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ASK family in cardiovascular biology and medicine

Tingting Liu¹, Huanjiao Jenny Zhou², and Wang Min^{1,2}

¹Center for Translational Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

²Department of Pathology and the Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT, USA

Abstract

Cardiovascular disease is a major cause of death worldwide. Mitogen-activated protein kinase (MAPK) signal cascades signaling pathways play crucial roles in cardiovascular pathophysiology. Apoptosis signal-regulating kinase (ASK) family members ASK1, ASK2 and ASK3 are the key molecules in MAPK signal cascades and are activated by various stresses. ASK1 is the most extensively studied MAPKKK and is involved in regulation of the cellular functions such as cell survival, proliferation, inflammation and apoptosis. The current review focuses on the relationship between ASK1 and cardiovascular disease, while exploring the novel therapeutic strategies for cardiovascular disease involved in the ASK1 signal pathway.

1. Introduction

Cardiovascular disease remains the leading cause of death worldwide. Cellular stresses such as reactive oxygen species (ROS), endoplasmic reticulum (ER) stress and calcium influx are among risk factors that are responsible for the pathogenesis of cardiovascular diseases. And the adaptive responses to stresses, which are important to maintain the cellular function, are involved in complicated signal transduction.

Many signal pathways play crucial role in adult cardiovascular pathophysiology. The human mitogen-activated protein kinase (MAPK) cascade, including a MAPKKK, a MAPKK and a MAPK, conduct wide range of cellular functions such as proliferation, differentiation, migration, apoptosis, immune responses and stress responses. Upon activation, MAPKKK can phosphorylate specific residues on MAPKK and activate the MAPKK, sequentially the MAPK are phosphorylated and activated. In mammals, three major subgroups of MAPKs: extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 kinase have been identified, which conduct distinct function. ERK is generally participated

Correspondence: Dr. Wang Min, Interdepartmental Program in Vascular Biology and Therapeutics, Department of Pathology, Yale University School of Medicine, 10 Amistad St., 401B, New Haven, CT 06520. Tel: 203-785-6047; Fax: 203-737-2293; wang.min@yale.edu.

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in cell proliferation and differentiation, whereas JNK and p38 are involved in the cell death and inflammation in response to various stresses (Nagai et al., 2007).

Apoptosis signal-regulating kinase (ASK) family members: ASK1 (MAP3K5), ASK2 (MAP3K6) and ASK3 (MAP3K15) are one of the most important molecules in MAPK signal cascades. The ASK1 is the most extensively studied MAP3K that activates MKK4-JNK and MKK3/6-p38JNK pathways in response to different stress, and control wide variety of cellular processes (Takeda et al., 2008). ASK2 is highly homologous to ASK1 in its kinase domain, and associates with ASK1 C-terminus; the formation of a complex with ASK1 is required to stabilize and activate ASK2 protein. Thus, ASK2 exerts its function as a MAP3K only in the presence of ASK1 (Takeda et al., 2007). It has been found that many cardiovascular diseases such as cardiac hypertrophy, cardiac remodeling after myocardial infarction, atherosclerosis and vascular restenosis are linked to the regulation of ASK1. Little is known about the functions of ASK2 and ASK3 in cardiovascular biology. There have been excellent reviews on the role of ASK family in neurodegeneration, cancer and signaling mechanism (Guo et al., 2017; Nishida et al., 2017; Ryuno et al., 2017; Shiizaki et al., 2013). In this review, we focus on the roles of ASK1 in the cardiovascular biology and discuss the potential as therapeutic targets.

2. Regulation of ASK1

The structure of ASK1 is highly conserved among different species. Some of the mammalian ASK family members have been identified in *Drosophila melanogaster* and *Caenorhabditis elegans*, *Mus musculus* denominated as DASK1, NSY-1 and mASK1, respectively (Kuranaga et al., 2002; Tobiume, 1998). Human ASK1 is a polypeptide of 1,374 residues consisting of N-terminal coiled-coil domain, a serine/threonine kinase domain and C-terminal regulatory domain (Bunkoczi et al., 2007). In an inactive state, ASK1 forms a high molecular mass complex by heteromeric interacting with a reduced form of thioredoxin (Trx-(SH)₂) through the N-terminal coiled-coil domain. The Trx family members Trx1 and Trx2 bind to Cys-250 and Cys-30 in the N-terminal domain of ASK1 in cytoplasm and mitochondria, respectively (Zhang et al., 2004). Under oxidative stress, the reduced form of Trx is converted to the oxidized form Trx-S₂, which leads to the dissociation of ASK1 from Trx (Fujino et al., 2007). Meanwhile, the tumor necrosis factor- α receptor-associated factor (TRAF) 2 and 6 are reciprocally recruited to the N-terminal domain of ASK1 and accelerates ASK1 oligomerization through the C-terminal domain. The oligomerization of ASK1 induces autophosphorylations of ASK1 at Thr838 in human (Thr845 in mouse) within the catalytic domain, leading to full ASK1 activation (Liu et al., 2000).

The duration of the ASK1 activity is also determined by the inactivation of ASK1. Several proteins have been reported to play vital role in the dephosphorylation of Thr838 in ASK1, resulting in ASK1 inactivation (Morita et al., 2001; Sekine et al., 2012). Under a basal condition, 14-3-3 protein inhibits the ASK1 activity in a phosphor-dependent manner by binding to ASK1 specifically via a site involving Ser-967 of ASK1 (Zhang et al., 1999). In response to oxidative stress, protein serine/threonine phosphatase 5 (PP5) deactivates ASK1 by dephosphorylating the Thr838 directly, resulting in a negative feedback. (Morita et al., 2001). In the previous study, we have identified that ASK1-interacting protein (AIP1)

mediates tumor necrosis factor (TNF)- α -induced ASK1 activation by facilitating dissociation of inhibitor 14-3-3 from ASK1, leading to enhanced ASK1-JNK signaling (Zhang et al., 2003). Later we demonstrated that AIP1 recruits protein phosphatase 2A (PP2A) to ASK1 resulting in TNF-induced ASK1-JNK apoptotic signaling activation (Min et al., 2008). It has been found that heat shock protein 72 (Hsp72) inhibits the activity of the ASK1 (Park et al., 2002; Zhang et al., 1999). Hsp72 binds directly to ASK1 and blocks the oligomerization of ASK1 and ASK1-dependent apoptosis, suggesting that Hsp72 functions as an endogenous inhibitor of ASK1 (Park et al., 2002).

Ubiquitination is also an important regulation mechanism for ASK1 activity. We have previously shown that overexpression of Trx1 in endothelial cells induces ASK1 ubiquitination and degradation, leading to inhibition of ASK1-induced apoptosis (Liu and Min, 2002). ASK1 is ubiquitinated in a ROS-dependent manner, and ubiquitin-specific peptidase 9 and X-linked (USP9X) blocks the proteasomal degradation of ASK1 by removing ubiquitin under oxidative stress (Nagai et al., 2009). On the other hand, the E3 ubiquitin ligase Roquin-2 ubiquitinates ASK1 and accelerates the proteasomal degradation of ASK1 after ASK1 activation (Maruyama et al., 2014).

3. Cellular function of ASK1 in cardiovascular disease

ASK1 is activated in various cardiovascular diseases from hypertension to heart failure. It has been found that the cellular function of ASK1 has pivotal role in the pathology mechanism of cardiovascular diseases. In this review, we focus on the relationship between the ASK1 and cellular function of cardiomyocyte, vascular endothelial cell, vascular smooth muscle cell (VSMC) and platelet.

3.1. ASK1 and cardiomyocyte

Cardiomyocytes, which are the major contracting cells in the heart, play pivotal role in maintain cardiac function. In the previous study, we have revealed that ASK1 via its C-terminal ASK1 domain specifically binds to cardiac troponin T (cTnT) in cardiomyocytes. We have also found that a constitutively active form of ASK1 (ASK1- N) induces cTnT phosphorylation in cardiomyocytes, shortening degree of cardiomyocytes and decreasing in rate and amplitude of Ca^{2+} transients, which could be evaluated as cardiac contractile dysfunction. These results suggest that ASK1 plays crucial role in regulation of cardiac contractile function by phosphorylating cTnT (He et al., 2003).

It has been elucidated that wild-type ASK1 or a constitutively active form of ASK1 induces apoptosis in various cells including cardiomyocytes by activating the JNK and p38 MAP kinase pathway. Conversely, oxidative stress or TNF-induced apoptosis is suppressed in *ASK1*^{-/-} cells (Xia et al., 2016). Similar results has been observed in isolated rat neonatal cardiomyocytes (Yamaguchi et al., 2003) (Murdoch et al., 2006). Trx1 suppresses ASK1-induced JNK activation and JNK-dependent apoptosis (Liu and Min, 2002). However, Trx2 deletion in cardiomyocytes could induce ASK1 activation with increased cellular ROS generation and cardiomyocytes apoptosis without JNK activation, suggesting that ASK1 in mitochondria mediates cardiomyocyte apoptosis is independent of JNK pathway (Huang et al., 2015). Besides apoptosis, ASK1 is also involved in non-apoptotic cardiomyocyte death.

In one study, the necrotic injury caused by ischemia-reperfusion (I/R) was detected in wild-type heart, but was drastically reduced in the *ASK1*^{-/-} heart. The evidence of apoptosis such as an increase in TUNEL-positive cells, DNA fragmentation or the activation of caspase-3 were not accompanied with the necrotic injury. In an *in vitro* study, *ASK1*^{-/-} cardiomyocytes were more resistant to H₂O₂- or Ca²⁺-induced apoptotic and non-apoptotic cell death compared with wild type cells (Watanabe et al., 2005). All these studies show that ASK1 plays an important role in regulating the cardiac function by promoting cell death in cardiomyocytes.

Most adult cardiomyocytes are unable to proliferation. However, in response to diverse hypertrophic stimuli such as overloaded pressure or volume, hypertension, ischemic heart disease, the cardiac tissue adapts themselves by growth of individual cardiomyocytes (hypertrophy) and result in the cardiac hypertrophy (Lorell and Carabello, 2000). Although ASK1 is activated by hypertrophic stimuli such as pressure overload, I/R, and agonist treatments *in vitro* and *in vivo*, several studies have found that ASK1 does not directly promote the cardiac growth and hypertrophy. Transgenic mice with cardiac-specific overexpression of ASK1 in the heart showed no significantly growth of the heart or stimulus-induced hypertrophy (Liu et al., 2009). *c-Raf-1*-knockout mice showed a high level of ASK1 activity with left ventricular systolic dysfunction and heart dilatation but without cardiac hypertrophy or lethality (Yamaguchi et al., 2004). The role of intracellular signaling pathway in the development of pathological cardiomyocyte hypertrophy is in part regulated by the calcineurin-NFAT (nuclear factor of activated T-cells) signaling circuit (Wilkins and Molkenstein, 2002). The NFAT transcription factor family proteins are directly dephosphorylated by calcineurin in the cytoplasm and translocate into the nucleus to perform the function of genes transcription (Hogan et al., 2003). Transgene mice with an activated mutant of NFAT3 or calcineurin in the heart showed a significantly hypertrophy characterized by two- to three- fold increase in heart growth. Meanwhile, cardiac dysfunction in calcineurin transgenic mice could be attenuated by injection of CsA, a pharmacologic calcineurin inhibitor (Molkenstein et al., 1998). ASK1 does not phosphorylate calcineurin but it inhibits the NFATc1 activation and translocation indirectly through JNK and p38 activation (Liu et al., 2006). Previous studies have shown JNK and p38 activations function to antagonize calcineurin A-induced cardiac hypertrophy, and this ability may be explained by the functions of JNK and p38 as NFAT kinases, thereby preventing their nuclear translocation (Braz et al., 2003; Liang et al., 2003). Calcineurin is able to dephosphorylate serine 967 in ASK1, resulting in 14-3-3 dissociate from ASK1 and activation of ASK1 (Goldman et al., 2004). It seems that the ASK1 play a protection role in cardiomyocyte hypertrophy by constituting a regulatory circle in which calcineurin up-regulates ASK1 through direct dephosphorylation while ASK1 down-regulates calcineurin-NFAT signaling through p38- and JNK-mediated NFAT phosphorylation (Liu et al., 2006) (Figure 1). However, other studies lead to a contrary conclusion that ASK1 activation promotes the cardiomyocyte hypertrophy (Izumiya et al., 2003). And this may be partly explained by the evidence that ASK1 in cardiac fibroblasts also contributes to pathological cardiomyocyte hypertrophy (Piek et al., 2016). A co-culture study using adult rat fibroblasts, myofibroblasts and cardiomyocytes showed that both fibroblasts and myofibroblasts directly reduce cardiomyocyte cell viability and increase cardiomyocyte volume through paracrine

interaction (Cartledge et al., 2015). Cathepsin B (CTSB), a lysosomal cysteine protease, is expressed in murine and human hearts and was upregulated in response to hypertrophic stimuli (Wu et al., 2015). Knockout of CTSB in mice attenuated pressure overload-induced cardiac hypertrophy, fibrosis, apoptosis, dysfunction and it is confirmed in *in vitro* study that angiotensin II (Ang-II) induced TNF- α /ASK1/JNK signaling pathway was blunted by CTSB deficiency (Wu et al., 2015). Inhibition of cardiomyocytes ASK1 expression in a hamster cardiomyopathy model not only attenuated the apoptosis, but also prevented dilation and preserved left ventricular (LV) systolic and diastolic function. Cardiac fibrosis was also inhibited significantly (Hikoso et al., 2007).

Taken together, the ASK1 activation regulates the apoptosis, necrotic death, hypertrophy and fibrosis of cardiomyocyte, which participate in the cardiac remodeling and result in the cardiac dysfunction.

3.2 ASK1 and vascular endothelial cell

Vascular endothelial cells (ECs) are intimately involved in several key functions such as keeping blood fluid homeostasis, regulating macromolecule and fluid exchange with the tissues, preventing leukocyte activation, and aiding in immune surveillance for pathogens (Pober et al., 2009). Assembly of these vascular functions involves proliferation, migration and sprouting of ECs. Injury or death of ECs will lead to dysfunction of the vasculature.

ECs exhibit similar processes in apoptosis and responses to injurious stimuli as observed in cardiomyocytes with some unique characteristics. Inflammatory cytokines such as TNF, oxidative stress and endoplasmic reticulum (ER) stress combined with contribute to injury and apoptosis of ECs through ASK1-JNK/p38 signaling pathway.

3.2.1 Endothelial cell and Oxidative Stress—Among all the stimuli, the oxidative stress in ECs has been the most investigated. ROS-producing systems in ECs consist of various nicotinamide-adenine dinucleotide phosphate (NADPH) oxidases (NOXs), xanthine oxidase, the uncoupling of NO synthase as well as mitochondria (Ballinger et al., 2002; Cai, 2005; Nishikawa et al., 2000). Excess ROS induce EC injury, death or dysfunction by reducing NO bioavailability and activating apoptotic signaling (Dai et al., 2009). Compared to control ECs, ROS-induced EC dysfunction was attenuated in ASK1-deficient ECs, with elevated endothelial NO synthase (eNOS) and reduced NOX activities. Consistently, ASK1 deletion in mice prevents high-salt diet-induced vascular endothelial dysfunction by suppressing NOX2-associated oxidative stress (Kataoka et al., 2011). These data support that ASK1 mediates EC dysfunction by enhancing NOX and reducing eNOS activities (Yamashita et al., 2007) (Figure 2a). As observed in cardiomyocyte, ASK1 is localized in both the cytoplasm and the mitochondria in ECs; ASK1 in cytoplasm induces apoptosis through JNK-Bid/Bax-cytochrome c pathway, while ASK1 in mitochondria induces apoptosis independent of JNK activation in response to TNF and oxidative stress (Zhang et al., 2004). Bid is a cytosolic member of the Bcl-2 gene family, a prototypic antiapoptotic family. JNK activation induces Bid cleaved and the cleaved Bid inserts into mitochondrial membrane to interact with Bax (a proapoptotic Bcl-2 family member),

resulting in cytochrome c release from mitochondria and caspase-9 and caspase-3 activation and EC apoptosis(Pober et al., 2009)(Figure 2b). Several cellular factors have been identified that regulate apoptotic processes in ECs. Trx2 protects EC function by scavenging ROS to increase NO bioavailability and inhibiting ASK1 activity to enhance EC survival(Dai et al., 2009). Protein kinase D (PKD) is an important regulator of different intracellular signaling pathways. We have reported that that H₂O₂ induces PKD phosphorylation and subsequent translocation from the EC plasma membrane to the cytoplasm, leading to association of PKD with JNK upstream activator ASK1. Inhibition of PKD blocks H₂O₂-induced ASK1-JNK activation and EC apoptosis(Zhang et al., 2005). Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and a key regulator of normal and abnormal angiogenesis(Ferrara, 2004). Therefore, VEGF significantly attenuates H₂O₂-derived endothelial cells apoptosis by inhibiting ASK1 activation(Nako et al., 2012).

3.2.2 TNF Pathway—TNF is one of the most important proinflammatory cytokines. In ECs, TNF activates ASK1-JNK/p38 signaling via TNF receptor 1(TNFR1), resulting in ECs dysfunction and apoptosis. TNFR1 is a type I transmembrane protein which has been extensively characterized in ECs(Pober et al., 2009). In resting ECs, ASK1-interacting protein-1(AIP1), a novel member of the Ras-GTPase-activating protein(GAP) family proteins, binds to TNFR1 in resting ECs. Upon TNF engagement, trimerized TNFR1 recruits TRADD (TNF receptor-associated death domain-containing protein), RIPK1(receptor interacting protein kinase 1) and the TRAF2 to form a complex and release of AIP1 from TNFR1. Meanwhile, AIP1 binds recruits phosphatase PP2A to AIP1-ASK1 complex to dephosphorylate ASK1 at pSer967, facilitating the dissociation of ASK1 from inhibitors Trx and14-3-3, leading to ASK1-JNK activation(Min et al., 2008). We have previously shown that TNF induces deubiquitination and stabilization of ASK1, leading to ASK1 activation. Therefore, the stabilization of ASK1 is also important way for ASK1 activation. SOCS1 (suppressor of cytokine signaling 1), a member of suppressor of cytokine signaling, binds to the phosphotyrosine residues of ASK1 in resting EC, leading to ASK1 rapid degradation. TNF induces dephosphorylation of ASK1 and dissociation of ASK1 from SOCS1, resulting in activation of ASK1-JNK signaling (He et al., 2006). However, TNF-induced cell death through ASK1-JNK/p38 MAPK is limited in ECs and it is believed that the activity of the enzymes is shut off by specific phosphatases regulation, providing negative feedback control(Pober et al., 2009).

3.2.3 Endoplasmic Reticulum Stress—The endoplasmic reticulum (ER) stress refers to a condition that unfolded proteins accumulation in ER in response to stimuli such as oxidative stress, metabolic and ischemic insult. ER stress triggers the unfolded protein response (UPR) and activate PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1), to maintain ER homeostasis (Minamino et al., 2010). It has been found that the IRE1, a serine/threonine protein kinase/endonuclease can promote apoptosis in cultured ECs through the activation of ASK1-JNK signaling pathway, linking the ER stress to the activation of ASK1-JNK pathway(Pober et al., 2009). The cytoplasmic part of IRE1 binds to TRAF2 which couples to ASK1-JNK activation(Urano et al., 2000). AIP1 is also critical in transducing IRE1-mediated ER stress-induced ASK1-JNK activation. In AIP1-KO EC, the ER stress-induced IRE1 activation and

ASK1-JNK activation are blunted. At the molecular level, AIP1 binds to IRE1 in response to ER stress and enhances IRE1 dimerization(Luo et al., 2008).

3.3 ASK1 and Vascular Smooth Muscle Cell

The unbalanced apoptosis, inflammation, migration, proliferation and phenotypic switching of vascular smooth muscle cell (VSMC) have been linked to the atherosclerotic lesion and vascular remodeling after injury(Bennett et al., 2016). It has been suggested that the JNK and p38 signaling pathway are linked to the apoptosis of VSMC. However, the role of ASK1 in VSMC apoptosis is still debatable(Al Ghouleh et al., 2013; Zhang et al., 2014). It has been demonstrated that ASK1 is involved in the vascular remodeling by regulating VSMC migration, proliferation and hypertrophy(Al Ghouleh et al., 2013; Izumi et al., 2003). Neointimal hyperplasia is primarily a disorder of VSMCs, characteristic by VSMC migration, proliferation and extracellular matrix deposition in the intima (Koyama et al., 1998; Yu et al., 2007). By gene transfer of a dominant-negative mutant of ASK1 (DN-ASK1) to mice, it was shown that the DN-ASK1 gene transfer remarkably suppressed VSMC proliferation in both intima and media at 7 days after injury, and prevented neointimal formation at 14 days after injury. Significant apoptotic cells in intima and media cells were observed, and the DN-ASK1 gene transfer did not significantly decrease the apoptosis (Izumi et al., 2003). It leads to the conclusion that ASK1 directly involves in VSMC proliferation and migration, but not apoptosis. However, an opposite result was reported recently. In a carotid artery ligation model, ASK1-deficient (*ASK1*^{-/-}) mice exhibited augmented intimal formation with fewer apoptotic SMCs. Furthermore, TNF- α induced apoptosis was prevented in cultured aortic SMCs from *ASK1*^{-/-} mice(Tasaki et al., 2013). The discrepancy could be due to different animal models and different ASK1 manipulations (DN-ASK1 vs ASK1 deficiency). Recently, it is demonstrated that ASK1 accompanied with aquaporin (Aqp) 1 and NOX1 are responsible for extracellular H₂O₂-induced VSMC hypertrophy. At pathophysiological concentrations, extracellular H₂O₂ enters rat aortic SMCs (rASMC) via Aqp1 channels and activates NOX1, leading to increased NOX-derived superoxide (O₂⁻) and subsequent ASK1 activation and rASMC hypertrophy without proliferation of rASMC(Al Ghouleh et al., 2013).

4. ASK1 in Cardiovascular Diseases

Cardiovascular diseases (CVD) include coronary artery disease (CAD), stroke, hypertension, heart failure, congenital heart disease and peripheral vascular disease(Cervantes Gracia et al., 2017). In this part, we will focus on the links between the ASK1 with CAD and cardiomyopathy, which are the major causes of mortality and morbidity worldwide.

4.1 ASK1 and Coronary Heart Disease

Coronary heart disease is the major cause of mortality and morbidity worldwide(Lopez et al., 2006). The most important pathologic process underlying coronary heart disease is atherosclerosis, which is characterized by plaque formation in the inner coronary artery wall (Mehta et al., 1998). Atherosclerotic plaques formation is a process involved in ECs, VSMCs and macrophages in the vasculature. ASK1 also plays pivotal role in the pathologic process of atherosclerosis.

The EC is the first barrier, which limits the inflammatory and atherosclerotic process in vessel. EC dysfunction is an early event during atherosclerosis progression (Libby, 2002). As we have reviewed above, ASK1 participates in the process of stress-induced EC dysfunction and apoptosis, which leads to the barrier breakdown. Our recent study shows that ASK1 mediates degradation of endothelial junction protein VE-cadherin via a lysosomal pathway to promote EC barrier disruption. Moreover, a pharmacological ASK1 inhibitor prevents TNF-induced vascular leakage (Guo et al., 2017; Nishida et al., 2017; Ryuno et al., 2017; Shiizaki et al., 2013; Yin et al., 2017). Active ASK1 was significantly increased in ApoE-deficient mice (ApoE), a widely used model for atherosclerosis (Zhang et al., 2007). ASK1 also plays a key role in ischemia-induced angiogenesis (a process of new blood vessel formation) by inducing VEGF and monocyte chemoattractant protein-1 (MCP-1) expression (Izumi et al., 2005). While excessive angiogenesis links atherosclerosis, defects in angiogenesis directly contribute to myocardial infarction (Dai et al., 2009).

ASK1 also participates in the process of atherosclerosis by regulating macrophage biology. It has been reported that apoptotic macrophages, SMCs and ECs are aggregating in atherosclerotic lesions. And the majority of apoptotic cells are macrophage foam cells (Libby, 2002). It has been found that ASK1 prevents hyperlipidemia-induced atherosclerosis through increased macrophage apoptosis and that ASK1 may cause plaque vulnerability through necrotic core development (Yamada et al., 2011). This conclusion coincides with a previous study that apoptotic macrophages suppress plaque progression in early lesions because macrophage apoptosis reduces cellularity by phagocytotic clearance (Seimon and Tabas, 2009). Macrophage-derived foam cell formation is another crucial step in the process of atherosclerosis. The formation of foam cells involves in the uptake of oxidized low-density lipoprotein (oxLDL) by scavenger receptors such as scavenger receptor-A (SR-A) and CD36. A recent study shows that endophilin-A2 promotes oxLDL uptake by up-regulating expression of scavenger receptors CD36 and SR-A through activation of ASK1/JNK/p38 pathway (Huang EW, 2016).

Vascular thrombosis is a crucial event leading to myocardial infarction and unstable angina pectoris (Sakurai et al., 2004). Studies show that ASK1 is also essential for platelet activation and thrombus formation. Specifically, several platelet agonists trigger the phosphorylation of ASK1 in platelets, correlated with platelet aggregation; the aggregation of platelets from *ASK1*^{-/-} mice is defective in response to agonists stimuli. Besides, the platelet ASK1 activates MKKs 3/6 and 4, leading to p38 activation, subsequently phosphorylates the key enzyme cytoplasmic phospholipase A2 (cPLA2) in thromboxane A2 (TxA2) generation, resulting in platelet granule release and thrombus formation (Naik et al., 2017) (Kamiyama et al., 2017).

Heart undergoes remodeling after myocardial infarction, characterized by cardiomyocyte death in the infarct border zone with resultant infarct extension, fibrosis at the site of infarct and in the unaffected myocardium, dilation of the left ventricle and hypertrophy of the unaffected myocardium. Cardiac remodeling has adaptive features to maintain cardiac function, but eventually leads to functional decompensation and heart failure (Muslin, 2008). The role of ASK1 in pathological cardiac remodeling has been investigated. When *ASK1*^{-/-} and wild-type mice were subjected to myocardial infarction by coronary artery ligation,

the initial infarct sizes were the same in the two groups. However, ASK1^{-/-} mice had reduced cardiac remodeling, with reduced fibrosis in the border zone and remote myocardium, smaller increases in left ventricular end-diastolic and end-systolic ventricular dimensions, and smaller decreases in fractional shortening compared with wild-type mice. Activation of JNK1/2, but not p38, in the infarct boarder zone was reduced in ASK1^{-/-} mice after myocardial infarction (Yamaguchi et al., 2003). Recent reports have shown that the inhibition of ASK1 could also reduce myocardial infarct size in rat and mouse models of ischemia/reperfusion(Gerczuk et al., 2012; Toldo et al., 2012). These data suggest that ASK1 is a potential therapeutic strategy for the treatment of coronary heart disease and myocardial infarction.

4.2 ASK1 and Cardiomyopathy

The major cardiomyopathies, including dilated cardiomyopathy, hypertrophic cardiomyopathy, diabetic cardiomyopathy and alcoholic cardiomyopathy, can all eventually result in heart failure. ASK1 is one of the important regulator in the development of cardiomyopathy and heart failure. Since we have reviewed the role of ASK1 in the pathogenesis of cardiomyocyte hypertrophy, we focus on the other types of cardiomyopathies in this part. Recently, our group have found that mice with cardiac-specific deletion of Trx2 (Trx2-cKO) develop a significant reduction in left ventricular (LV) fractional shortening and progressive increases in LV systolic and diastolic dimensions, resulting in LV contractile dysfunction. The Trx2-cKO mice had 100% mortality by 4 months of age. Interestingly, a highly selective ASK1 inhibitor GS-444217 could prevent cardiac remodeling and cardiac dysfunction in Trx2-cKO mice(Huang et al., 2015). Long-term ethanol exposure triggers the onset and development of alcoholic cardiomyopathy characterized by cellular and structural changes within the myocardium(Piano and Phillips, 2014). The enzyme cytochrome P450 2E1 (CYP2E1), which catalyze ethanol metabolism, involves in ethanol-induced cardiomyocyte contractile defect(Aberle and Ren, 2003). Chronic ethanol exposure leads to elevated levels of CYP2E1, cardiac fibrosis, cardiac contractile and apoptosis accompanied with increased phosphorylation of JNK and ASK1, and the effects was attenuated or ablated by inhibition of CYP2E1. Moreover, inhibitors of JNK and ASK1 obliterated ethanol-induced myocardial injury(Zhang et al., 2013).

5. Clinical Translations

We have described that ASK1 is involved in various cardiovascular diseases. The clinical application of these findings is always the final destination. Many clinical studies focus on the ASK1 inhibitors as a therapeutic in several diseases that are exacerbated by ASK1 activation. It has been shown that a new chemical compound, succinobucal (AGI-1067), was able to reduce atherosclerosis in several animal models, including hypercholesterolemia cynomolgus monkeys, LDL receptor-deficient mice and ApoE^{-/-} mice(Sundell CL, 2003). A study showed that AGI-1067 inhibits LPS activation of ASK1 and JNK1/2 and p38 in both monocytes and endothelial cells, resulting in the inhibition of inflammatory mediators and tissue factor (TF) expression(Luyendyk et al., 2007). The effect of AGI-1067 on major cardiovascular events was assessed in a larger clinical trial called The Aggressive Reduction of Inflammation Stops Events (ARISE). It was disappointed that AGI-1067 had no effect on

the primary endpoint, even though the composite secondary endpoint of cardiovascular death, cardiac arrest, myocardial infarction, or stroke occurred in fewer patients in the AGI-1067 group compared to the placebo group (Tardif et al., 2008). Recently, we report that AGI-1067 prevents the LPS and Toll-like Receptor-4 (TLR4, the primary receptor for LPS)-induced dissociation ASK1 from its inhibitor Trx1, but not from 14-3-3, leading to inhibition of inflammation. As we described above, the highly selective ASK1 inhibitor GS-444217 can effectively block cardiomyopathy in a mouse model (Huang et al., 2015). From the cases, it seems that ASK1 inhibitors have potential as potential therapeutics for cardiovascular diseases.

6. Conclusion

In this review, we summarize the studies investigating the role of ASK1 in cardiovascular diseases from the cellular functions to the diseases. First, the structure and regulatory mechanisms of ASK1 have been well documented. However, the interactions between ASK1 and other proteins need further exploration. Second, ASK1 is a crucial regulator of cellular functions such as apoptosis, necrosis, migration, proliferation in ECs, hypertrophy in cardiomyocytes and SMCs. Besides, recent studies found that ASK1 is also expressed in the platelet, which is essential for the development of cardiovascular disease. Third, although the mechanism of ASK1 in cardiovascular disease had been studied for 20 years, the therapeutic application has not yet proceeded very far.

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Figure 1.

The role of ASK1 in cardiomyocyte function. ASK1 regulates cardiac contractile function by phosphorylating cTNT. Hypertrophic stimuli induce activation of ASK1-JNK/p38 MAPK pathway and calcineurin-NFAT signal pathway, resulting in cardiomyocytes death and hypertrophy, respectively. NCC: N-terminal coiled-coil domain; CCC: C-terminal coiled-coil domain; ROS: reactive oxygen species; TNF: tumor necrosis factor; NFAT: nuclear factor of activated T-cells; cTNT: cardiac troponin T.

Figure 2.

The role of ASK1 in endothelial cell. **a:** ASK1 regulates eNOS and NADPH oxidase activity, leading to EC dysfunction. **b:** ASK1 in cytoplasm induces apoptosis through JNK-Bid/Bax-cytochrome c pathway, while ASK1 in mitochondria induces apoptosis independent of JNK activation in response to TNF and oxidative stress. eNOS: endothelial NO synthase; NADPH: nicotinamide-adenine dinucleotide phosphate.