

Review

Therapeutic targets in the ASK1-dependent stress signaling pathways

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Abstract: Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates downstream MAP kinases (MAPKs), c-Jun N-terminal kinases (JNKs) and p38 MAPKs, in response to various stresses, such as reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, lipopolysaccharide, and calcium overload. Activation of the JNK and p38 pathways induces stress responses such as cell death, differentiation, and the production of inflammatory cytokines. A series of studies using ASK1-deficient mice have indicated that ASK1 plays important roles in many stress-related diseases, including cardiovascular and neurodegenerative diseases, suggesting that small compounds that inhibit ASK1 activity could possibly be used for the amelioration of the development and/or progression of these diseases. In this review, we provide an overview of the pathophysiological roles of ASK1-dependent signaling pathways and discuss the mechanistic basis for how these could serve as potential therapeutic targets.

Keywords: ASK1, inhibitors, MAP kinase, signal transduction, stress

1. Introduction

Our bodies are constantly exposed to many stressors, including environmental stressors such as bacterial or viral infection, ultraviolet (UV) exposure, and temperature changes; extracellular stressors such as osmolality changes and inflammatory cytokines; and intracellular stressors such as reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress, which are generated through cellular activities such as respiration and protein synthesis. To cope with these stresses, cells have developed various measures to maintain homeostasis. When the strength and/or duration of the stresses encountered exceed the cellular capacity to deal with them, homeostasis is disturbed, causing various diseases.

The stress-activated mitogen-activated protein kinase (MAPK) pathways have been widely adopted

as the defence systems against these stressors in eukaryotes. MAPK pathways are protein kinase cascades in which signals are relayed through phosphorylation of downstream kinases by activated upstream kinases, leading to the appropriate cellular responses. ASK1 is a member of the large MAPK kinase kinase (MAP3K) family that activates downstream MAPKs, c-Jun N-terminal kinases (JNKs) and p38 MAPKs, and it plays a pivotal role in various stress responses, including cell death, differentiation, and production of inflammatory cytokines^{1,2} (Fig. 1). ASK1 is activated in response to various stresses, such as ROS, ER stress, lipopolysaccharide (LPS), and calcium overload (Fig. 2). A series of studies using ASK1-deficient mice whose phenotype is different from that of other MAP3K-deficient mice³ have highlighted the importance of stress-dependent ASK1 activation *in vivo*. For example, ASK1-deficient mice show decreased infarct size and increased resistance to myocardial cell death when subjected to myocardial ischaemia-reperfusion injury in which ROS play a critical role (see 3.1). The analysis of ASK1-deficient mice has also demonstrated the requirement of the ASK1-p38 pathway for immune responses (see 3.4).

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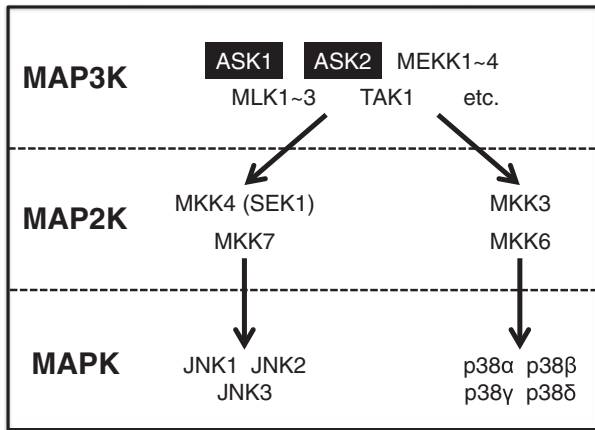


Fig. 1. Mammalian stress-activated MAPK cascades. ASK1 and ASK2 are among a number of MAP3Ks that activate three isoforms of JNK and four isoforms of p38 through activation of MKK4/MKK7 and MKK3/MKK6, respectively.

Many recent studies using inhibitor compounds have revealed that the components of the ASK1-MAPK pathways have potential as therapeutic targets for the diseases in which ASK1 is involved.⁴⁾⁻⁷⁾ Among such compounds, p38 inhibitors have been extensively studied for the treatment of inflammatory diseases, including rheumatoid arthritis.⁸⁾⁻¹⁰⁾ Although p38 inhibitors show a definite efficacy in animal models, accumulating evidence suggests that they are not necessarily suitable for clinical use due to their toxicity.¹¹⁾⁻¹⁵⁾ Upstream kinases such as ASK1 have been proposed as alternative therapeutic targets because control of such kinases may facilitate the precise regulation of stress responses through the JNK and p38 pathways and thus provide less toxic pharmaceutical treatment options.

In this review, we briefly summarise the regulatory mechanisms behind ASK1 signaling (reviewed

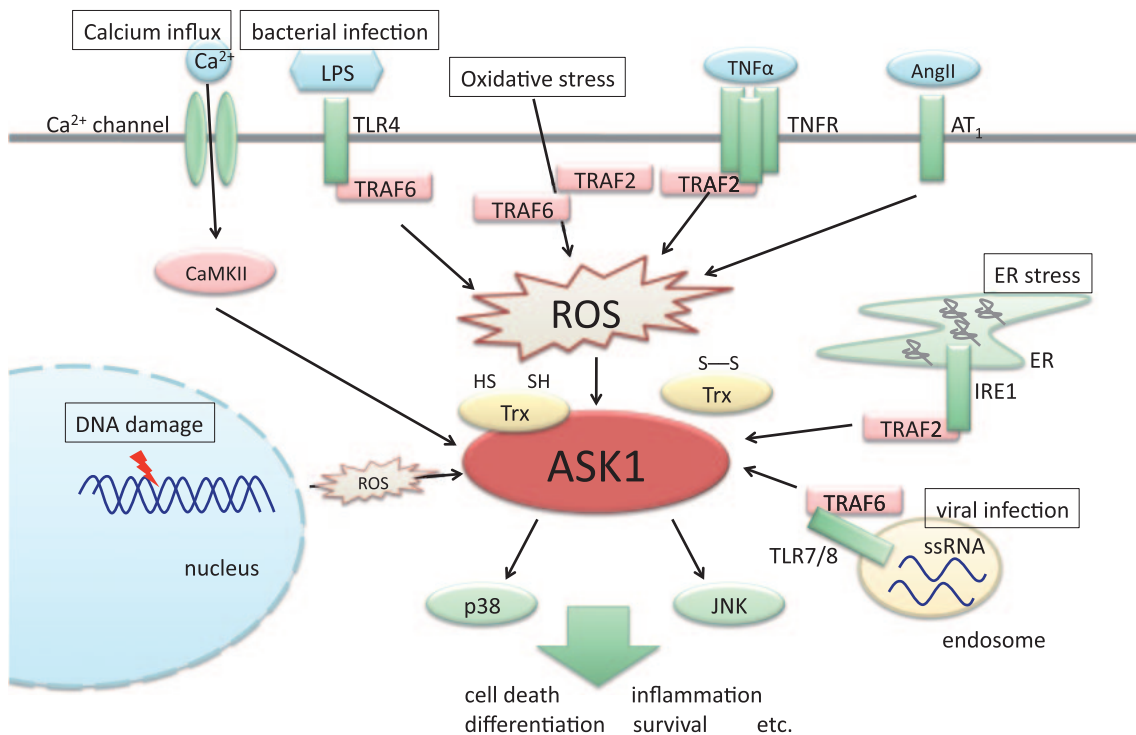


Fig. 2. Overview of the functions of ASK1. ASK1 is activated by various stimuli, such as oxidative stress, ER stress, calcium influx, DNA damage-inducing agents, and receptor-mediated signaling through TNF receptor (TNFR), AngII receptor type 1 (AT₁), and Toll-like receptors TLR4, 7, and 8. Intracellular signaling molecules, such as TNFR-associated factor (TRAF) family proteins, TRAF2 and TRAF6, and CaMKII, act as activators of ASK1. In addition to such ASK1-activating molecules, many stimuli that activate ASK1 use reactive oxygen species (ROS) as signaling intermediates. Thioredoxin (Trx) is a redox protein that changes its structure depending on the cellular redox state. Only the reduced form of Trx binds to the N-terminus of ASK1 and inhibits ASK1 activity. Upon ROS stimulation, Trx is converted to the oxidized form and is dissociated from ASK1, leading to ASK1 activation. Activated ASK1 in turn activates the downstream p38 and JNK pathways and induces various cellular responses, including cell death, inflammation, differentiation, and survival.

in detail elsewhere²⁾) and then focus on the roles of ASK1 in various diseases, mainly as demonstrated by analyses of ASK1-deficient mice.

2. Mechanisms of ASK1 activation

ASK1 was identified as a MAP3K that activates the MAP kinase kinase 4 (MKK4)/MKK7-JNK and MKK3/MKK6-p38 pathways.¹⁾ Activation of these pathways induces cellular responses such as apoptosis, differentiation, cell survival, and production of inflammatory cytokines.^{16)–18)} ASK1 is activated in response to various stresses, such as oxidative stress, ER stress, calcium overload, and inflammatory signals, including those induced by tumour necrosis factor α (TNF α) and LPS.^{19)–22)} Sustained activation of ASK1 induces apoptosis mainly through mitochondria-dependent caspase activation,^{19),23),24)} and mouse embryonic fibroblasts (MEFs) derived from ASK1-deficient mice undergo less apoptosis in response to oxidative stress or TNF α . Indeed, TNF α -induced apoptosis requires ROS-dependent activation of the ASK1-JNK/p38 pathways. These findings suggest that ASK1 is required for the execution of ROS-induced apoptosis.¹⁷⁾ ASK1 also mediates signaling involved in cell fate determination, such as differentiation and survival. For example, angiotensin II (AngII) activates ASK1 via the AngII type I receptor and induces cardiac hypertrophy²⁵⁾ (see 3.1.2).

ASK1 is highly conserved among eukaryotes. Invertebrates, such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, have a single homologue of ASK1 named NSY-1 and DASK1, respectively.^{26),27)} Mammals also express ASK2, which is another MAP3K highly homologous to ASK1.²⁸⁾ The activation of ASK1 is tightly regulated by phosphorylation of a threonine residue (Thr838 in human ASK1) within the activation loop of the kinase domain, which appears to be a common activation mechanism among the ASK family of proteins, *i.e.*, ASK1, ASK2, NSY-1, and DASK1.^{2),29)} The coiled-coil domain in the C-terminus of ASK1 is also essential for the homo-oligomerization and activation of ASK1.²⁹⁾ ASK1 mutant lacking this C-terminal coiled-coil domain fails to form a homo-oligomer and displays lower kinase activity than wild-type ASK1 under unstimulated conditions. On the other hand, artificial homo-oligomerization of this mutant leads to autophosphorylation and activation, suggesting homo-oligomerization through the C-terminal coiled-coil domain is important for ASK1 activation.

It has been reported that ASK1 activity is regulated by many ASK1-interacting proteins,²⁾ among which thioredoxin (Trx) plays an important role.¹⁹⁾ Trx is a redox protein that changes its structure depending on the cellular redox state. Only the reduced form of Trx binds to the N-terminus of ASK1 and inhibits ASK1 activity by inhibiting homophilic interaction through the N-terminal coiled-coil domains in the preexistent ASK1 oligomer under unstimulated conditions. Upon ROS stimulation, Trx is converted to the oxidized form and is dissociated from ASK1, which allows tight oligomerization through the N-terminal coiled-coil domain and thereby activates ASK1 by inducing autophosphorylation of ASK1. ASK1 activation in response to TNF α and LPS signaling has been reported to depend on ROS generation, suggesting that ROS play a key role in the regulation of ASK1 activity.^{17),30)}

ASK2 was originally identified as an ASK1-interacting protein. ASK2 is unstable and inactive in ASK1-deficient cells, suggesting that ASK1 is necessary for ASK2 stabilisation and activation. Once ASK2 is stabilised in a heteromeric complex with ASK1, ASK2 in turn directly activates ASK1 by phosphorylation of ASK1 at Thr838. These findings suggest that ASK1 and ASK2 form a stable heteromeric complex in which they activate one another.²⁸⁾

Posttranslational modifications, including phosphorylation, play a critical role in regulation of ASK1 activity. For example, Akt negatively regulates ASK1 activity by direct phosphorylation of ASK1 on Ser83,^{31),32)} while calcium/calmodulin-dependent protein kinase type II (CaMKII) likely activates the ASK1-p38 pathway in response to calcium influx through phosphorylation of an unidentified residue in ASK1.²¹⁾ Thus far, MAP3K kinases (MAP4Ks) that directly activate ASK1 by phosphorylating Thr838 of ASK1 have not been identified. In addition to phosphorylation, a recent study suggests that ubiquitination of ASK1 is important for its stability and thus plays a role in the net activation of ASK1 and ROS-induced cell death.³³⁾

3. ASK1 in diseases

In this section, we describe various mammalian diseases in which the ASK1-MAPK pathways are reported to be involved (summarized in Table 1).

3.1. Cardiovascular diseases and ASK1.

3.1.1. Ischaemia/reperfusion injury. It is well known that ROS are key factors involved in the pathogenesis of ischaemia reperfusion (IR) injury

Table 1. Diseases related to ASK1

Disease	Stress	Cell/pathology	Ref.
Vascular diseases			
Ischemia/reperfusion injury (cardiac muscle)	ROS, Ca ²⁺	myocardial cell death	34),37),38),42),51)
Ischemia/reperfusion injury (kidney)	Hypoxia, ROS?	renal tubule epithelial cell apoptosis; leukocyte infiltration	44),131)
Ischemia/reperfusion injury (other tissues)	ER stress, ROS?	retinal neurons apoptosis, spinal cord apoptosis	45),46)
Cardiac remodeling	ROS, mechanical stress, inflammatory cytokines, AngII	cardiomyocyte death; cardiomyocyte hyperplasia and fibrosis	25),51),52)
Vascular injury	AngII	apoptosis and eNOS dimer disruption	53),54)
Atherosclerosis	disturbed blood flow, TNF α , oxidized LDL	inflammation	132),133)
Brain ischemia	oxidative stress	apoptosis	134)
Neurodegenerative disorders			
PolyQ disease	ER stress	neuronal cell death	20),55)–59)
ALS	ER stress	motor neuron death	61)–64)
AD	oxidative stress	neuronal cell death	68),69),73),135)
PD	oxidative stress, ER stress	dopaminergic neuron death	75)–80),136)–138)
Normal tension glaucoma	oxidative stress	retinal ganglion cell apoptosis	81),82)
Mesial temporal lobe epilepsy	ER stress	neuronal cell death?	83)
Progressive cervical cord compression	mechanical stress?	spinal cord apoptosis	84)
Sensorineural deafness/retinal dystrophy	light, oxidative stress	sensory cell death	139)
Inflammatory diseases			
Multiple sclerosis	TLR	chemokine production	4)
RA	TNF α , LPS	cytokine production	93)
Tumor			
Skin cancer	DNA damage, ROS	inflammation; apoptosis	103)
Colon cancer	TLR?	inflammation; innate immunity	104)
Gastric cancer	?	proliferation	105)
Breast cancer	DNA damage	cell migration; apoptosis	140),141)
Liver cancer	DNA damage?	apoptosis	106),142),143)
melanoma	?	?	107)

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(IRI). Reperfusion to ischaemic tissues, which is caused by infarction, induces ROS production and results in cell death. However, ASK1-deficient cardiomyocytes are resistant to H₂O₂- or calcium-induced cell death.³⁴⁾ In cardiomyocytes of neonatal rats, overexpression of heat shock transcription factor-1 (HSF-1), which is known to protect against cardiovascular diseases including IRI,^{35),36)} inhibits H₂O₂-induced JNK activation and apoptosis. This inhibitory effect of HSF-1 overexpression is cancelled by ASK1 overexpression, suggesting that ASK1 is negatively regulated by HSF-1 in H₂O₂-induced cardiomyocyte death.³⁷⁾ Compared to young animals, IR-induced cardiomyocyte apoptosis and infarct size

are increased in ageing animals. This appears to be caused by the increased nitration of Trx, a modification that inhibits Trx activity, and subsequent disruption of Trx-ASK1 interactions in the ageing heart.³⁸⁾ 14-3-3, which binds to phosphorylated ASK1 and suppresses ASK1 activity,^{39)–41)} has also been implicated in IRI.⁴²⁾ These findings suggest that ASK1 plays an important role in the pathogenesis of myocardial IRI.

ASK1 may also play a role in the kidney, where IR has been shown to induce kidney injury, as ASK1 is activated in response to hypoxia and induces apoptosis in renal tubule epithelial cells.⁴³⁾ Consistently, infiltration of leukocytes and cell death in

Continued.

Disease	Stress	Cell/pathology	Ref.
Infection			
Influenza virus	TNF α , IL-1 β	apoptosis	95),96)
HIV-1	FasL	macrophage apoptosis	97)
Japanese encephalitis virus	ROS	apoptosis	98)
Sepsis	TLR, ROS	inflammatory cytokine production; TF expression	22),99)
<i>Mycobacterium tuberculosis</i>	TLR, ROS	monocyte macrophage apoptosis	100),144)
Chronic hepatitis C virus	HCV core protein?	cytokine production; angiogenesis?	102)
BCG	TLR, ROS	macrophage cytokine production	101)
Other diseases			
Fanconi anemia	TNF α , ROS	hematopoietic cell apoptosis	108),109)
Asthma	LTD ₄ , NO	pulmonary vascular EC apoptosis; airway smooth muscle cell remodeling	110),145)
Hepatic steatosis	AngII, TNF α , ROS	hepatocyte apoptosis; fibrosis	54),115)
Diabetes	TNF α , ROS	pancreatic β cell apoptosis; inflammation; insulin resistance	116),118),119)
Liver injury induced by drug	ROS?	hepatocyte apoptosis	120),121)
Aging	ROS?	?	122),123)
Membranous nephropathy	complement, ROS	glomerular epithelial cell apoptosis	146)
Necrotizing enterocolitis	TNF, ROS	intestinal EC apoptosis	147)

renal tubules decreased after IRI in ASK1-deficient mice compared to wild-type (WT) mice. IR-injured ASK1-deficient mice had lower blood urea nitrogen and creatinine levels than WT mice, indicating that the kidney was less damaged in ASK1-deficient mice after injury.⁴⁴⁾ ASK1 has also been reported to be involved in IRI of the retina and spinal cord.^{45),46)}

3.1.2. Cardiac remodelling. During myocardial infarction, some cardiomyocytes undergo necrosis due to a shortage of oxygen and nutrition, which causes low cardiac output. To compensate for this loss of cardiac function, surviving cardiomyocytes undergo changes in size and location, which is referred to as cardiac hypertrophy.⁴⁷⁾ Sustained hypertension and diabetic cardiomyopathy can also induce cardiac hypertrophy. In ventricular hypertrophy, gene reprogramming and accumulation of extracellular matrix proteins are critically involved in ventricular fibrosis and remodelling.⁴⁸⁾

AngII is a key factor in the renin-angiotensin system that regulates blood pressure and has been suggested to have an important role in cardiovascular diseases.^{49),50)} AngII binds to the AngII receptor type I (ATI) and activates ASK1, which causes cardiac remodelling, including cardiomyocyte hyperplasia and fibrosis, and cardiomyocyte death.²⁵⁾ ASK1-

deficient mice have smaller infarct size than WT mice.³⁴⁾ It has also been reported that ASK1-deficient mice show limited enlargement of the heart after left coronary artery ligation compared to WT mice.⁵¹⁾ In addition, the number of apoptotic cells is decreased in ASK1-deficient hearts. These findings suggest that ASK1 plays a critical role in the pathogenesis of ventricular remodelling by promoting apoptosis or cardiomyocyte hypertrophy. ASK1 has also been suggested to be involved in aldosterone-induced cardiac inflammation and fibrosis through induction of monocyte chemoattractant protein (MCP)-1 and transforming growth factor (TGF)- β 1 expression, respectively.⁵²⁾

3.1.3. Vascular endothelial dysfunction. Vascular endothelial cells maintain microcirculatory homeostasis, and their dysfunction leads to arteriosclerosis or thrombus formation. Many factors can induce vascular endothelial dysfunction, such as hypertension and obesity. AngII-induced endothelial cell apoptosis and inhibition of eNOS activity occur in Dahl salt-sensitive hypertensive rats (DS rats), resulting in vascular endothelial dysfunction and hypertensive diastolic heart failure. ASK1 deficiency or inhibition of ASK1 by administration of valsartan, an ATI blocker, diminishes endothelial apoptosis

and inhibition of eNOS.⁵³⁾ Likewise, another ATI blocker, olmesartan, also suppresses ASK1 activation, vascular endothelial dysfunction and remodeling, and cardiac inflammation and fibrosis in high fat diet (HFD)-fed obese and diabetic mice. ASK1 deficiency also attenuates HFD-induced vascular endothelial impairment by preserving eNOS activity.⁵⁴⁾ These findings suggest that activation of ASK1 by AngII accelerates vascular endothelial dysfunction by promoting apoptosis and inhibiting eNOS activity.

3.2. Neurodegenerative disorders and ASK1.

3.2.1. PolyQ diseases. Polyglutamine (PolyQ) diseases are inherited neurodegenerative disorders, such as Huntington's disease (HD), spinobulbar muscular atrophy, and some types of spinocerebellar ataxia (SCA), including SCA3/Machado-Joseph disease (MJD). In these diseases, insoluble aggregations of pathogenic proteins with expanded polyQ repeats are thought to disturb the ubiquitin-proteasome system and cause ER stress, resulting in neuronal dysfunction and/or cell death.²⁰⁾

HD is an autosomal, dominantly inherited disorder that is representative of polyQ diseases and is characterized pathologically by the degeneration of striatal and cortical neurons and the appearance of neuronal inclusions. HD is caused by the expansion of polyQ repeats in the N-terminus of the huntingtin (htt) protein. ASK1 has been demonstrated to play a role in the pathogenesis of polyQ diseases, including HD. Primary neurons from ASK1-deficient mice show resistance to expanded polyQ repeat- and ER stress-induced neuronal cell death through JNK activation.²⁰⁾ HD model mice, which express exon 1 of the human HD gene, have increased ASK1 expression and ER stress in the striatum and cortex,⁵⁵⁾ while a rat model of HD has been shown to have high levels of phosphorylated c-Jun, a major substrate of JNK.⁵⁶⁾ These findings suggest that ER stress activates the ASK1-JNK pathway *in vivo*. Consistently, inhibition of ASK1 activity using an anti-ASK1 antibody prevents atrophy of striatal neurons and improves motor dysfunction in HD model mice.⁵⁵⁾ In addition, injection of mice with the mitochondrial complex II inhibitor 3-nitropropionic acid (3-NP), which is known to induce HD-like brain lesion, elevates ASK1 activity in an age- and oxidative stress-dependent manner, and infusion of ASK1 siRNA prevents 3-NP-induced neuronal cell death.⁵⁷⁾ Moreover, genetic allelic analysis of HD patients demonstrates that sequence variations of the *MAP3K5* and *MAP2K6* genes, which encode ASK1

and MKK6, respectively, appear to modify the age of onset of HD.⁵⁸⁾

Another polyQ protein, ataxin-1 (ATXN1), has been suggested to contribute to neuronal cell death in SCA1. ATXN1 activates the ASK1-JNK pathway, and the activation of JNK promotes the sumoylation and aggregation of ATXN1, which is supposed to have a crucial role in the pathogenesis of SCA1.⁵⁹⁾ Together, these findings suggest the importance of ASK1 in polyQ diseases.

3.2.2. ALS. Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterized by the selective loss of motor neurons in the spinal cord, brain stem, and cerebral cortex. One of the genes responsible for inherited familial ALS (FALS) is Cu/Zn-superoxide dismutase 1 (SOD1).⁶⁰⁾ Mutant SOD1 protein specifically causes motor neuron death, but the mechanism remains controversial.⁶⁰⁾

Immunohistochemical analysis revealed that FALS model mice, which express the ALS-linked SOD1 mutant (SOD1(mut)), exhibit activation of ASK1 and p38 concomitant with motor neuron death.^{61),62)} One of the mechanisms by which the SOD1(mut) activates ASK1 and causes neuronal cell death is through interaction with the putative ER translocon Derlin-1 and inhibition of ER-associated degradation (ERAD), which in turn evokes ER stress and ASK1 activation, resulting in cell death.⁶³⁾ This hypothesis is supported by the fact that a polypeptide of the cytosolic region of Derlin-1 that disrupts the SOD1(mut)-Derlin-1 interaction can inhibit SOD1(mut)-induced cell death. Moreover, ASK1 deficient FALS model mice exhibit attenuated motor neuron loss and have longer life spans. In addition, the p38 inhibitor semapimod mitigates SOD1(mut)-induced motor neuron degeneration.⁶⁴⁾ Thus, the ASK1-p38 pathway could be a good target for the treatment of ALS.

3.2.3. Alzheimer's disease. Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid β ($A\beta$) accumulation in cerebral senile plaques and neurofibrillary tangles containing the microtubule-associated protein tau.⁶⁵⁾ $A\beta$ is generated by the sequential cleavage of the amyloid precursor protein (APP) by two intramembrane proteases, β - and γ -secretases. Under physiological conditions, $A\beta_{40}$ is mainly generated, whereas $A\beta_{42}$ is produced under pathological conditions; mutations of the substrate APP and the protease presenilin 1/2 have been suggested to be involved in this process.^{66),67)}

APP itself, through dimerization, can activate the ASK1-MKK6-p38 pathway and induce hyperphosphorylation of tau, which is a main component of neurofibrillary tangles in AD.⁶⁸⁾ It has also been suggested that APP and ASK1 form a complex with MKK6, JNK, and JIP1 in the brain of APP transgenic mice.⁶⁹⁾

ROS are also likely to play important roles in the pathogenesis of AD. A β impairs mitochondrial redox activity and increases ROS generation, and A β -induced neuronal cell death is attenuated by antioxidant treatment, suggesting that oxidative stress is involved in the pathogenesis of AD.^{70)–72)} It has been shown that A β activates ASK1 through ROS production, rather than through ER stress. Thus, ROS-mediated ASK1 activation may be one of the key mechanisms for A β -induced neurotoxicity.⁷³⁾

3.2.4. Parkinson's disease. Parkinson's disease (PD) is a common neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of Lewy bodies in the brain. The dysfunction of proteins, such as parkin, PTEN-induced putative kinase 1 (PINK1), and DJ-1, has been implicated in the pathology of autosomal recessive juvenile parkinsonism (AR-JP).⁷⁴⁾

DJ-1 plays a neuroprotective role by antagonizing oxidative stress, and accumulating evidence suggests that DJ-1 negatively regulates ASK1. DJ-1 appears to change its conformation upon exposure to oxidative stress and then binds to and inhibits ASK1.⁷⁵⁾ Another mechanism by which DJ-1 inhibits ASK1 is dependent on the death-associated protein Daxx, which activates ASK1;⁷⁶⁾ DJ-1 binds and sequesters Daxx in the nucleus and prevents it from translocating to the cytosol, where it activates ASK1.⁷⁷⁾ Administration of the neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) leads to PD-like dopaminergic neuronal death, and therefore, mice treated with these compounds are used as PD models. MPTP administration activates ASK1, decreases the amount of DJ-1 protein, and permits the translocation of Daxx from the nucleus to the cytosol.⁷⁸⁾ Intriguingly, it has been reported that DJ-1 and redox enzymes such as Trx are more abundant in female mice than in male mice. Consistent with this finding, MPTP administration activates ASK1 more strongly in male mice than in female mice, and the progressive dopaminergic cell loss in the substantia nigra is attenuated in female mice compared to male mice. These finding might account for the

fact that incidence of PD is lower in women than in men.^{76),78),79)} Paraquat is another known parkinsonism toxin, and ASK1 is reported to also be involved in paraquat-induced neuronal death.⁸⁰⁾

3.2.5. Normal-tension glaucoma. The classical definition of glaucoma is compression of the optic nerve due to elevation of intraocular pressure, resulting in visual-field constriction. In contrast, normal-tension glaucoma is a progressive disease with neuronal atrophy and visual-field constriction without increased intraocular pressure. Experiments using ASK1-deficient mice have revealed that ischaemia- or oxidative stress-induced ASK1-p38 activation is involved in apoptosis of retinal ganglion cells (RGCs).⁸¹⁾ Mice deficient in the glutamate/aspartate transporter (GLAST), a major glutamate transporter in the retina, display optic nerve degeneration and are used as model mice for normal-tension glaucoma.⁸²⁾ ASK1 deficiency prevents optic nerve degeneration and improves visual-field parameters in GLAST-deficient mice. In ASK1-deficient Müller glial cells, TNF α -induced p38 activation and inducible nitric oxide (NO) synthase production are attenuated. In addition, TNF α -induced cell death is suppressed in ASK1-deficient RGCs.⁸¹⁾ These findings suggest that ASK1 activity in both neuronal and glial cells is crucially involved in normal-tension glaucoma.

3.2.6. Other neurodegenerative diseases. ASK1 is also reported to play important roles in other neuronal diseases. Expression of ASK1 and activation of JNK and the ER stress-associated kinase IRE1 α are upregulated in the hippocampi of patients with refractory mesial temporal lobe epilepsy.⁸³⁾ Tiptoe-walking Yoshimura (TWY) mice are a mouse model of progressive cervical cord compression, which is manifested by degenerative spinal cord changes, such as myelin destruction, loss of axons and oligodendrocytes in the white matter, and loss of neurons in the gray matter. It has recently been shown using TWY mice that the ASK1-JNK/p38 pathways are activated in both neurons and oligodendrocytes in compressed spinal cords.⁸⁴⁾

3.3. Inflammatory diseases and ASK1.

3.3.1. Multiple sclerosis. Multiple sclerosis (MS) is a common neurological disease characterized by demyelination, axonal degeneration, and neuronal loss in the brain and spinal cord. Although the pathogenic mechanism that underlies MS is not fully understood, an autoimmune response to components of the central nervous system (CNS) is thought to cause the destruction of myelin sheaths and axons.⁸⁵⁾

Experimental autoimmune encephalomyelitis (EAE) is a model for diseases associated with brain inflammation, including MS, because the clinical features of EAE, such as inflammation and demyelination, resemble those of MS.⁸⁶⁾ EAE is evoked by immunization with CNS homogenates or myelin-derived antigens such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and myelin proteolipid protein (PLP). Chemokines from astrocytes, such as MCP-1; RANTES (regulated upon activation, normal T-cell expressed, and secreted); and macrophage inflammatory protein-1 α (MIP-1 α), induce migration of inflammatory cells, such as T lymphocytes and microglia, to the CNS.^{87)–89)} Moreover, inhibition of glia cell activation suppresses the release of chemokines from astrocytes and attenuates the severity of EAE.⁹⁰⁾ Recently, it has been revealed that the ASK1-p38 pathway is necessary for toll-like receptor (TLR)-mediated production of chemokines from astrocytes, and that ASK1-deficiency in EAE mice results in a less severe phenotype than in WT EAE mice.⁴⁾ Consistent with these findings, oral administration of a specific, low-molecular-weight inhibitor of ASK1, MSC2032964A (its molecular characteristics will be described in the section 4), suppresses EAE-induced inflammation in both the spinal cord and optic nerves. Importantly, MSC2032964A ameliorates the severity of EAE in WT mice to the same degree as ASK1-deficiency, strongly suggesting that MSC2032964A specifically inhibits ASK1 activity *in vivo*.⁴⁾ Thus, treatment with compounds that inhibit ASK1 may be a promising approach for the treatment of inflammatory diseases.

3.3.2. Rheumatoid arthritis. Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial cytokine production, inflammation in the synovium, and joint destruction. Because p38 has been strongly suggested to be involved in the production of cytokines, such as TNF α and IL-1, and consequently in the pathogenesis of RA,⁹¹⁾ some small-molecule p38 inhibitors have been subjected to clinical trials.^{12)–14)} Nevertheless, even though they are effective against inflammatory diseases in animal models, none have been successful in humans, perhaps due to their low efficacy and high toxicity.¹⁵⁾

In the K/BxN serum transfer model, a well-established model of RA,⁹²⁾ ASK1-deficiency, or administration of the p38 inhibitor SD-0006 to WT mice leads to attenuation of edema, cartilage destruction, bone erosion, and general inflammatory responses.⁹³⁾ Transcriptional induction of many

inflammatory genes, such as those encoding cytokines, chemokines, and extracellular matrix degradative enzymes, is also attenuated in ASK1-deficient mice compared to WT mice.⁹³⁾ Moreover, treatment with the JNK inhibitor SP600125 or knockdown of ASK1 partially inhibits the induction of IL-6 in human synovial fibroblasts isolated from RA patients (RASf) following stimulation with TNF α .⁹³⁾ In addition, treatment of RASf with both p38 and JNK inhibitors almost completely abolishes TNF α -induced IL-6 production.⁹³⁾ These findings suggest that the ASK1-JNK/p38 pathways play a critical role in RA and that inhibition of ASK1 may have a positive therapeutic effect on RA through suppression of both the p38 and JNK pathways.

3.4. Infectious diseases and ASK1.

3.4.1. Influenza virus. Cells infected with influenza virus (IV) undergo apoptosis, which is thought to be a pivotal mechanism to inhibit virus amplification. Induction of the expression of death receptors, such as Fas, DR4, and DR5, is one mechanism of IV-induced apoptosis.⁹⁴⁾ ASK1 also contributes to apoptosis of IV-infected cells as the ASK1-JNK/p38 pathways are activated in IV-infected human bronchial epithelial cells. Moreover, ASK1-deficient MEFs show little activation of JNK and p38 and are resistant to IV-induced cell death.⁹⁵⁾ TLR7 and TLR8 are pattern-recognition receptors that recognise single strand RNA, which constitutes the IV genome, and have been reported to activate ASK1.⁹⁶⁾ The death of human myeloid cells, which has been associated with ligand-induced TLR7/8-mediated inflammatory stress, depends on activation of ASK1, although ASK1 may not be required for the production of pro-inflammatory cytokines such as TNF α and IL-1 β in this particular setting.⁹⁶⁾

3.4.2. HIV-1. Human immunodeficiency virus type 1 (HIV-1) causes acquired immunodeficiency syndrome (AIDS) through destruction of immune cells, especially CD4-positive helper T-cells. In virally infected T-cells, the Nef protein of HIV-1 induces FasL, which facilitates apoptosis of neighbouring T-cells that express Fas. Conversely, Nef protects the infected cells from death signals by *cis* ligation of Fas and FasL through binding to and inactivation of ASK1. This mechanism seems to be important for immune evasions and subsequent survival of HIV-1.⁹⁷⁾

3.4.3. JEV. It has recently been reported that Japanese encephalitis virus (JEV) induces apoptosis in human promonocyte cells through ASK1 activation.⁹⁸⁾ The downregulation of Trx and upregulation

of intracellular ROS are proposed to be involved in this process.

3.4.4. Sepsis. Sepsis is a systemic inflammatory response syndrome, which is caused by bacterial toxins (*e.g.*, LPS) or excess cytokines in the blood resulting from bacterial infection. Sepsis evokes dysfunctions of multiple organs, such as the lungs, kidney, and liver. ASK1-deficient splenocytes and bone marrow-derived dendritic cells show decreased LPS-induced p38 activation and production of inflammatory cytokines, such as TNF α , IL-6, and IL-1 β , compared to WT cells.²²⁾ LPS-induced ASK1-p38 activation is attenuated by treatment with the antioxidant N-acetyl-L-cysteine (Nac), suggesting the involvement of ROS. Interestingly, upon ligation of LPS to its receptor, Toll-like receptor 4 (TLR4), ROS is generated in the cells and triggers the dissociation of Trx from ASK1 and facilitates p38-dependent production of inflammatory cytokines through ASK1 activation. Consistent with this molecular mechanism, ASK1-deficient mice are resistant to LPS-induced septic shock and consistently show low levels of TNF α and NO in the serum.²²⁾ LPS-induced tissue factor (TF) expression also requires ASK1.⁹⁹⁾ Pulmonary microvascular expression of TF is suggested to initiate the activation of the blood coagulation cascade, resulting in acute lung injury by LPS.

3.4.5. Other infectious diseases. *Bacillus Calmette-Guérin* (BCG)- or mycobacterium tuberculosis-infected macrophages undergo apoptosis and induce inflammatory response through ASK1-p38 activation.^{100),101)} Infection with hepatitis C virus (HCV) leads to production of cytokines, such as TGF β and VEGF, which is thought to mediate the development of hepatic angiogenesis in patients with chronic HCV infection and depends on JNK, p38 and possibly ASK1 signaling.¹⁰²⁾

3.5. Tumorigenesis and ASK1. How ASK1 is involved in tumorigenesis is still not fully understood. Nevertheless, accumulating evidence suggests that the diverse functions of ASK1 and ASK2 in apoptosis, inflammation, proliferation, and migration are involved in tumorigenesis. Regulation of apoptosis in damaged cells and precancerous cells plays an important role in tumorigenesis. Suppression of apoptotic signals confers resistance to death signals and various cellular stresses leading to malignant transformation. Cytotoxic T lymphocytes (CTLs) also contribute to the elimination of cancer cells. Some cancer cells protect themselves from CTLs by highly expressing FasL, which evokes apoptosis of

CTLs. In addition, inflammation plays important roles in tumorigenesis by promoting the proliferation of transformed cells mainly through production of inflammatory cytokines.

3.5.1. Skin cancer. Papillomas can be developed in two-stage skin tumorigenesis experiments in which the dorsal skin of mice is treated once with 7,12-dimethylbenz(a)anthracene (DMBA; tumour initiation) and then continuously with 12-*O*-tetradecanoylphorbol-13-acetate (TPA; tumour promotion). ASK2-deficient mice develop increased papillomas compared to WT mice in this model.¹⁰³⁾ The observation that ASK2-deficient keratinocytes show resistance to DMBA-induced apoptosis suggests that ASK2 suppresses tumorigenesis by eliminating damaged cells during the tumour initiation phase. The tumour-suppressive role of ASK2 is also supported by the finding that ASK2 expression is reduced in some human cancer cell lines and tissues.¹⁰³⁾ In ASK1-deficient keratinocytes, DMBA-induced apoptosis is also suppressed to a similar level as in ASK2-deficient keratinocytes, consistent with the fact that ASK2 only functions in a heteromeric complex with ASK1.²⁸⁾ Intriguingly, however, ASK1-deficient mice develop fewer papillomas than ASK2-deficient mice, suggesting that ASK1 has a tumour-promoting role in addition to its tumour-suppressive role. In fact, TPA-induced production of inflammatory cytokines, such as TNF α and IL-6, is suppressed in ASK1-deficient mice, but not in ASK2-deficient mice, suggesting that ASK1 exerts its tumour-promoting function through production of inflammatory cytokines. These findings suggest that ASK1 and ASK2 are critically involved in tumorigenesis by differentially regulating apoptosis and inflammation.

3.5.2. Colon cancer. Patients with inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, are prone to colitis-associated cancer, which exemplifies the critical involvement of inflammation in carcinogenesis. ASK1-deficient mice are more susceptible to colonic inflammation than WT mice in mouse models in which colitis is induced by dextran sodium sulfate (DSS) or the Gram-negative pathogen *Citrobacter rodentium*.¹⁰⁴⁾ ASK1-deficient macrophages are defective in their ability to kill bacteria and undergo increased bacterial-induced apoptosis, probably due to the impairment of p38-dependent expression of anti-apoptotic genes. Bone marrow transplantation experiments indicate that ASK1 deficiency in myeloid cells promotes severe colonic inflammation. In addition, ASK1-deficient mice developed more numerous and larger tumours

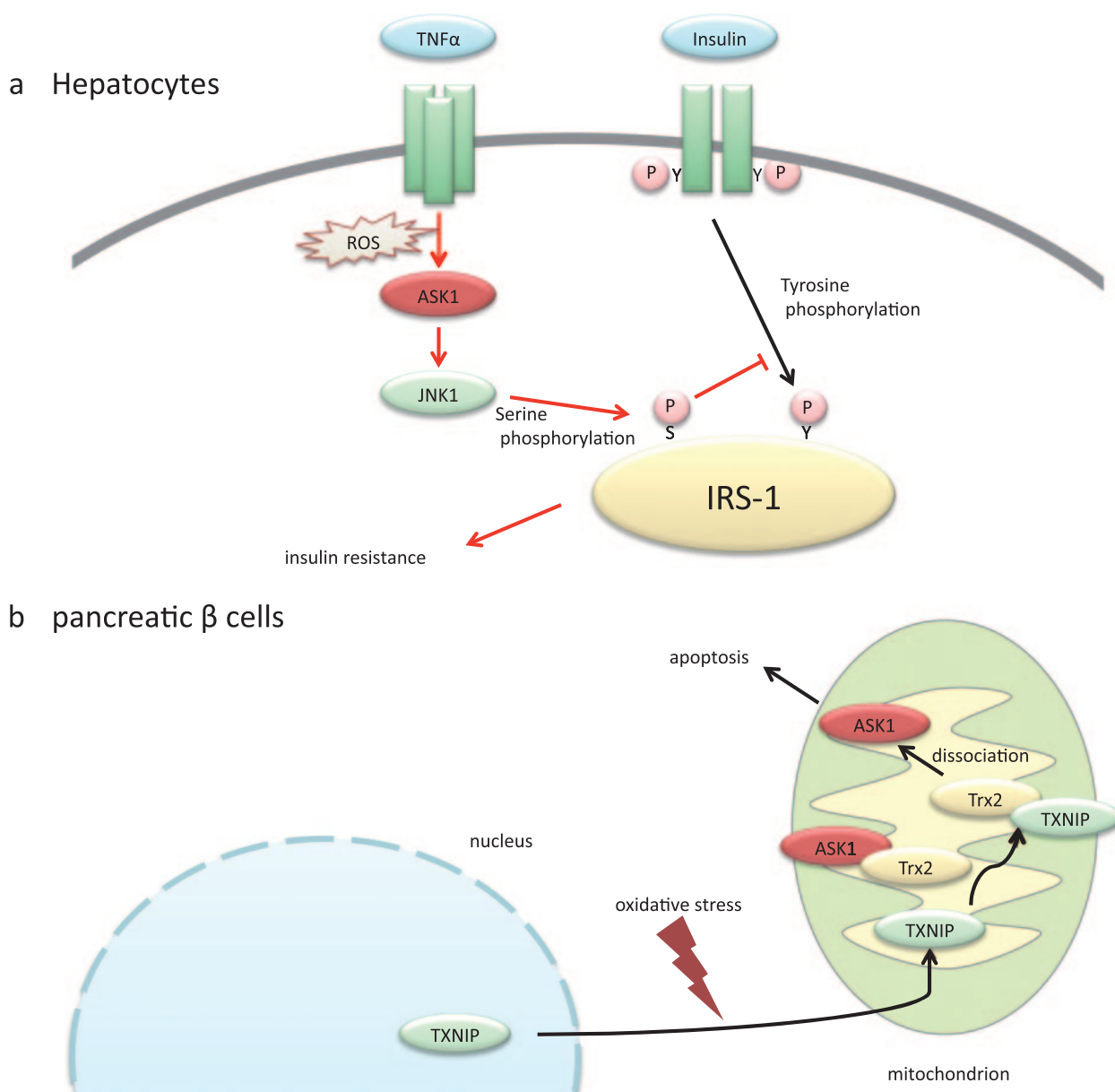


Fig. 3. Possible involvement of ASK1 in insulin resistance and pancreatic β cell apoptosis. a) The ASK1-JNK pathway is involved in insulin resistance. In liver cells, TNF α activates the ASK1-JNK pathway through ROS production, which induces serine phosphorylation of insulin receptor substrate-1 (IRS-1) and subsequent inhibition of tyrosine phosphorylation of IRS, thereby inducing insulin resistance. b) ASK1 is involved in TXNIP-dependent pancreatic β cell apoptosis. In pancreatic β cells, TXNIP is localised mainly in the nucleus. Upon oxidative stress, TXNIP translocates into the mitochondria, binds to Trx2, and dissociates Trx2 from ASK1, leading to ASK1-dependent apoptosis.

than WT mice in the azoxymethane (AOM)/DSS colitis-associated cancer (CAC) model. These findings suggest that ASK1 contributes to the suppression of intestinal inflammation and CAC mainly by regulating innate immunity.¹⁰⁴⁾

3.5.3. Gastric cancer. Recently, a link between ASK1 and gastric cancer (GC) has also been

reported.¹⁰⁵⁾ GC cells have increased levels of ASK1 protein compared to non-GC cells. Studies using a mouse model of GC, in which *N*-methyl-*N*-nitrosourea (MNU) is administered, have revealed that ASK1 deficiency leads to a decrease in the number and size of tumours. Protein expression of ASK1 and c-Jun increases in these experimental tumours,

whereas increased apoptosis is not observed. Knockdown of ASK1 suppresses cell proliferation in various GC cell lines but not in other cancer cell lines such as colon, pancreatic, and lung cancer cell lines. These data suggest that ASK1 positively regulates gastric carcinogenesis by promoting cell proliferation. In this process, ASK1 and cyclin D1 appear to form a positive feedback loop to promote cell proliferation.¹⁰⁵⁾

3.5.4. Liver cancer. Administration of diethylnitrosamine (DEN) is a well-established method used to create a mouse model for liver cancer. In this model, it has recently been reported that the number of detected tumours is higher in ASK1-deficient mice than in WT mice.¹⁰⁶⁾ Whereas cell proliferation is comparable in cancerous tissues from both types of mice, cancer cell apoptosis is suppressed in cancerous tissues in ASK1-deficient mice, suggesting that ASK1 suppresses hepatocellular carcinogenesis through its pro-apoptotic activity.

3.5.5. Melanoma. Recently, exome sequencing of metastatic melanoma cells reveals somatic mutations in the genes encoding ASK1 and another MAP3K, mixed lineage kinase 1 (MLK1).¹⁰⁷⁾ In the prevalent screen, mutations in the protein-coding regions of ASK1 and MLK1 are found in 9% and 15% of 85 melanoma cell lines examined, respectively, and are almost mutually exclusive. In addition, 85% and 67% of melanoma cell lines have the loss of heterozygosity for the *ASK1* and *MLK1* genes, respectively, suggesting that the mutations are inactivating. In fact, some mutations of ASK1 and MLK1 are shown to inactivate their kinase activity, and overexpression of ASK1 and MLK1 mutants in HEK293T cells reduces the phosphorylation of downstream MAPKs. Although the role of ASK1 mutations in melanoma remains unclear at present, inactivation of ASK1-mediated signaling may critically contribute to melanomagenesis and metastasis.

3.6. Other diseases and ASK1.

3.6.1. Fanconi anaemia. Fanconi anaemia (FA) is an autosomal recessive genetic disorder typified by reduction of haematopoietic stem cells in the bone marrow. FA patients are prone to chromosomal abnormalities, tumorigenesis, and apoptosis of haematopoietic stem cells. Hypersensitivity of haematopoietic progenitors to oxidative stress and cytokines such as TNF α and IFN γ contributes to the pathogenesis of bone marrow failure in FA. Cells deficient in the murine Fanconi anaemia, complementation group C (Fancc) protein, one of the genes responsible for FA, are susceptible to H₂O₂ and cytokines. It has

been suggested that apoptosis of Fancc-deficient cells treated with H₂O₂ or TNF α is dependent on ASK1 activation.^{108),109)}

3.6.2. Asthma. ASK1 is involved in NO-induced activation of the transcription factor activator protein-1 (AP-1), which is thought to induce airway inflammation, resulting in bronchial asthma.¹¹⁰⁾ ASK1 also plays an important role in airway remodelling, an irreversible hypertrophic change that occurs in chronic bronchitis. Leukotriene D₄ has been suggested to activate ASK1 and induce AP-1 activation in airway smooth muscle cells, leading to airway remodelling.¹¹⁰⁾

3.6.3. Hepatic steatosis. Non-alcoholic fatty liver disease (NAFLD) is a common liver disease characterized by fat accumulation in hepatocytes that is not linked to excessive alcohol intake and is correlated with obesity, insulin resistance, and cardiac diseases.¹¹¹⁾ NAFLD is categorised into simple steatosis and non-alcoholic steatotic hepatitis (NASH), the latter of which can lead to hepatic fibrosis, hepatic cirrhosis, and liver cancer.¹¹²⁾ HFD is used to induce hepatic steatosis in mouse models. HFD causes fat accumulation and fatty acid oxidation, which leads to ROS generation and subsequent hepatocyte dysfunction and cell death in the liver.^{113),114)} TNF α -deficient mice show reduced hepatic steatosis, indicating that proinflammatory cytokines including TNF α are required for liver injury. TNF α -induced apoptosis of hepatocytes is mediated by ASK1-JNK activation.¹¹⁵⁾ ASK1-deficient mice have reduced HFD-induced hepatic steatosis, fibrosis, and TGF β expression, which is responsible for hepatic fibrosis.⁵⁴⁾ Olmesartan, an ATI blocker, also improves HFD-induced hepatic steatosis by inhibiting ASK1. Moreover, olmesartan or ASK1 deficiency can also attenuate HFD-induced cardiac inflammation and fibrosis, and vascular endothelial dysfunction and remodelling. These findings suggest that ASK1 is involved in obesity-associated cardiovascular complications and hepatic steatosis.

3.6.4. Diabetes. TNF α is one of the factors that aggravate insulin resistance. In hepatocytes, TNF α induces ROS production in the mitochondria and activates JNK via ASK1, which leads to insulin receptor substrate-1 (IRS-1) serine phosphorylation. Such phosphorylation decreases tyrosine phosphorylation of IRS-1 resulting in insulin resistance and eventually causing type 2 diabetes¹¹⁶⁾ (Fig. 3a).

Thioredoxin-interacting protein (TXNIP) is required for glucotoxicity-induced pancreatic β cell apoptosis and development of type 1 and 2

diabetes.¹¹⁷⁾ Upon oxidative stress, TXNIP translocates from the nucleus to the mitochondria, where it deprives ASK1 of Trx2, the mitochondrial-localised isoform of Trx, leading to ASK1 activation and initiation of the apoptosis cascade in pancreatic β cells¹¹⁸⁾ (Fig. 3b).

Variant analysis in Pima Indians, a Native American tribe with high susceptibility to type 2 diabetes, indicates that genetic variants in the *ASK1* gene that alter ASK1 expression affect insulin resistance.¹¹⁹⁾ Three point mutations in the promoter or intron regions of the *ASK1* gene are associated with decreased expression of ASK1 in the skeletal muscle and development of type 2 diabetes. This report suggests that ASK1 expression accelerates insulin action in the skeletal muscle and antagonises the development of type 2 diabetes. Considering the role of ASK1 in hepatocyte insulin resistance described above, ASK1 may have diverse effects on insulin signaling depending on the cell type and cellular context, although further investigation will be needed for the understanding of the role of ASK1 in diabetes.

3.6.5. Liver injury. An overdose of acetaminophen, a widely used analgesic and antipyretic agent that is usually safe at therapeutic doses, is known to cause liver injury. In ASK1-deficient mice, acetaminophen-induced, sustained activation of JNK is suppressed and resistance to liver injury increased, indicating that the ASK1-JNK pathway plays a critical role in acetaminophen-induced liver injury.¹²⁰⁾ ASK1 has also been reported to be involved in liver injury induced by troglitazone, a first-generation thiazolidinedione insulin sensitizer that has been linked to an unacceptable risk of liver injury in patients.¹²¹⁾

3.6.6. Ageing. ROS is thought to be one of the major causes of ageing. Consistent with this notion, long-lived mouse models, such as Snell dwarf mice, Ames dwarf mice, and Klotho overexpressing mice, are known to be resistant to oxidative stress. MEFs derived from Ames dwarf mice possess a larger amount of the Trx-bound form of ASK1 and have less p38 activity than those derived from WT mice, suggesting that activity of the ASK1-p38 pathway is attenuated in Ames dwarf mice.¹²²⁾ Also, in the livers of Klotho overexpressing mice, the activity of the ASK1-p38 pathway and the amount of Trx-bound ASK1 are decreased and increased, respectively, whereas the opposite is observed in liver extracts from Klotho-deficient mice.¹²³⁾ These findings suggest that ROS-induced ASK1 activity contributes to regulation of ageing-related cellular functions.

4. Inhibitors of ASK1

In this section, we will review the emerging small molecule inhibitors of ASK1. Two classes of compounds have been identified as ASK1 ATP-competitive inhibitors by virtual screening. One class includes 3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones. Further *in vitro* experiments have revealed that, among their derivatives, ethyl 2,7-dioxo-2,7-dihydro-3*H*-naphtho[1,2,3-*de*]quinoline-1-carboxylate (NQDI-1) shows the strongest inhibitory effect against ASK1 with a K_i of 500 nM and an IC_{50} of 3 μ M.¹²⁴⁾ NQDI-1 specifically inhibits ASK1, but hardly inhibits other kinases, such as casein kinase 2 (CK2), JNK3, and Aurora A. Another class includes 2-thioxo-thiazolidines, among which derivatives, 5-bromo-3-(4-oxo-2-thioxo-thiazolidine-5-ylidene)-1,3-dihydro-indol-2-one is the most active compound with an IC_{50} of 2 μ M.¹²⁵⁾

Another virtual screening approach has identified a number of compounds that inhibit ASK1. This screening consisted of two approaches, ligand-based and structure-based virtual screening. The ligand-based screening used the ASK1 inhibitors that had been reported by Takeda Pharmaceutical Co. Ltd. (Uchikawa, O., Sakai, N., Terao, Y. and Suzuki, H. WO 2008016131 A1, 2008; EP 2058309 A1, 2009; US 20100029619, 2010) as queries for a similarity search. On the other hand, the structure-based screening used a protein-ligand complex model as a query. In this screening, X-ray crystal structure of the ASK1 kinase domain was adopted.¹²⁶⁾ Among a total of 845 compounds identified by this screening, 20 hit compounds were identified by *in vitro* ASK1 inhibition assays. Moreover, using these compounds as queries, a similarity search was carried out. As a result, five compounds were identified with higher inhibitory activity. These compounds commonly have a purine ring, which is assumed to make a bond with the hinge region of the ASK1 kinase domain. These compounds exhibit an IC_{50} of 13.3 μ M at the most.¹²⁷⁾

MSC2032964A is a lead compound optimized from a hit compound MSC1946002A, which was identified by high-throughput screening. This compound shows a high potency on ASK1 (IC_{50} of 93 nM), a high selectivity, and a good *in vitro* absorption, distribution, metabolism, and excretion (ADME) profile. MSC2032964A can block LPS-induced ASK1 and p38 activation *in vitro*, and the administration of it to EAE mice can also reduce demyelination, decrease astrocyte and microglia

activation, and suppress chemokine production, leading to attenuation of the disease course in EAE mice, as described in the section 3.3.1.⁴⁾

5. Summary and outlook

The evidence described in this review strongly suggests that ASK1 and, in some cases, ASK2 play pivotal roles in the pathogenesis and pathology of a wide range of diseases in which ROS and/or ER stress may be common pathogenic factors. On one hand, ASK1 may prevent stress-induced disorders and protect the whole body from bacterial and viral infection under physiological circumstances. On the other hand, ASK1 appears to exert adverse effects, possibly through the aberrant induction of cellular apoptosis and differentiation and the exaggerated production of inflammatory cytokines, under some pathological conditions, such as those found in neurodegenerative disorders, cardiovascular diseases, inflammatory diseases, and chronic inflammation-induced carcinogenesis.

Among the therapeutic approaches to these diseases, p38 inhibitors have received much attention as promising drugs. However, p38 inhibitors were shown to have high toxicity and insufficient clinical efficacy in clinical trials for RA.¹²⁸⁾ One possible reason for this limited efficacy is that p38 has been reported to display not only proinflammatory properties but also anti-inflammatory properties through production of anti-inflammatory cytokines.¹²⁹⁾ Alternatively, limited efficacy of p38 as a drug treatment has been proposed to be due to the redundancy of signaling networks. Inhibition of the p38 pathway can lead to the compensatory upregulation of redundant pathways, including the JNK pathway, which has also been implicated in RA pathogenesis.¹⁵⁾ In this regards, ASK1 may be an alternate therapeutic approach because inhibition of ASK1 efficiently suppresses stress-induced activation of both p38 and JNK.

The high toxicity of p38 inhibitors implies that complete inhibition of p38 activity is deleterious, and in fact, p38 α -deficient mice exhibit embryonic lethality.¹³⁰⁾ Considering that JNK1/2 double knockout mice also exhibit embryonic lethality, JNK inhibitors would likely induce similar adverse effects. However, ASK1 could be a good alternate therapeutic target because inhibition of ASK1 is expected to selectively suppress the stress-induced activity of p38 and JNK without profoundly affecting their basal activity. It may be important to selectively inhibit the stress-induced activation, but not the basal

activity, of p38 and JNK, because complete inhibition of p38 or JNK, including their basal activity, causes adverse effects as exemplified by the lethality of mice deficient in p38 or JNK as described above. This notion is supported by the finding the ASK1-deficient mice have no obvious abnormalities at least under unstimulated conditions.¹⁷⁾ Nevertheless, it should be noted that ASK1 inhibitors may still cause adverse effects, such as increased susceptibility to infection and exacerbation of precancerous cells. To avoid such adverse effects and achieve therapeutic effects by inhibiting ASK1, further analysis of the precise regulatory mechanisms of ASK1 activity and a more complete understanding of how ASK1 is involved in human diseases are required.

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Profile

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