Arg and Arg-Lys) to produce a range of C-terminal fragments, including APELIN-36, −17, −13, and the post-translationally modified (Pyr1) APELIN-13. These peptides are all agonists of the APJ receptor but may present different tissue distributions and potencies [9, 14] [15]

Both APELIN and the APJ receptor are widely expressed in the heart, brain, pancreas, lung, liver, kidney and placenta [9, 16, 17], and all are highly expressed in the endothelial cells of various vessels [18–20]. Consistent with their prominent expression in the cardiovascular system, APELIN/APJ has well-documented vasoactive functions [21, 22]. Systemic infusions of APELIN lower blood pressure in humans and rodents [23, 24] and cause vasorelaxation of glomerular arterioles, resulting in increased diuresis [25]. Mechanistically, APELIN stimulates the expression and activity of eNOS, increases endothelial production of NO [18, 25, 26], and antagonizes the vasoconstriction activity of angiotensin II by upregulating angiotensin-converting enzyme 2 (ACE2) expression [27, 28]. In cerebral artery vascular smooth muscle cells, APELIN controls the vascular tone of the cerebral artery by inhibiting large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel via a PI3K-dependent signaling pathway [29].

The APELIN-APJ system also possesses angiogenic potential. In vitro, APELIN treatment stimulates endothelial cells and vascular smooth muscle cells to proliferate via the PI3K-Akt, PKC, ERK and NOTCH signaling pathways [30–33]. In the postmyocardial infarction heart, APELIN enhances the homing of vascular progenitor cells, increases angiogenesis and improves cardiac repair [34], while APELIN deficiency compromises in vivo myocardial angiogenesis [35]. A recent study showed that APJ is highly enriched in tumor blood vessels and that pharmacological blockage of Apelin-APJ signaling inhibits tumor angiogenesis and growth [36].

APELIN is a potent stimulator of cardiac contractility by activating the PKCε and ERK1/2 signaling pathway [37, 38]. Systemic APELIN treatment increases coronary blood flow [23], increases cardiac contractile reserve and improves the hemodynamic profile [39], whereas mouse knockout of *Apelin* or *APJ* leads to impaired cardiac contractility under aging or stress conditions [40]. Furthermore, loss of *Apelin* exacerbates myocardial infarction while APELIN perfusion protects the heart from ischemia–reperfusion injury by suppressing the oxidative damage to the sarcoplasmic reticulum function [35, 41].

ELABELA is a non-redundant ligand for APJ

APJ-null, but not *Apelin*-null, mice display overt congenital cardiac anomalies [40, 42]. Such phenotypic inconsistency was explained by the recent discovery of ELABELA, the second endogenous ligand of APJ [43, 44]. *ELABELA* was initially annotated as a non-coding RNA but was later confirmed to contain a 54–amino-acid open reading frame with a predicted signal sequence [43]. The mature peptide includes ELABELA-32, ELABELA-22 and ELABELA-11 isoforms as a result of differential cleavage, with the shortest isoform 11 being highly conserved across vertebrate species [45]. *ELABELA* mRNA or peptide has been detected in induced pluripotent stem cells (iPSCs), human embryonic stem cells, and mouse embryonic endoderm. In the adults, ELABELA expression is detected in the kidney, prostate, placenta and plasma but not as widely as APELIN and APJ [33, 44, 46]. Like APELIN, ELABELA is also abundantly expressed in the endothelial cells of the heart and various blood vessels [47, 48].

Like APELIN, ELABELA peptide increases cardiac contractility and induces coronary vasodilation by activating ERK in the cardiac tissues, but its mechanism is independent of PKC activation [47, 48]. ELABELA also improves cardiac function by downregulating angiotensin-converting enzyme (ACE) expression in stressed hearts [49]. Injection of ELABELA increases diuresis and water intake in rats [7]. ELABELA is also essential for mouse embryonic angiogenesis [11].

ELABELA also plays a non-redundant role to APELIN. During zebrafish embryogenesis, Elabela is the earliest ligand for APJ before the onset of gastrulation, whereas *apelin* expression begins 5 h later, during gastrulation [44]. Loss of either *Elabela* or *APJ* impair endoderm differentiation and mesodermal cell migration and severely disrupts cardiac development, whereas the *Apelin* knockout has no such dramatic effect [43, 44, 50]. The *Elabela* null, but not *Apelin* null, mouse is associated with PE symptoms [11]. Moreover, ELABELA also functions as an endogenous growth factor that sustains hESC self-renewal via the PI3K/Akt pathway. Interestingly, hESCs do not express

APJ, suggesting that ELABELA may signal through an alternate unknown receptor in supporting stem cell self-renewal [46].

Functions of ELABELA and APJ signaling in the pathophysiology of PE

Ho et al. reported that *Elabela* knockout pregnant mice exhibited PE-like symptom such as kidney glomerular endotheliosis, proteinuria, and hypertension and that these symptoms can be normalized by systemic infusion of recombinant ELABELA peptide, indicating that ELABELA signaling is necessary for regulating maternal-placental vascular homeostasis to prevent PE [11]. A lack of ELABELA causes damages to the placenta and the maternal cardiovascular system, both of which are linked to PE. In the placenta, *Elabela* is predominantly expressed in villous cytotrophoblasts and syncytiotrophoblasts. Loss of *Elabela* expression delays syncytiotrophoblast differentiation, impairs APJ signaling in the neighboring fetal endothelial cells, and disrupts placental angiogenesis. These changes result in an overall pathological change to the placenta, as evidenced by elevated hypoxia and inflammation and a thinner labyrinth. On the maternal side, *Elabela* knockout results in endothelial damage and higher systolic blood pressure, while ELABELA peptide infusion normalizes the blood pressure, consistent with the vasorelaxation function of ELABELA. It is currently unclear whether the development of PE-like symptoms is primarily due to placental defects or maternal vascular dysfunction or both as a result of ELABELA deficiency. It is also unclear through what mechanism does ELABELA regulate trophoblast differentiation and invasion and placenta angiogenesis. As mentioned earlier, ELABELA may have a second receptor in addition to APJ, and this second receptor might also mediate specific anti-PE functions of ELABELA in the placenta.

As discussed earlier, APJ signaling activates the PI3K/Akt pathway and NO production in endothelial cells, promoting vasodilation and angiogenesis. Competent NO signaling has protective effects on placental function and maintenance of vascular tone. In the placenta, eNOS is abundantly expressed in the syncytiotrophoblasts and endothelial cells [51]. The NO level is often decreased in the placenta and plasma of humans with PE [52, 53]; long-term NOS inhibition produces PE-like syndrome in animal models [54], while glyceryl trinitrate-induced release of NO can improve utero-placental perfusion [55], suggesting that abnormal NO signaling may be involved in the pathology of PE. As eNOS activity is regulated by PI3K/Akt signaling [56], which is downregulated in the hypoxic [57] and PE placenta [58, 59], APJ signaling may regulate placental development partly through modulating PI3K/Akt-eNOS activity in the trophoblast or fetal endothelial cells.

Increased reactive oxygen species (ROS) level or ROS-mediated damage has been observed in human PE placenta [60–62]. ROS can scavenge NO, forming the reactive nitrogen species $ONOO^{-\cdot}$ that further induces eNOS uncoupling and compromises NO production and NO-mediated vasorelaxation [63, 64]. Thus, placental oxidative stress has been proposed as an important cause of PE [65, 66]. In cultured adipocytes, APELIN treatment can inhibit the production and release of ROS by modulating the expression of anti-oxidant and pro-oxidant enzymes in an ERK-, AMPK- and Akt-dependent manner [67]. In cultured cardiomyocytes, APELIN treatment can inhibit the generation of ROS and apoptosis following ischemic/reperfusion injury by enhancing superoxide dismutase activity and ERK and Akt signaling [68]. Furthermore, Heme oxygenase-1 (HO-1) is an important enzyme that catabolizes ROS to prevent hypertension [69]. HO-1 expression and HO-1-mediated inhibition of sEng release from the placenta are dependent on Akt [59]. Thus, APJ signaling may play an important protective role in vascular integrity by suppressing oxidative damage in the hypoperfused placenta.

The anti-apoptotic activity of Apelin has been observed in various cell types, including cardiomyocytes, vascular smooth muscle cells, endothelial cells and osteoblasts [70–73], and this effect is induced by the activation of ERK and Akt [74]. In the central nervous system, APELIN can stimulate Akt phosphorylation after hypoxic/ischemic injury, and inhibiting PI3K reverses the phosphorylation and attenuates the protective effects on apoptosis [75]. Inhibition of APJ signaling by *Elabela* knockout results in increased apoptosis in the placenta [11]. Thus, competent APJ signaling is required to prevent apoptosis, which is frequently observed in placentas with PE. [62, 76–78]

During normal pregnancy, the invasion of extravillous cytotrophoblasts into the uterine spiral arteries converts them from small, high-resistance vessels to wide caliber, low-resistance vessels. In the placenta with PE, this process is incomplete, and shallow trophoblast invasion is observed [1, 79]. The transformation of villous cytotrophoblasts into migratory and invasive extravillous cytotrophoblast at the tip of the chorionic villi requires epithelial to mesenchymal transition (EMT) [80]. Ho et al. reported that ELABELA can stimulate trophoblast-like JAR choriocarcinoma cells to acquire a more invasive phenotype in vitro, indicating that ELABELA-APJ may positively regulate trophoblast invasion, though the mechanism is unknown [11]. TGF-β is a well-known inducer of EMT during many physiological and pathological processes, such as embryonic development, cancer progression and metastasis, and post-injury organ fibrosis [81]. However, in the placenta, TGF-β inhibits rather than promotes trophoblast invasion. Its mechanism has not been fully defined but may involve inhibiting the activity and expression of multiple extracellular proteolytic

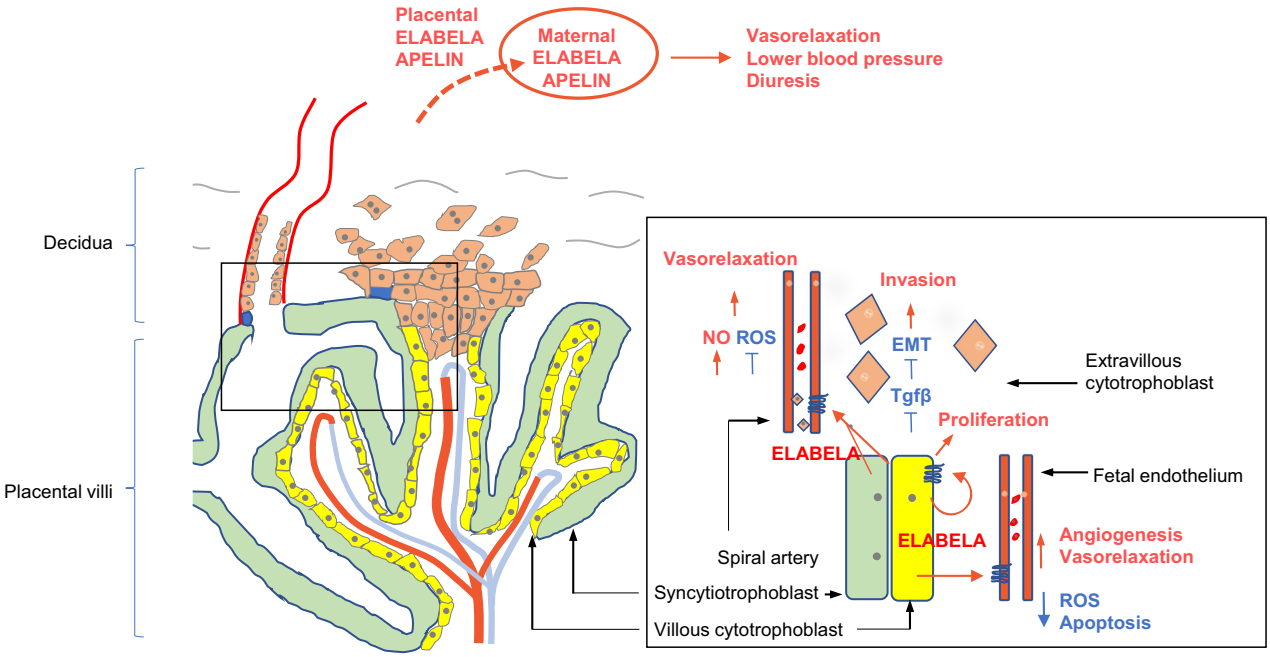

Fig. 1 The biological function of ELABELA and APJ signaling in the placental development and maternal cardiovascular homeostasis. In the placenta, ELABELA- and APELIN-APJ promote cytotrophoblast invasion/proliferation and fetal vessel angiogenesis, support NO production to increase uterine blood flow, reduce oxidative stress, and suppress apoptosis. Systemically, ELABELA and APELIN lower blood pressure and enhance cardiac function through their vasodilation effects. Loss of ELABELA or APJ signaling perturbs the placental and maternal vascular function, and contributes to PE.

enzymes [82–84] and suppressing vascular endothelial cadherin (VE-cadherin) expression [85]. One study provided in vitro evidence that TGF-β may inhibit trophoblast EMT by upregulating epithelial-cadherin and beta-catenin expression [86]. Increased levels of TGF-β1/3 [87, 88] and E-cadherin [87, 89, 90] and a reduced level of VE-cadherin have been detected in the trophoblasts of patients with PE [89]. APJ activation can inhibit TGF-β signaling in a number of in vivo and in vitro fibrosis models [91–94]. Therefore, APJ activity may be required in the normal placenta to counteract the inhibitory effect of TGF-β on trophoblast invasion, which may explain the impaired trophoblast invasion in the *Elabela*-null placenta, as reported by Ho et al (2017) [11].

Conclusion and prospects

ELABELA- and APELIN-APJ signaling regulate important aspects of placental development and maternal cardiovascular homeostasis (Fig 1). ELABELA- and APELIN-APJ promote angiogenesis and cytotrophoblast invasion, support NO production to increase uterine blood flow, reduce oxidative stress, and suppress apoptosis in the placenta. Systemically, ELABELA and APELIN lower blood pressure and enhance cardiac function through their vasodilation effects. Many of these actions are executed through the activation of ERK and Akt and inhibition of TGF-β. Experiments on mouse models have demonstrated that ELABELA deficiency promotes PE and cardiovascular disease in mice. However, the clinical relevance of ELABELA, APELIN and their receptor APJ remains an open question. A recent study did not find altered expression of ELABELA in placentas from humans with PE, though the findings from 82 patients with PE need to be verified in larger cohort studies [95]. Assuming that the loss of ELABELA-APJ signaling may not account for a significant number of patients, given its widespread vasorelaxation activity, would APJ agonist treatment alleviate PE syndrome due to other causes? Wang et al (2017) found that systemic APELIN treatment significantly ameliorated the symptoms of PE in a rat model of PE induced by reduced uterine perfusion pressure [96]. Whether APELIN, or perhaps even more importantly ELABELA, has a similar therapeutic efficacy in human PE remains to be tested in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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