REVIEW ARTICLE

Biological functions of Elabela, a novel endogenous ligand of APJ receptor†

Running title: Elabela and Biological functions

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Abstract:
The G protein-coupled receptor APJ and its cognate ligand, apelin, are widely expressed throughout human body. They are implicated in different key physiological processes such as angiogenesis, cardiovascular functions, fluid homeostasis and energy metabolism regulation. Recently, a new endogenous peptidic ligand of APJ, named Elabela, has been identified and shown to play a crucial role in embryonic development. In addition, increasing evidences show that Elabela is also intimate associated with a large number of physiological processes in adulthood. However, a comprehensive summary of Elabela has not been reported to date. In this review, we provide an overview of the biological functions of Elabela. Collectively, Elabela, a potential therapeutic peptide, exerts diverse biological functions in both embryos and adult organisms, such as dysontogenesis, self-renewing of human embryonic stem cells, endoderm differentiation, heart morphogenesis, cardiac dysfunctions, blood pressure control, angiogenesis, blood pressure control, regulation of food intake and water intake, bone formation and kidney diseases. This article is protected by copyright. All rights reserved

Key words: APJ, Elabela, apelin
Introduction of apelinergic system

The apelin receptor, also named APJ or angiotensin receptor-like 1, was first cloned in 1993. APJ shares 54% homology in transmembrane domains and 31% homology for the entire sequence with the angiotensin II receptor (AT1). Nevertheless, APJ cannot bind angiotensin II (O’Dowd et al., 1993). In 1998, Apelin, the APJ receptor’s first endogenous ligand, is identified in bovine stomach extracts by Tatemoto (Tatemoto et al., 1998). Pre-pro-apelin is a 77 amino acid precursor peptide. Several forms of apelin peptides are obtained by cleavage of Pre-pro-apelin. These peptides include apelin-36, apelin-17, apelin-13, apelin-12 and pyr-apelin-13. Pyr-apelin-13 is the most binding with high affinity to APJ (Tatemoto et al., 1998).

Both Apelin and APJ receptor are widely expressed in various tissues including heart, brain, limbs, retina, liver, lung, skin, kidney, adipose tissue and so on (Dray et al., 2010; He et al., 2015). The importance of apelinergic signalling is well delineated in the cardiovascular system, energy metabolism, fluid homeostasis, angiogenesis, human immunodeficiency virus-1 (HIV-1) infection, and the neuroendocrine stress response (Masri et al., 2005; O’Carroll et al., 2013; Pitkin et al., 2010). At the cellular level, the activation of APJ activates various G-protein-dependent signaling pathways, including the suppression of adenylate cyclase, calcium mobilization, phosphorylation of extracellular signal regulated kinases 1/2 (ERK1/2) and p70S6K, and nitric oxide synthase (NOS) activation (D’Aniello et al., 2009; Medhurst et al., 2003; Murza et al., 2016; Yue et al., 2011).

Physiological functions of APJ receptor

It has been shown that APJ receptor plays an important role in the cardiovascular system. Apelin is known to be inotropic agents (Szokodi et al., 2002). In response to mechanical stretch, APJ receptor acts in an apelin-independent manner (Scimia et al., 2012). APJ knockout (KO) mice lead to decreased risk of cardiac hypertrophy. Apelin-APJ signalling is also reported to have cardioprotective effects on myocardia infraction.

Another key function of APJ receptor is their regulation of energy metabolism. Both APJ receptor and Apelin are found in adipose tissue (Wei et al., 2005). APJ
receptor also presents at the membrane of islet β-cells (Boucher et al., 2005), with its activation by apelin, resulting in suppressed insulin secretion, and impairment of glucose elimination. Furthermore, apelin-13 can enhance pancreatic islet cell mass and β-cell insulin content in mice with type I diabetes to alleviate the symptoms of diabetes (Chapman et al., 2014; Chen et al., 2011). Furthermore, a number of studies indicate that APJ receptor has an important role in maintaining fluid homeostasis. Apelin appears to a clear diuretic effect via the inhibition of vasopressin expression which exerts an anti-diuresis effect (De Mota et al., 2004; Reaux et al., 2001). In contrast, anti-diuresis mediated by APJ is reported in a study that APJ knockout mice fail to decrease urine volume in response to water deprivation (Roberts et al., 2010).

APJ receptor also involves the process of angiogenesis. Apelin, binding to the APJ receptor, is reported to be angiogenic in normal physiology and the pathology of cancer. The presence of both receptor and ligand has a key function in the normal development of frog and mouse heart vasculature, and loss of them results in vessel disruption in most of embryos (Cox et al., 2006). On the other hand, apelin and the APJ trigger premature angiogenesis in Xenopus, and both are increased in microvascular proliferations of malignant gliomas (Kalin et al., 2007).

All these above discussion show some important physiological functions of APJ receptor. Although physiological importance of APJ receptor is well described, comprehensive and systematic investigations remain to be further described the dual effects of APJ and the exact mechanisms of signal transduction via APJ remain essential.

**Discovery of Elabela, a novel endogenous ligand of APJ receptor**

Different studies show the possible existence of another ligand for APJ receptor. APJ is detected early during gastrulation and throughout the subsequent development stages (Devic et al., 1996), whereas apelin expression initiates only at the end of gastrulation (Zeng et al., 2007). Apelin knock-out mice but not APJ knock-out mice accord with a Mendelian inheritance pattern (Kang et al., 2013). Moreover, APJ receptor knock-out mice display different phenotype compared with apelin knock-out mice. NO deficiency is found in early embryonic development of Apelin knock-out...
mice (Kuba et al., 2007). However, loss of APJ receptor causes variable embryonic lethality because of growth retardation and cardiac malformations (Charo et al., 2009). In line with the above finding, Scott et al and Zeng et al. find that knock down of apelin does not phenocopy loss of APJ receptor in zebrafish (Scott et al., 2007; Zeng et al., 2007). All these studies indicate the possible existence of another unknown ligand for APJ.

Human Elabela consists of three exons on chromosome 4, which generates a transcript (AK092578) that is annotated as a noncoding RNA. Recently, two different research groups identify the new endogenous peptide ligand for APJ receptor, called Elabela (Chng et al., 2013; Pauli et al., 2014). Chng et al. also report that this gene contains a conserved open reading frame which can predict to express a conserved vertebrate protein of 54 amino acids (aa) consisting of a secretory signal and a mature 32-aa peptide, named Elabela (Chng et al., 2013). Elabela is highly enriched during gastrulation and its knockdown in zebrafish shows the phenotype of loss of APJ expression (Chaves-Almagro et al., 2015). Moreover, severe developmental defects, such as rudimentary or absent heart formation, reminiscent of the apelin receptor phenotypes, are also observed in Elabela mutant zebrafish. In addition, Elabela and APJ receptor spatiotemporal expression pattern, mutant rescue, as well as receptor internalisation experiments also indicate that Elabela is likely to be a ligand of APJ receptor (Chaves-Almagro et al., 2015; Chng et al., 2013; Pauli et al., 2014).

Additionally, Elabela is further demonstrated to signal via APJ receptor. Phenotypes caused by loss of function of Elabela are very similar to that induced by Apelin receptor removing. In addition, Elabela protein in human binds to cells engineered to APJ receptors (Chng et al., 2013), and over-expression of the ligand in embryos results in internalization of the labeled receptor (Pauli et al., 2014), marking the interaction between a ligand and its GPCR (Reichman-Fried and Raz, 2014).

A role for Elabela beyond development is described in adult human heart and blood vessels, where Elabela is localized in endothelium and Elabela has an affinity for the human APJ receptor. In addition, Elabela is also detected in human stem cells, prostate and kidney (Chng et al., 2013; Wang et al., 2015). Elabela activates the signal...
transduction pathways downstream of overexpressed human APJ receptor, facilitates angiogenesis, and enhances vasodilatation in mouse aorta (Wang et al., 2015). More interesting, Elabela inhibits angiotensin II-induced pressor effect in mouse (Yang et al., 2015). Elabela, new APJ ligand, plays a crucial role not only in heart morphogenesis, mesendodermal cells movement and endoderm differentiation, but also in adults’ organs. Therefore, in this paper, we review expression profile, cellular and tissular functions of Elabela both in embryos and adult organs.

**Elabela is biologically functional in embryos**

Elabela, a secreted peptide hormone, is expressed in undifferentiated human embryonic stem cells and is immediately downregulated during differentiation (Miura et al., 2004). Elabela has biologically functional effects on the growth of embryos.

1. **Elabela has a key function in early embryonic development**

   Elabela is the basis of early embryonic development. Most of the homozygous ela mutant animals induced by zinc-finger (ZFN) (Chng et al., 2013) and transcription activator-like effector nuclease (TALEN) appear to death at the end of embryogenesis in zebrafish (5–7 days of development). The survival from childhood through adulthood in mutant embryos with the wild-type gene product during early embryogenesis suggests that the function of Elabela is essential only during embryonic stages. Moreover, Elabela mutant in embryos lead to delayed blastopore closure and thickened notochord (insets) and deferred embryos relative to WT embryos. Of note, Elabela plays an essential role in early embryonic development.

2. **Elabela is critical to cell motility during gastrulation**

   Elabela elicits a biological function in cell motility. Pauli et al. (Pauli et al., 2014) show that loss of Elabela function impacts the process of gastrulation. Pauli et al. observe normal early cell specification, but defective cell movement during gastrulation. Specifically, cell movement in the gastrulating embryos is postponed in the process of epiboly (Pauli et al., 2014). Additionally, ubiquitous and localized expression of Elabela effectively resumes cell movement in Elabela mutants, suggesting that Elabela is an important contributing factor for elevating cell motility.

3. **Elabela holds a key to endoderm differentiation and heart morphogenesis**
Elabela is known to play an important role in endoderm differentiation and heart morphogenesis. Elabela expression is found to significantly downregulate during human embryonic stem cell (hESC) differentiation (Miura et al., 2004). In line with it, Elabela mutant gastrulae results in specific defects in the mesendodermal lineage. Elabela is reported to be essential for mesendoderm differentiation during embryogenesis in zebrafish (Chng et al., 2013). Interestingly, this is confirmed by the fact that Elabela, in concert with the TGF-β pathway, is involved in endoderm differentiation via maintaining hESCs primed toward the mesendoderm lineage (Chng et al., 2013). Furthermore, the expression of SRY (sex determining region Y)-box 17 (SOX17) and Fork head box protein A2 (FOXA2) in Elabela null embryos, which marks the definitive endodermal precursors, exhibits a decline in its marginal, but not axial, component, suggesting a reduced endoderm differentiation potential. Remarkably, Elabela plays a prominent role in the proper differentiation of endodermal precursors. In addition, differentiation of endodermal precursors is crucial for guiding the overlying cardiac progenitors toward the heart-forming region (Chng et al., 2013). Elabela deficiency impairs heart development and tail elongation. Moreover, Elabela heterozygous fish are phenotypically normal, whereas Elabela null fish displays severe cardiac dysplasia ranging from a rudimentary heart to no heart. Null Elabela larvae also appear to pericardial edema and excess accumulated erythrocytes, and minimal blood circulation at the intermediate cell mass. Moreover, due to heart dysgenesis, loss of Elabela causes embryonic lethality and variable posterior anomalies, such as loss of ventral fin, tailbud duplications, and extreme tail/trunk truncations. These results indicate that, Elabela, acting though APJ, maintains the proper endoderm differentiation and subsequent cardiogenesis via regulating SOX17 and FOXA2.

4. Elabela is a crucial contributor to self-renewing human embryonic stem cells

Elabela is shown to underlie self-renewing human embryonic stem cells (hESCs). Embryonic stem cells have a key function in maintaining genome stability. hESCs do not express APJ receptor, but can secrete Elabela abundantly. Similarly, Elabela is highly detected in mouse ESCs (mESCs). Additionally, Elabela, as a regulatory RNA,
is demonstrated to regulate p53-mediated DNA damage induced apoptosis (DIA) of mESCs via interacting with heterogeneous nuclear ribonucleoprotein L (hnRNPL) (Li et al., 2015). Consistent with its role in maintaining growth and self-renewal, Lena Ho et al. show that ELA facilitates hESC cell-cycle progression and protein translation, suppresses stress-induced apoptosis, and activates PI3K/AKT/mTORC1 signaling, which is important for growth, viability and the self-renewal capacity of hESCs (Armstrong et al., 2006; Zhou et al., 2009). Decreased cell growth, cell death, and loss of pluripotency in hESC caused by the inhibition of Elabela suggest that Elabela acts as an important endogenous growth factor in human embryos and hESCs plays a paramount role in maintaining growth and self-renewal in response to cellular stress (Ho et al., 2015). In addition, depletion of Elabela in both single-cell and colony format leads to reduced growth rates, a loss of hESC colony morphology and reduced pluripotency markers. Thus, it is proposed that ELA may accelerate the growth of hESCs via regulation of PI3K/mTOR-dependent activation of protein translation and cell-cycle progression.

5. **Elabela is associated with the skeletal development, bone formation and bone homeostasis**

Elabela is essential to the skeletal development, bone formation and bone homeostasis. Formation of three embryonic germ layers is critical to eliciting differentiation including bone formation. Pluripotency factor Pou5f3 (homologous to mammalian Oct4) in zebrafish plays a role in cell fate determination. At the blastula stage, Pou5f3 in zebrafish interacts with Nanog to form Pou5f3–Nanog complexes, which, however, can be restricted by Sox32 via binding either Pou5f3 or Nanog in dorsal endoderm during gastrulation. Interestingly, Pou5f3–Nanog complexes regulate the bone morphogenetic protein (BMP) signaling in the mesendoderm lineage. Elabela is able to inhibit expression of Sox32. Therefore, Elabela/APJ pathway may have impact on the skeletal development, bone formation and bone homeostasis via inhibiting Sox32 levels to modulate the proportion of Pou5f3–Nanog complexes in ventrolateral endodermal cells (Perez-Camps et al., 2016).

Overall, the above lines of evidence suggest the Elabela/APJ pathway exerts
many biological functions in embryos including proper endoderm differentiation, skeletal development, bone formation, bone homeostasis, cardiogenesis, hESC self-renewal and especially early embryonic development (Perez-Camps et al., 2016) (Figure1).

**Elabela is biologically functional in adult organs**

1. Elabela is another endogenous molecule(s) acting though APJ signaling pathway in a biased agonism manner

Elabela and apelin is reported to activate the same receptor APJ. Moreover, Elabela and apelin relax the blood vessel in different manners. In addition, Elabela acts though a less-endothelium-dependent manner whereas apelin though a predominantly endothelium-dependent fashion. Apelin-KO mice accelerate TAC-induced hypertrophy, whereas APJ-KO mice inhibit TAC-induced hypertrophy, revealing that APJ rather than apelin signaling takes part in the TAC-induced hypertrophy. More importantly, APJ-KO mice but not apelin-KO mice attenuates water intake and fails to concentrate urine. Apparently, the distinctive cardiac responses to trans-aortic constriction (TAC) in mice indicate that Elabela is biased agonism(Yang et al., 2015). Therefore, we make a comparison between Apelin-KO mice, APJ-KO mice, and Elabela -KO zebrafish (Table1).

2. Elabela is required for vasculogenesis/angiogenesis

A key role for Elabela has been unraveled in vasculogenesis/angiogenesis. Elabela is expressed in kidney (Deng et al., 2015) and prostate tissues in adults (Wang et al., 2015), and induces angiogenesis, and relaxes aortic blood vessel in mice (Wang et al., 2015). It is thus evident that Elabela serves as a regulator for the circulation system (Chng et al., 2013; Wang et al., 2015; Xie et al., 2014). The migration of angioblasts towards the midline is the core of the formation of the large axial vessels, namely the dorsal aorta (DA) and the cardinal vein (CV), which are the basis of the developing cardiovascular system. Vascular endothelial growth factor (Vegf), a main regulator of vascular growth in the embryonic and adult organism, is previously reported to modulate the migration of angioblasts towards the midline (Coultas et al.,
2005; Verma et al., 2010). However, Christian S.M. and Helker et al. (Helker et al., 2015) report that the deficiencies in the Vegfa signaling pathway in zebrafish embryos has no effect on angioblast migration, suggesting that there exist another endogenous signal(s) guiding angioblast migration. Angioblast migration to the midline is reported to depend on APJ receptors. But, endogenous Apelin, ligand for APJ, is not enough to guide angioblast migration. Elabela, which can bind and activate APJ (Chng et al., 2013; Pauli et al., 2014), is demonstrated to guide to help angioblast migration to the midline. Moreover, Elabela plays a leading role and Apelin has a minor additive role in regulating angioblast migration. These result leads to a proposal that the ligands Elabela or Apelin and their receptors are a key step to regulating vasculogenesis. In accordance with the role of Elabela in vasculogenesis, Wang et al. demonstrate that Elabela promotes the angiogenesis (tubular formation) in HUVECs (Wang et al., 2015), which is increased by overexpression of APJ. In addition, synthetic human Elabela leads to cAMP suppression, ERK activation and intracellular calcium mobilization via internalization of human APJ. cAMP regulates calcium influx, which in turn affects the cAMP pathway (Peverelli et al., 2014). Alteration of intracellular Ca$^{2+}$ homeostasis is known to be related to angiogenesis in tumor (Cui et al., 2017). TGF-β1, a gene related to the NODAL/TGF-β pathway, is increased in Elabela - pulsed hESCs (Ho et al., 2015). TGF-β1 is reported to promote angiogenesis (Goumans and Ten Dijke, 2017; Ito et al., 1995). TGF-β can phosphorylate Smad2 and Smad3 by binding to transforming growth factor-βreceptor-2 (TβRII). Elabela activates pSMAD3 (Ho et al., 2015). In addition, SMADs, cooperating with other transcription factors, enhances angiogenesis via endoglin and VEGF induction(Euler-Taimor and Heger, 2006). Taking together, it is noteworthy that Elabela may promote angiogenesis via disturbing the intracellular Ca$^{2+}$ homeostasis or acting through TGF-β/SMADs pathway.

3. Elabela plays an essential role in regulating vascular and cardiac functions

Emerging evidence supports the view that Elabela is critical to making vascular and cardiac functions normal. Elabela peptide is found in human plasma. Expression of Elabela is highest in the embryonic heart, declining toward adulthood. Additionally,
Elabela is predominantly detected in the fibroblasts and endothelial cells in the adult heart. Apelin and APJ receptor are abundantly expressed in the endothelial cells. It is likely that Elabela gene expression in the diseased hearts can be induced in fibroblasts and endothelial cells. Apelin is shown to decrease in severe heart failure although Apelin mRNA levels have little change in the infarcted mouse hearts (Chong et al., 2006; D'Aniello et al., 2009). Elabela, very similar to apelin, have an essential role in regulating cardiac inotropy and vascular function. Moreover, upregulation of both Elabela and APJ in MI hearts appears to better preserved LV systolic function, suggesting that Elabela–APJ system exerts a central role in modulating cardiac function in the diseased myocardium. Perjes et al. also report that Elabela, predominantly enriched in the noncardiomyocyte fraction in the adult rodent heart, binds to APJ receptors in the heart. In addition, similar to apelin, Elabela and its active (19–32) fragment, smaller bioactive fragment of Elabela, are found to in the isolated-perfused rat heart (Wang et al., 2015). Elabela strengthens cardiac contractility and left ventricular contractility, enhances left ventricular developed pressure (LVDP) (Wang et al., 2015), and results in coronary vasodilation in the nanomolar level with the activation of extracellular signal-regulated kinase (ERK) 1/2, an established regulator of cardiac development (Perjes et al., 2016). Interestingly, the increase in cardiac contractility or inotropy induced by Elabela is inhibited by suppression of ERK1/2 activation and MEK inhibitor U0126 markedly decreases the Elabela-induced inotropy. The acute cardiovascular effects of Elabela in rat enhance cardiac contractility, ejection fraction, cardiac output and vasodilatation. Crucially, Elabela is decreased in human disease. Rodent PAH models can remit the severity of changes in cardiopulmonary function/histology in the monocrotaline (MCT) rat model of PAH (Yang et al., 2017). These studies reveal that, Elabela is not only increased in post-infarction cardiac remodeling, but also plays a key role in increasing cardiac contractility in an ERK1/2-dependent manner by stimulation of the apelin receptor. Thus, it is possible that stimulation of the Elabela–APJ receptor system and subsequent activation of ERK1/2 signaling may also remit hemodynamic stress in the failing heart through directly improving cardiac contractility (Perjes et al., 2016).
Furthermore, Elabela exerts cardioprotective effects in failing hearts and attenuates Ang II induced hypertension and cardiac dysfunction in mice (Sato et al., 2017). Elabela suppresses the H/R- or I/R-induced increase of TGF-β1 (Chen et al., 2017). In addition, the TGF-β inhibition suppresses left ventricular hypertrophy as well as interstitial fibrosis, and attenuates left ventricular remodeling or heart failure (Ikeuchi et al., 2004). Based on above findings, it is, therefore, likely that Elabela may regulate vascular and cardiac functions possibly through the inhibition of TGF-β (Figure 2).

4. Elabela relaxes aortic blood vessel and may exert an anti-hypertension effect

Elabela exerts an anti-hypertension effect. Apelin-13 induces hypotensive effects both in normal Sprague–Dawley and in spontaneously hypertensive rats. Elabela shares its receptor with apelin. Thus, Elabela is also likely to regulate vascular tone. Recently, Elabela is also demonstrated to relax precontracted mouse aortic rings (Wang et al., 2015). In addition, administration of Elabela in Sprague–Dawley rats lowers blood pressure similar to apelin-13. By coincidence, Wang et al demonstrate that Elabela leads to the relaxation of mouse aortic blood vessels by the stimulation of APJ (Helker et al., 2015; Wang et al., 2015). In contrast to apelin, Elabela-mediated vascular relaxation is independent on NO. Elabela is previously reported to have vasodilatory effects. Moreover, cardiac Elabela overexpression decreases blood pressure. In addition, ERK activity is enhanced in vasculature and kidneys in various models of hypertension including spontaneously hypertensive rat (Touyz et al., 2001). ERK may elicit excessive vasoconstriction (Watts, 2000). Therefore, overactivity of ERK is associated with the pathogenesis of various forms of hypertension including obesity-associated hypertension (Banday et al., 2007; Wojcicka et al., 2008). In addition, synthetic human Elabela peptide leads to ERK activation. PD98059, an ERK inhibitor, has vasodilating and hypotensive effects in animal models of hypertension (Touyz et al., 2002), suggesting that Elabela exerts strong hypotensive effects in vivo in rats. In addition, Lena Ho et al. demonstrate that infusion of exogenous Elabela improves hypertension (Ho et al., 2017). Therefore, ELA may exert an anti-hypertension effect via suppressing ERK activation (Schreiber et al., 2016).
5. Elabela may inhibit renal remodeling and has direct anti-renal fibrosis

Elabela is involved in renal fibrosis. Elabela is predominantly found in renal collecting ductal cells. Elabela treated rats display less glomerular and tubulointerstitial injuries along with lower transcript levels of profibrotic genes, TGF-β, collagen type 1α, fibronectin and metalloproteinase inhibitor 1 in the kidney. Elabela suppresses renal remodeling, decreases renal fibrosis and reduces the expression of fibrosis associated genes in the kidneys in salt-induced hypertensive DS rats (Schreiber et al., 2016). TGF-β is considered as a key contributing factor of renal fibrosis (Bottinger, 2007). Therefore, all these results hint that Elabela may exert anti-fibrotic effects via regulating TGF-β (Figure 3).

6. Elabela alleviates food intake and elicit arginine vasopressin

Many researches indicate that Elabela is negatively related to food intake. Apelin is extensively/ubiquitously found in various organs, such as the heart, lung, kidney, gastrointestinal, and brain (Cheng et al., 2012; Higuchi et al., 2007; Lv et al., 2013; Masaki et al., 2012; Reaux et al., 2001). Peripheral apelin is shown to pass through the blood–brain barrier (BBB) (Higuchi et al., 2007). Furthermore, apelin exerts an anorexigenic effect via impacting the feeding regulation mechanism in the brain (Masaki et al., 2012; Reaux-Le Goazigo et al., 2011) and brain distribution of the rat apelin receptor (Lv et al., 2012), which may be due to activation of anorexigenic neuropeptide arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) neurons in the hypothalamus, as well as stimulation of hypothalamic α-melanocyte-stimulating hormone release (De Mota et al., 2004; Lv et al., 2012; Reaux-Le Goazigo et al., 2011; Taheri et al., 2002). APJ receptor is shown to be involved in the process. Moreover, APJ is detected in anorexigenic neurons in the hypothalamus, including CRH and AVP neurons (Reaux et al., 2001). Recently, ELA is identified as an endogenous ligand of APJ. Additionally, Elabela shares some similar sequences with the apelin (Xie et al., 2014). Thus, Elabela, similar to apelin, may also cross the BBB to make anorexia via regulating the ELA-APJ signaling system. Putra Santoso et al. further demonstrate that administration of Elabela in AVP and CRH neurons of the paraventricular nuclei (PVN) of adult mice displays...
an anorexic effect, along with the increase of c-Fos expression and [Ca\textsuperscript{2+}], which can regulate cellular activities of the AVP and CRH neurons, resulting in decreased food intake. In other words, Elabela may suppress food intake via activation of AVP and CRH neurons and the increase of c-Fos expression and [Ca\textsuperscript{2+}] in PVN (Santoso et al., 2015).

7. **Elabela plays a key role in regulating water homeostasis.**

Elabela, binding to APJ, plays an important role in regulating the balance of water intake. Apelin and arginine vasopressin (AVP) exert diuretic and antidiuretic effects, respectively, and apelin suppresses AVP release (De Mota et al., 2004; Flahault et al., 2017). Increasing plasma osmolality results in elevated circulating AVP and reduced apelin levels (Azizi et al., 2008). Elabela shares its receptor with apelin, a neuropeptide with a wide array of functions involved in maintaining the body fluid and cardiovascular homeostasis (Chong et al., 2006). One study reports that Elabela elicits a diuretic effect in adult rats. Similarly, Elabela administration affects fluid homeostasis by increasing diuresis and water intake in the adult rats (Wang et al., 2015). Elabela regulates fluid homeostasis by binding to the APJ receptor to activate Gi signaling, while another study shows that intracerebroventricular Elabela injection activates AVP (Santoso et al., 2015). Application of apelin-17 in lactating rats enhances diuresis with a decrease in urine osmolality. Both apelin-13 and Elabela is further demonstrated to elevate daily urinary output in Sprague–Dawley Rats. Application of Elabela in line with apelin-17, are also reported to display a significant suppression in urine osmolality and electrolyte excretion, such as concentrations of Na\textsuperscript{+} and K\textsuperscript{+} (Murza et al., 2016). Sustained Elabela is demonstrated to have no notable effect, but block sodium and chloride percent fractional excretion in pre-HSD DS rats. Although the effect of Elabela on regulation of water homeostasis remains contentious, it is clear that Elabela holds a key to regulate osmolality via NaCl reabsorption (Schreiber et al., 2016).

Elabela in the adult kidney appears to a 5-fold higher maximal response than apelin in regulating diuresis and water intake, and 2-fold higher plateau responses in stimulating phosphorylation of ERK1/2 than apelin. In addition, application of
Elabela -PA, the newly designed Elabela antagonist, suppresses the urine flow rate, water intake as well as ERKs activation of both Elabela and apelin in adult rats, indicating that Elabela of kidney origin is more important than apelin in regulating APJ functions in the kidney and fluid homeostasis by binding to the APJ receptor to activate Gi signaling serving as aquaretic agents (Deng et al., 2015).

8. Conclusion

APJ receptor is shown to exert various physiological effects on organ, such as the regulation of fluid homeostasis, food intake, glucose metabolism, regulation of cardiovascular function, angiogenesis, cardiac development, cardiac contractility, vascular tone, cardiac hypertrophy, type 2 diabetes (T2D) and obesity. Due to sharing some homology with the first ligand apelin, Elabela is likely to exert similar effects. Accumulating evidences demonstrate that Elabela has a critical function not only in embryonic development, but also in adulthood, such as vasculogenesis, alleviating food intake, regulating vascular and cardiac functions, promoting the angiogenesis, relaxing mouse aortic blood vessel and exerting an anti-hypertension effect, inhibiting renal remodeling, suppressing fibrotic effects and regulating water homeostasis. The finding of a second peptide, Elabela, explains why apelin knock-out mice shows significant difference in early embryonic development, phenotype, and pathogenesis of the cardiovascular disease, compared with apelin knock-out mice. Going forward, increasing evidence has underscored the role of Elabela in embryonic development, and in adulthood, yet little is known about molecular mechanisms and precise functions of Elabela in different pathophysiological events. Therefore, a comprehensive and systematic biological study of Elabela still needs to be done.

Contributors

Jin Xu and Linxi Chen designed and wrote the paper. Lan-fang Li and Zhisheng Jiang reviewed and edited the manuscript. All authors read and approved the manuscript.

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Figure 1. Elabela is involved in embryonic development. Elabela–mediated increase of foxa2 and sox17 promotes heart development by endoderm differentiation. Elabela–mediated repression of Sox32 enhances bone formation via facilitating Pou5f3–Nanog complexes, along with subsequent enhanced BMP. Elabela can promote cell motility. Elabela inhibits the process of delayed embryos, eventually suppressing severe embryonic development embryonic defects. Elabela–mediated phosphorylation of PI3K and AKT explains the up-regulation of mTOR/CCND1 to augment the hESC self-renewal.

Figure 2. Elabela has a key function in physiological process in adult. Elabela attenuates TGF-β, causing decrease of hypertension induced by AngII and cardiac dysfunction. Elabela elicits cAMP and further promotes intracellular Ca²⁺ mobilization, ultimately causing tubule-like formation, which is also induced by phosphorylation of SMAD3/ TGF-β1 signaling pathway. Elabela mediated-ERK1/2 phosphorylation inhibits blood pressure, exerting anti-hypertension effect.

Figure 3. Elabela takes part in biological process in adult. Elabela reduces TGF-β, resulting in the inhibition of renal fibrosis. Elabela increases Ca²⁺ in activated AVP and activated CRH neurons, leading to reduced food intake. Elabela suppresses AVP and subsequent blocks water intake, which is increased by reduced Na⁺ and Cl⁻ excretion induced by Elabela.
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<tr>
<td>Embryo</td>
<td>-</td>
<td>Vascular Defects↑</td>
<td>Defects in functional heart↑</td>
</tr>
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<td></td>
<td></td>
<td>IEL↑, and CDD↑ (Kang et al., 2013)</td>
<td>No blood circulation↑ (Pauli et al., 2014)</td>
</tr>
<tr>
<td>CVD</td>
<td>Systolic dysfunction↑ (Kuba et al., 2007)</td>
<td>Cardiac contractility↑</td>
<td>Rudimentary</td>
</tr>
<tr>
<td></td>
<td>Exercise capacity↑ (Charu et al., 2009)</td>
<td></td>
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<tr>
<td></td>
<td>Myocardial dysfunction↑ (Wang et al., 2013)</td>
<td>Exercise capacity↑ (Hamada et al., 2015)</td>
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<tr>
<td></td>
<td>Platelet adhesion↑</td>
<td>Platelet aggregation↑ (Adam et al., 2016)</td>
<td></td>
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<tr>
<td>Heart failure</td>
<td>heart failure↑ (Wang et al., 2013)</td>
<td>Dox-induced heart failure↑ (Hamada et al., 2015)</td>
<td>-</td>
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<tr>
<td>Hypertrophy</td>
<td>TAC-induced hypertrophy↑ (Yang et al., 2015)</td>
<td>TAC-induced hypertrophy↑ (Yang et al., 2015)</td>
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<tr>
<td>Hypertension</td>
<td>Pulmonary hypertension↑ (Tatin et al., 2017)</td>
<td>-</td>
<td>Hypertension↑ (Ho et al., 2017)</td>
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<tr>
<td>Blood vessel</td>
<td>Neointima formation↑ (Kojima et al., 2010)</td>
<td>Neointima formation↑ (Kojima et al., 2010)</td>
<td>SEVM↑ (Ho et al., 2017)</td>
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<td>ALV↑ (Tatin et al., 2017)</td>
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<tr>
<td>Angiogenesis</td>
<td>Retinal angiogenesis↑ (Kasai et al., 2008)</td>
<td>Retinal angiogenesis↑ (del Toro et al., 2010)</td>
<td>Tip cell markers↑</td>
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<td>Angiogenic genes↑ (Ho et al., 2017)</td>
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<tr>
<td>Inflammation</td>
<td>Proinflammatory status↑</td>
<td>-</td>
<td>Hypoxic response↑ (Ho et al., 2017)</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
<td>AVP levels↑ and water intake↑ (Roberts et al., 2009)</td>
<td>Proteinuria↑ (Ho et al., 2017)</td>
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<tr>
<td>Others</td>
<td>AMPK↑ and eNOS↑ (Chandra et al., 2011)</td>
<td>JNK activation↑ (Yasuzaki et al., 2013)</td>
<td>Rapid demise of hESCs↑</td>
</tr>
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<td>DANF↑, and AOIA↑ (Sakimoto et al., 2012), differentiation of hESCs↑ (Ho et al., 2015)</td>
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<tr>
<td>BFR↑ and OBs↑↑ (Wattanachanya et al., 2013)</td>
<td></td>
<td>Autophagic dysfunction↑ (Hamada et al., 2015)</td>
<td>EDP↑ (Cheng et al., 2013; Chaves-Almagro et al., 2015)</td>
</tr>
</tbody>
</table>


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Figure 2

Ang II induced hypertension and cardiac dysfunction

TGF-β

TGF-β1

pSMAD3

Intracellular calcium mobilization

ERK1/2

Blood pressure

Hypotensive effects

Tubule-like formation

Cell membrane

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