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REVIEW PAPER

TAK1 signaling is a potential therapeutic target for pathological angiogenesis

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Abstract

Angiogenesis plays a critical role in both physiological responses and disease pathogenesis. Excessive angiogenesis can promote neoplastic diseases and retinopathies, while inadequate angiogenesis can lead to aberrant perfusion and impaired wound healing. Transforming growth factor β activated kinase 1 (TAK1), a member of the mitogen-activated protein kinase kinase kinase family, is a key modulator involved in a range of cellular functions including the immune responses, cell survival and death. TAK1 is activated in response to various stimuli such as proinflammatory cytokines, hypoxia, and oxidative stress. Emerging evidence has recently suggested that TAK1 is intimately involved in angiogenesis and mediates pathogenic processes related to angiogenesis. Several detailed mechanisms by which TAK1 regulates pathological angiogenesis have been clarified, and potential therapeutics targeting TAK1 have emerged. In this review, we summarize recent studies of TAK1 in angiogenesis and discuss the crosstalk between TAK1 and signaling pathways involved in pathological angiogenesis. We also discuss the approaches for selectively targeting TAK1 and highlight the rationales of therapeutic strategies based onTAK1 inhibition for the treatment of pathological angiogenesis.

Keywords Transforming growth factor β activated kinase 1 · Angiogenesis · Inflammation · Hypoxia · Oxidative stress

Introduction

Angiogenesis is the process by which new capillaries grow from preexisting blood vessels. It is fundamental in embryonic vascular development and reproduction, as well as wound healing and repair in adults. During embryonic development, angiogenesis is the basis for the maturation ofthe circulatory system. Angiogenesis starts with the proliferation of endothelial cells, followed by endothelial tube formation, a process enriched by smooth muscle cells, and later facilitates the formation of a specific vascular system [1]. Upon injury, severed vessels are elongated and anastomosed with each other, and the vessels then become tortuous with endothelial cell proliferation and pericyte coverage, eventually normalizing through vessel regression over a few months [2]. Angiogenesis is a highly regulated process that is activated under physiological stresses and inactivated when those stresses are relieved [3].

Angiogenesis is also central to several pathological conditions, such as solid tumors and neovascular eye diseases. Under pathological conditions such as tumor growth, host blood vessels are stimulated to grow into the vicinity of the tumor to maintain cell growth, and tumor vascularization is characterized by dilated, tortuous and disorganized blood vessels [4, 5]. Tumors constantly promote the growth of new blood vessels to ensure an adequate supply of nutrients for expansion, and these new vessels also provide potential routes for tumor metastasis [6]. In addition, pathological

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angiogenesis, a process responsive to inadequate perfusion or ischemia, occurs in some eye diseases, particularly in the retina. Such ocular neovascularization can result in severe impairment of vision. For instance, retinal neovascularization occurs in patients with advanced diabetic retinopathy, often leading to fundus hemorrhage and severe vision impairment due to invasion and leakage of fluid from abnormal blood vessels into the retina and vitreous.

Transforming growth factor β (TGF-β)-activated kinase 1 (TAK1) is a key regulator of immune and proinflammatory signaling pathways [7]. TAK1 activates nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways in response to a diverse range of stimuli, including inflammation, hypoxia and oxidative stress, the major causes of angiogenesis [8]. Activation of the NF-κB and MAPK pathways regulated by TAK1 promotes the expression of various inflammatory response proteins, including those encoding cytokines and chemokines, and participates in inflammasome regulation, all of which in turn facilitate angiogenic processes [9]. Recent studies have also found that, in addition to its role in mediating inflammatory signals, activated TAK1 can prevent endothelial apoptosis and maintain vascular integrity under inflammatory conditions [10]. In fact, TAK1 deficiency leads to embryonic lethality due to vascular destruction, which implies its crucial role in maintaining vascular integrity during embryogenesis [11]. Therefore, a better understanding of the mechanism that underlies TAK1-mediated signaling in angiogenesis is of great significance for developing therapeutic strategies for the management of pathological angiogenesis.

TAK1 and its activity

TAK1 was discovered in 1995 as a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family [12]. It is a critical signal transduction mediator that can be activated by cell membrane receptor interacting protein kinases or second messengers in cells after a variety of stimulations, including proinflammatory cytokines or antigens such as tumor necrosis factor α (TNF-α), IL-1, or lipopolysaccharides (LPS). Normally, TAK1 binds to adaptor proteins such as TAK-binding protein-1 (TAB1) and its homologs TAB2 and TAB3 to form heterotrimeric complexes consisting of either TAK1–TAB1–TAB2 or TAK1–TAB1–TAB3 [8]. TAB1 binds to the N-terminal kinase domain of TAK1, whereas the homologs TAB2 and TAB3 bind to the C-terminal region (Fig. 1). Through different signaling pathways, both TAB1 and TAB2 activate the TAK1 protein. TAB1 is essential for osmotic stress-induced TAK1 activation, whereas TAB2 or TAB3 is required for TNF-α- or IL-1-induced TAK1 activation (Fig. 2) [13].

Although TAB1 constitutively binds to TAK1, it possesses no phosphatase or other enzymatic activity. Pathak et al. recently reported that glycosylation with *N*-acetylglucosamine (O-GlcNAcylation) of a single residue (Ser395) on TAB1 can modulate the activation of TAK1 in response to IL-1 stimulation or osmotic stress [14]. *O*-GlcNAcylation of TAB1 substantially increases the autophosphorylation of TAK1, phosphorylation of inhibitory kappa B kinase (IKK) and translocation of NF-κB, which results in increased production of cytokines. Moreover, an E3 ubiquitin ligase X-linked inhibitor of apoptosis protein (XIAP) has also been found to directly interact with TAB1 and to further activate TAK1 as a downstream biological factor in TGF-β receptor (TGFBR) and bone morphogenetic protein (BMP) receptor (BMPR) activation through formation of the XIAP–TAK1–TAB1 complex. Activated TAK1 then upregulates the expression of NF-κB and transcription factor activator protein-1 (AP-1) by activating the NF-κB and MAPK (JNK and p38) pathways [15] (Fig. 2a). However, the detail mechanism by which TAK1 is activated by TAB1 remains unclear.

In contrast to TAB1, TAB2 and its analogous protein TAB3 have been extensively studied in TAK1-mediated signaling pathways in angiogenesis. When IL-1 and LPS bind to their receptors, interleukin-1 receptor kinase 1 (IRAK1) and IRAK4 recruit TNF receptor (TNFR)-associated factor-6 (TRAF6) and its associated enzymes ubiquitin conjugating enzyme 13 (Ubc13) and ubiquitin E2 variant

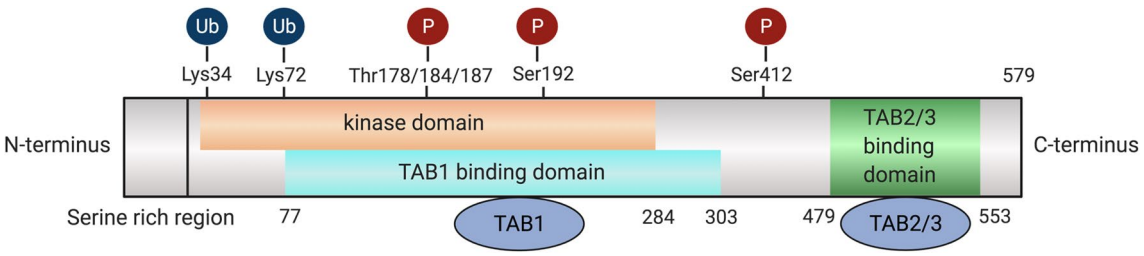


Fig. 1 Schematic illustration of the domain structures of human TAK1 and TABs. The kinase activity of TAK1 is mediated by binding interactions with TAB1 and its homologs TAB2/3. TAB1 binds to the N-terminal kinase domain of TAK1, whereas the homologs TAB2 and TAB3 bind to the C-terminal region, resulting in the activation of TAK1 catalytic activity

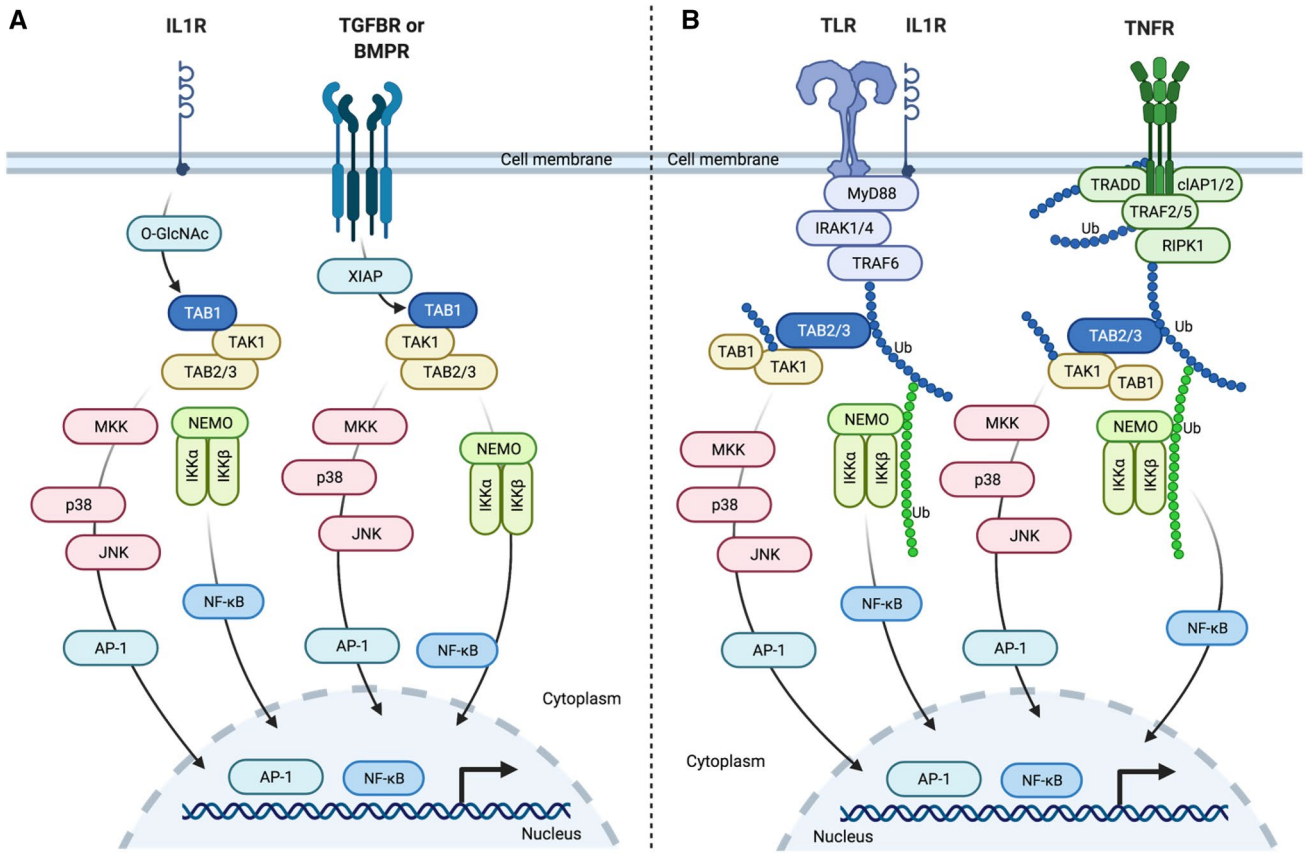


Fig. 2 Interaction between TAK1 and TABs. **a** Proinflammatory ligands bind to IL-1R, TGF-β receptor (TGFBR) and bone morphogenetic protein receptor (BMPR) to trigger interaction with TAK-binding protein-1 (TAB1) and further activate TAK1. Activated TAK1 activates IKK complex and MKKs/MAPKs (p38 MAPK and JNK), which further activates NF-κB and AP-1. **b** Proinflammatory ligands bind to IL-1R, Toll-like receptor (TLR) and TNF receptor (TNFR). All these interactions trigger the strong interaction of TAB2/3 with K63-linked polyubiquitin chains to activate TAK1, which subsequently activates IKK complex and MKKs/MAPKs (p38 MAPK and JNK) to activate NF-κB and AP-1, ultimately regulating inflammation, proliferation and angiogenesis processes. String of beads: polyubiquitination. Created with BioRender.com

1a (Uev1a). In a similar manner, when TNF-α binds to TNFR, receptor interacting protein kinase (RIPK1) recruits TRAF2/5 with its associated enzymes Ubc13 and Uev1b. TRAF2/5 complexes generate lysine 63 (K63)-linked polyubiquitin chains on either TAB2 or TAB3, thus activating TAK1 [16–18]. K63-linked polyubiquitin activates TAK1 by inducing conformational changes that lead to the autophosphorylation of Thr187, Thr178, Thr184, and Ser192 residues [19, 20]. Activated TAK1 then phosphorylates IKK, MKK4/7 and MKK3/6 to activate NF-κB, JNK and p38 MAPK, respectively (Fig. 2b). Collectively, these involved signaling pathways result in inflammation and immune responses, apoptosis and angiogenesis [8].

TAK1–TAB2 maintains vascular homeostasis under TNF-α stimulation by preventing endothelial apoptosis [21]. Morioka et al. reported that cell migration and tube formation were significantly affected in TAK1- and TAB2-deficient endothelial cells but not in TAB1-deficient endothelial cells, suggesting that TAB2 instead of TAB1 plays an important role in angiogenesis [11]. Furthermore, TAB2 deficiency in mouse embryos led to the abnormal growth of capillary blood vessels due to reduced TAK1 activity, revealing that TAB2 is crucial for maintaining normal vascular homeostasis [11]. Nonetheless, the activation of TAK1 is arguably regulated by both TAB1 and TAB2/3, and their respective contributions are complex and dependent upon the tissue type and cellular context [22].

Molecular mechanisms of TAK1 involved in angiogenic activities

Inflammation is a physiological response to harmful stimuli such as pathogens, damaged cells and toxic compounds with the overall aim of removing the source of injury and repairing damaged tissue to restore tissue architecture and maintain tissue homeostasis [23]. When inflammation lasts for an extended period of time, endothelial cells proliferate and

migrate to form new capillaries to restore nutrient supply, therefore facilitating the immune response [24]. Inadequate supply of vasculature and the resultant reduction in oxygen level leads to angiogenesis to fulfil the oxygen needs of the tissue [25]. Apart from tissue hypoxia and inflammation, oxidative stress plays a significant role in angiogenesis. Oxidative stress is the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the tissue under harmful stimuli. However, short exposure to ROS or low levels of ROS can also promote physiological angiogenesis and maintain healthy blood vessel homeostasis [26]. There is substantial evidence that inflammation, hypoxia and oxidative stress are three important inducers of angiogenesis, and each has a complicated molecular mechanism for promoting and inhibiting angiogenic activities [6, 27]. Given that TAK1 is an important mediator in many pathways involved in angiogenesis, its role and function in mediating inflammation, hypoxia and oxidative stress in angiogenesis are discussed below.

TAK1 activates the inflammatory response

There is increasing evidence that inflammation plays a central role in various pathophysiological processes, such as angiogenesis. Inflammation has been shown to be involved in angiogenesis via several physiological processes, such as embryonic development and tissue repair [28], as well as angiogenic diseases, such as a variety of tumors and neovascular eye diseases. TAK1 has been identified as a key mediator in inflammation and defense immune signaling pathways [29]. TAK1 is activated by several inflammatory signaling pathways, such as the IL-1β, TNF-α, Toll-like receptor (TLR), T-cell receptor (TCR) and B-cell receptor (BCR) signaling pathways, after TRAF6 and the ubiquitin-binding enzyme complex (Ubc13 and Uev1a) catalyzes the polyubiquitination of the Lys63 residue on TAB2 or TAB3 [30]. Activated TAK1 phosphorylates NF-κB-inducing kinase (NIK) and IKK or MAP kinase kinases (MKKs), which leads to the activation of NF-κB and AP-1 [31], ultimately resulting in the expression of inflammatory cytokines (e.g., IL-1 and IL-6), chemokines (e.g., CXCL1 and IL-8) or adhesion molecules (e.g., intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)) that participate in tissue inflammatory and angiogenic responses (Fig. 3). As such, these inflammatory proteins induce diverse physiological and pathological effects, such as tissue repair and tumor progression [32–36]. Therefore, TAK1, which is involved in inflammatory signaling pathways, has been found to play a vital role in the development of multiple physiopathological conditions, especially in angiogenic processes.

A number of studies have attempted to determine the role of TAK1-induced inflammatory signaling in angiogenic diseases. Singh et al. found that *arginyltransferase* 1 *(ATE1)* gene knockout in mouse embryos can cause contractile dysfunction, cardiovascular dysplasia and impaired angiogenesis due to blockage of the TAK1-dependent JNK1/2 signaling pathway [37]. Moreover, blocking TAK1 inhibited NF-κB by downregulating the phosphorylation of IKKα/β and NF-κB p65, resulting in the reduced expression of proinflammatory genes, such as IL-6, monocyte chemoattractant protein-1 (MCP-1) and ICAM-1, in vascular smooth muscle cells, ultimately leading to attenuation of neointimal formation in wire-injured femoral arteries [38].

TNF-α can induce endothelial cell death during inflammation via either caspase-dependent apoptosis or RIP1 kinase-dependent necrosis [39]. Naito et al. found severe bleeding in the liver and small intestine in endothelial-specific TAK1 knockout mice. The study also showed that TAK1 is essential for endothelial cell survival through inhibition of inflammatory apoptosis induced by TNF-α during acute inflammation [10]. In addition, inhibiting TAK1 alleviated joint inflammation and pannus caused by abnormal neovascularization in a collagen-induced mouse model of rheumatoid arthritis [40], suggesting that TAK1 plays a crucial role in promoting inflammation and angiogenesis in rheumatoid arthritis. Chang et al. further demonstrated that TAK1 phosphorylation is enhanced upon adenosine monophosphate-activated protein kinase (AMPK) activation in vivo and in vitro, leading to a proinflammatory phenotype in endothelial cells that facilitates angiogenesis via a downstream p38 MAPK signaling cascade [41]. Moreover, overexpression of TAK1 and TAB1 also enhances the phosphorylation of AMPK in cervical cancer cells [42], suggesting that TAK1 and AMPK are more likely to act together rather than alone to regulate these processes under different circumstances.

TAK1 signaling in hypoxia

Hypoxia is known as a reduction in oxygen supply that cannot meet cellular requirements. It is one of the key mechanisms involved in both physiological and pathological angiogenesis. Among several regulatory genes in hypoxia, hypoxia-inducible factor 1 (HIF-1) plays an important role in facilitating the response of cells to changes in systemic oxygen levels. HIF-1 is a heterodimeric transcription factor that consists of a constitutively expressed β-subunit (HIF-1β) and an oxygen-regulated α-subunit (HIF-1α) [43]. HIF-1α contains an oxygen-dependent degradation (ODD) domain that is hydroxylated by prolyl hydroxylase 2 (PHD2) under normoxic conditions to degrade HIF-1 through the ubiquitin–proteasome pathway [44]. However, hypoxia can strongly induce HIF-1 accumulation by preventing HIF-1α ubiquitination, which activates anaerobic metabolism and inflammation-related signaling pathways, including the

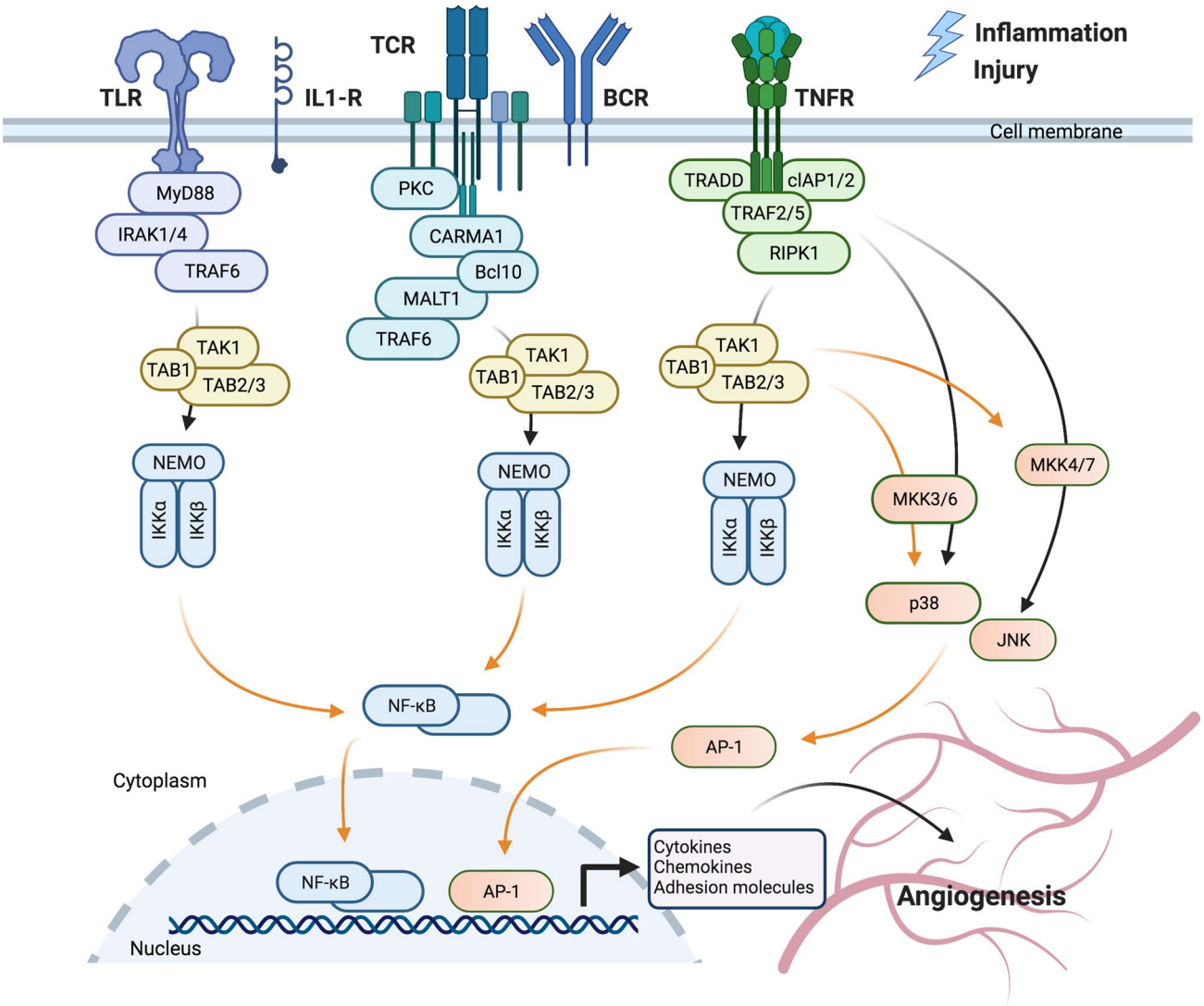


Fig. 3 Activation of TAK1 by injury and inflammation. Engagement of agonist with TNF receptor (TNFR) during inflammation and injury. The ubiquitin complex containing TRADD and TRAF activates TAK1, which subsequently activates IKK complex and MKKs/MAPKs (p38 MAPK and JNK) to activate NF-κB and AP-1. Both NF-κB and AP-1 increase the expression of various cytokines that contribute to angiogenesis. Ligands bind to IL-1R, BCR, TCR or TLR during inflammation and injury also trigger TAK1 activation. Created with BioRender.com

MAPK [45, 46], NF-κB [47] and AMPK [48, 49] pathways, in cells. Interestingly, TAK1 is closely related to these pathways [22], indicating that TAK1 may participate in hypoxia-induced angiogenesis (Fig. 4). Indeed, studies related to cancer progression and cardiomyocyte hypertrophy have shown that hypoxia can activate TAK1 via a mechanism that is dependent upon the activation of calcium calmodulin kinase (CaMK2) signaling and is mediated through the Ubc13–XIAP complex, resulting in the activation of NF-κB and the promotion of an inflammatory state in cells [50–52]. Such processes are mediated by NF-κB and MAPK signaling, both of which are downstream of TAK1 activation [53]. Unlike other signaling pathways, the role of TAK1 as a genuine upstream kinase of AMPK is still highly debated. In several studies, the potential role of TAK1 as an upstream mediator of AMPK activation was verified using various knockdown strategies [54–56]. Nagata et al. found that inhibition of AMPK signaling can inhibit endothelial cell migration and tube formation under hypoxic conditions and suppress the growth of blood vessels in mice subcutaneously implanted with Matrigel [57]. Although a number of studies have shown that TAK1 is closely related to hypoxia-induced angiogenesis, there is no clear evidence that TAK1 is causally involved in angiogenesis under hypoxic conditions.

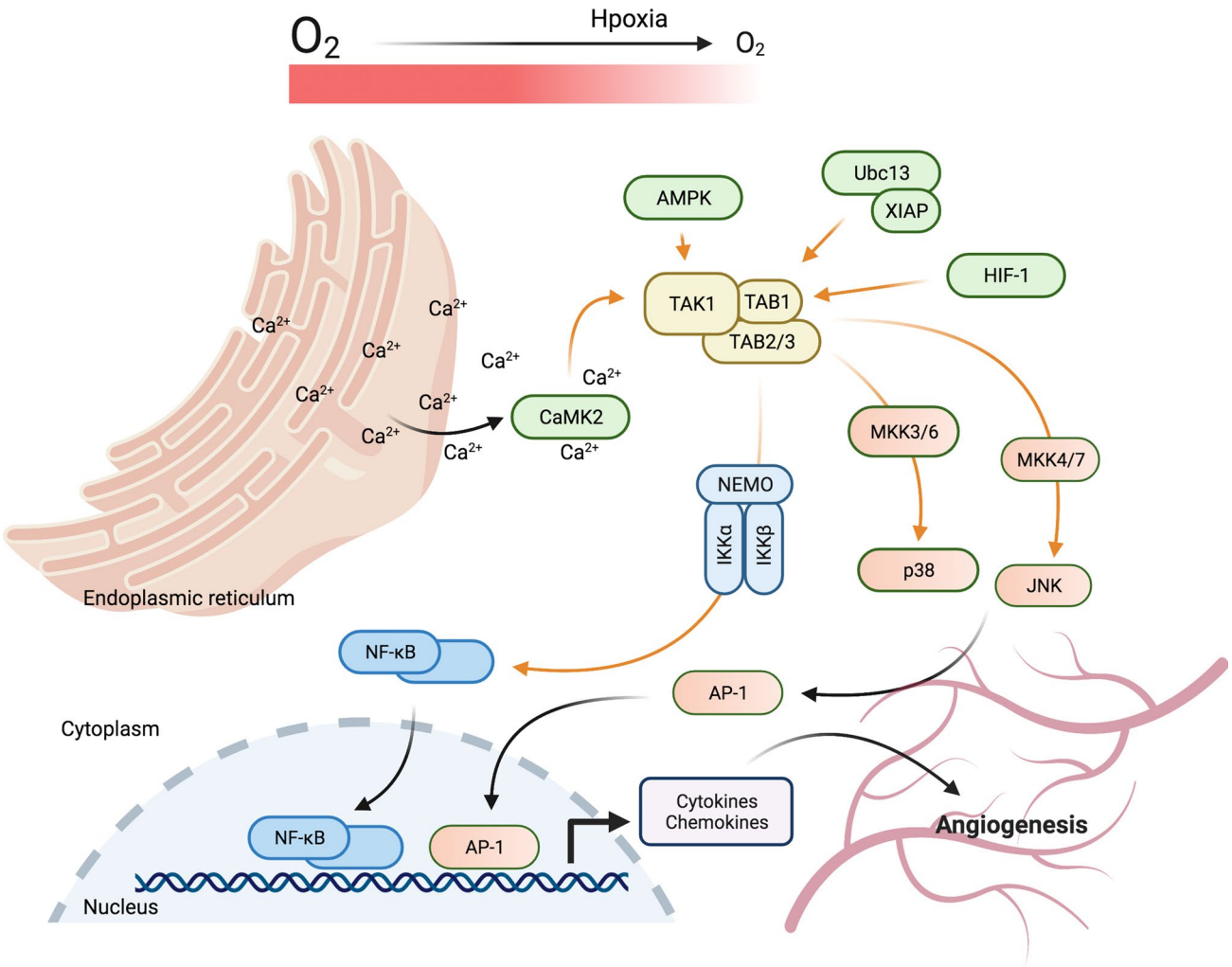


Fig. 4 Activation of TAK1 by hypoxia. Hypoxia activates TAK1 via the stimulation of CaMK2, AMPK and Ubc13–XIAP. Activated TAK1 activates IKK and MKKs/MAPKs, which further triggers the transcriptional activation of NF-κB and AP-1, leading to increased expression of various cytokines that contribute to angiogenesis. Created with BioRender.com

TAK1 signaling in oxidative stress

Angiogenesis can be affected by oxidative stress in different ways. When the degree of oxidation exceeds the oxide clearance rate, the oxidation system and antioxidant system become unbalanced, resulting in pathophysiological changes in tissue [27]. TAK1 also participates in redox regulation through various cellular signaling pathways [58], which may be related to pathological angiogenesis (Fig. 5). Zippel et al. reported that TAK1 knockdown by siRNA results in a significant change in the proteins that are involved in redox regulation in IL-1β-treated endothelial cells [59]. Kajino-Sakamoto et al. showed that ablation of TAK1 leads to the accumulation of ROS in the intestinal epithelium by reducing the expression of nuclear factor-erythroid 2 (NF-E2)-related factor 2 (NRF2), a key antioxidant transcription factor, and related antioxidant-responsive molecules [60]. ROS accumulation results in epithelial cell death, causing intestinal hemorrhage. NRF2 is known to promote angiogenesis by regulating NADPH oxidase 2 (NOX2) in several physical and pathological conditions, such as corneal neovascularization, ischemia-induced retinopathy, and tissue repair [61–64]. Reasonably, TAK1 is able to protect epithelial or endothelial cells from ROS-induced death by regulating NRF2 and NOX-related signals, promoting blood vessel formation. Indeed, Menden et al. reported that silencing NOX2 can suppress LPS-induced ICAM-1 expression through inhibition of TAK1 phosphorylation (Thr184/187) in human pulmonary microvascular endothelial cells, which limits macrophage-endothelial cell interactions and lung microvascular remodeling [65].

Superoxide dismutase (SOD), another endogenous antioxidant, also plays an important role in the oxidative stress response and is involved in TAK1-related angiogenesis.

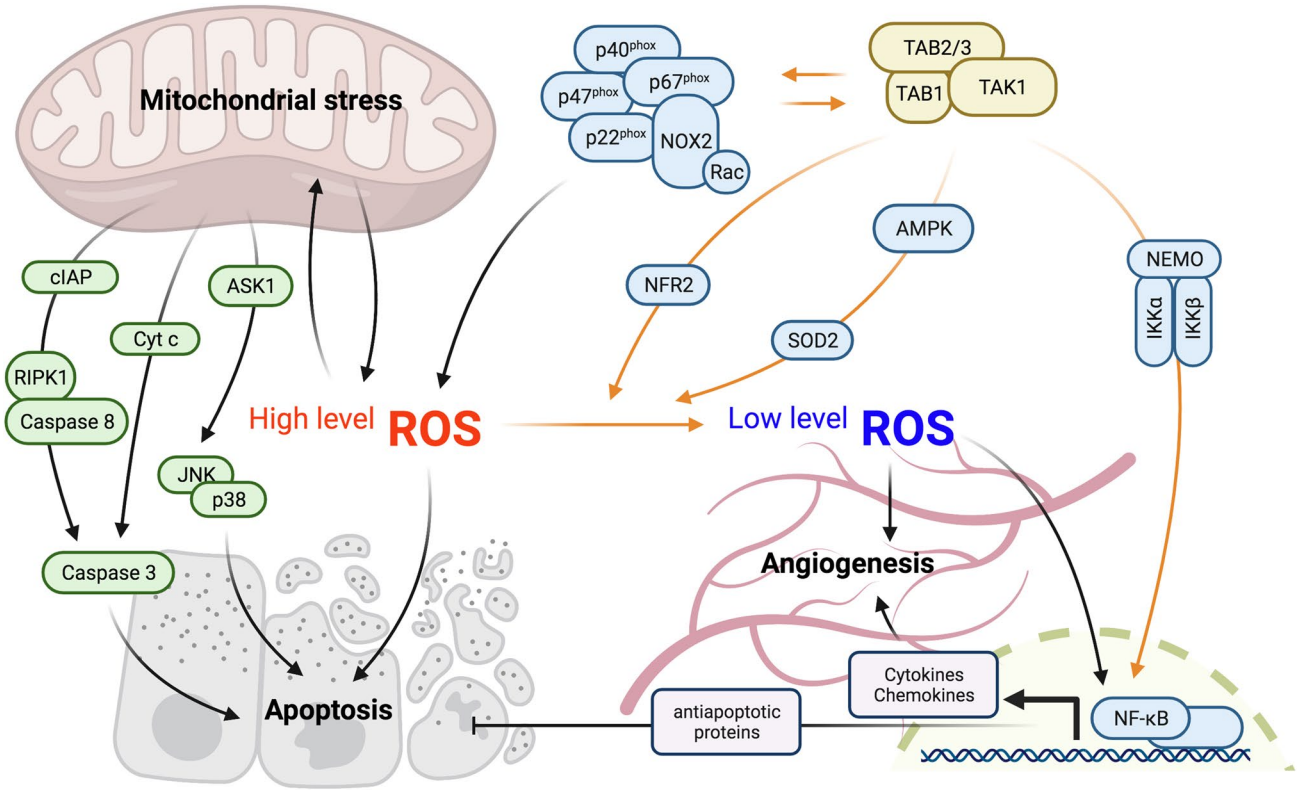


Fig. 5 TAK1 participates in redox balance. Activation of TAK1 prevents ROS accumulation, protects against ROS-induced apoptosis and enhances angiogenesis. TAK1 maintains ROS at levels that promote angiogenesis by activating NOX2 and upregulating endogenous antioxidants (such as NRF2 and SOD2). When TAK1 is active and the ROS level is low, triggers NF-κB transcriptional activation, leading to increased expression of angiogenic and antiapoptotic proteins, thereby promoting angiogenesis and inhibiting ROS-induced apoptosis. Created with BioRender.com

Zippel et al. found that TAK1 is an AMPK mediator that regulates angiogenesis by modulating SOD2 and redox signaling in endothelial cells [56]. Specifically, the dysregulated endothelial germination processes of ring and tube formation are normalized in the presence of polyethylene glycol-SOD under the condition of endothelial cell-specific TAK1 knockout in the aortic ring model. Similar rescue of angiogenesis was also observed in polyethylene glycol-SOD-treated aortic rings from AMPKα1 knockout mice [59]. Since AMPK can be activated under oxidative stress, can facilitate angiogenesis [66, 67], and closely interacts with TAK1, it can be implied that TAK1 plays a role in oxidative stress-induced angiogenesis. However, there is still a lack of evidence that redox signaling directly activates TAK1, and the crosstalk between TAK1 and oxidative stress signals in angiogenesis remains unclear.

Translational potentials in diseases associated with pathological angiogenesis

Angiogenesis is an important event in a variety of physiological settings, and it is also central to the pathogenesis of several pathological conditions. Activated TAK1 participates in crucial signaling pathways of inflammation, hypoxia and oxidative stress, which could lead to pathological angiogenesis under these conditions. We therefore discuss below the crosstalk between TAK1 and the signaling pathways involved in pathological angiogenesis processes, such as tumor angiogenesis and retinal neovascularization.

Tumor angiogenesis

Tumor growth and metastasis depend upon angiogenesis, which is usually stimulated by chemical signals from the tumor cells themselves [68]. Tumor cells may become necrotic or apoptotic without vascular support [69]. Therefore, tumors need to be supported by the rapid development of a new vascular network in order to progress [6]. TAK1 acts as a key mediator of angiogenic signaling in

tumor environments. TAK1 regulates MAPK signaling through P38 MAPK and JNK activation, which promotes the expression of VEGF, plasminogen activator inhibitor-1 (PAI-1) and MMPs, which are involved in vascular remodeling, angiogenesis and extracellular matrix degradation in tumors such as glioma [70, 71]. In addition to angiogenesis, TAK1 plays a significant role in preventing TNF-α-induced endothelial cell death. Knocking out or inhibiting TAK1 can induce the apoptosis of endothelial cells and destroy tumor vasculature, resulting in tumor regression [10]. Safina et al. showed that deletion of TAK1 can reduce the activity of NF-κB and the expression of MMP-9, thereby suppressing TGF-β-mediated tumor angiogenesis and metastasis [72]. Furthermore, studies also revealed that the inhibition of TAK1 with natural or artificial compounds such as cyclopeptide RA-V and triterpene celastrol suppressed angiogenesis and tumorigenesis.

In addition to endogenous angiogenic genes, hypoxia-related genes in the tumor microenvironment play a critical role in activating TAK1 and ultimately promoting tumor angiogenesis [73]. HIF-1α, a master regulator of the hypoxia response, can induce NF-κB activation in a TAK1-dependent manner [52]. Activation of this inflammatory pathway can in turn promote the expression of HIF-1α itself, forming a positive feedback loop. Such crosstalk between hypoxia and inflammation, which is centrally regulated by TAK1, further enhances tumor cell proliferation and angiogenesis [50]. Although there is still a lack of evidence that TAK1 directly leads to HIF-1 accumulation, it is reasonable to postulate that NF-κB might be a potential node between TAK1 and HIF-1.

Retinal neovascularization

Retinal neovascularization refers to abnormal vascular growth with increased permeability of blood vessels in the retina resulting in severe retinal hemorrhage and even blindness. Numerous studies have shown that VEGF signaling certainly plays a key role in retinal neovascularization [26, 74–76]. Hence, anti-VEGF drugs have been extensively studied and have been demonstrated to be effective in suppressing retinal neovascularization, thus ameliorating vision impairment. However, recent clinical trials showed that anti-VEGF therapy is not effective for all patients and that patients who benefit from treatment exhibit a high recurrence rate [77–79]. Therefore, it is important to look for other therapeutic target genes for retinal neovascularization.

Studies have shown that hypoxia and ischemia in the retina contribute to the progression of retinal neovascularization through HIF-1α- and NF-κB-related signaling involving TAK1 signaling [80]. TAK1 was found to be activated under hypoxic conditions, which stimulates the expression of proinflammatory and proangiogenic cytokines, including ICAM-1, IL-8 and TNF-α, through NF-κB [51, 52, 81]. Our recent study provided the first piece of evidence that TAK1 inhibition can significantly attenuate retinal neovascularization in a rat model of ischemia-induced retinopathy [82]. The data further suggest that selective inhibition of TAK1 by 5Z-7-oxozeaenol ameliorates the inflammatory response, which contributes to the promotion of aberrant retinal angiogenesis [82]. Furthermore, hypoxia and ischemia in the retina are accompanied by the production of ROS, including H_2O_2, and the induction of inducible nitric oxide 2 (NOS2). Hypoxia and ischemia promote retinal angiogenesis by upregulating the antioxidant transcription factor NRF2 and SOD and enhance the expression of epidermal growth factor (EGF), IL-8, platelet-derived growth factor (PDGF) and adhesion molecules under oxidative stress [64, 83, 84]. Interestingly, TAK1 has been found to be involved in compensatory cellular antioxidant responses, including NFR2- and SOD-related signaling pathways [59, 60]. TAK1 therefore appears to be crucial in pathological angiogenesis, suggesting that TAK1 could be a potential therapeutic target for retinal neovascularization.

Approaches for therapeutic targeting of TAK1 signaling

Inhibition of TAK1 activity with small molecule drugs

The role of TAK1 in multiple cellular pathways suggests that it might be a potential target for small molecule interventions against diseases, including cancer and inflammation- and angiogenesis-related diseases (Table 1).

(5Z)-7-oxozeaenol

(5Z)-7-Oxozeaenol, a kind of macrolide compound, is the 7-Oxo derivative of zeaenol (the 5Z stereoisomer) [85]. It is a natural product of fungal origin that functions as a TAK1-specific inhibitor through covalent interactions with TAK1 [86]. The therapeutic effects of (5Z)-7-oxozeaenol have been observed in a number of studies. For example, (5Z)-7-oxozeaenol was found to inhibit TAK1 activity and downregulate downstream signaling pathways, including p38 MAPK, IKK and JNK, and reduce chemokine receptor 7 (CCR7) expression, ultimately suppressing the lymphatic invasion and lung metastasis of breast cancer [87, 88]. In addition, other studies have shown that treatment with (5Z)-7-oxozeaenol effectively inhibits TAK1 and NF-κB activation and induces caspase-3 and -7 in colon and cervical cancer, resulting in enhanced apoptosis of cancer cells [89, 90]. In neurological

Table 1 Summary of pharmacological inhibition of TAK1

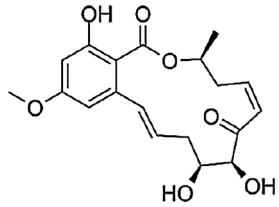
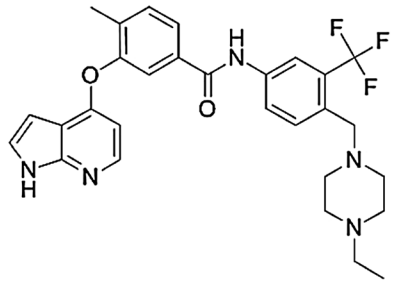
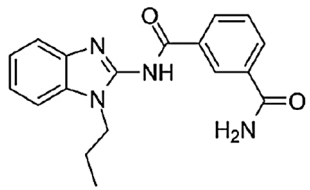
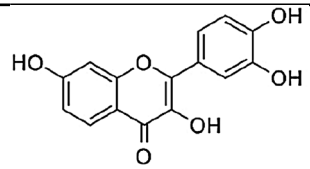
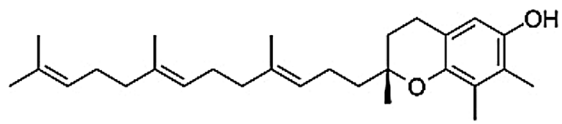
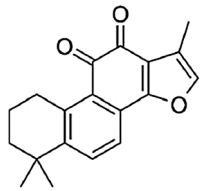
	Inhibitor	Structure	M.W	Origin	Solubility	Comments	Pre-clinical research	References
Common chemical inhibitors	5Z-7-Oxozeaenol		362.37	Natural product of fungal/resorcylic acid lactones	DMSO: > 10 mg/mL	Competitive inhibitor of ATP binding to TAK1 in irreversible manner. Inhibits the catalytic activity of TAK1 (IC50= 8.1 nM) Potently inhibits a panel of at least 50 other kinases Commercially available for research purposes	Tumor suppression (triple negative breast cancer, melanoma), rheumatoid arthritis	[22, 85, 134–137]
	NG25		537.58	Synthetic compound	H_2O: 5 mg/mL	Inhibits by binding to ATP binding pocket of TAK1 when TAK1 is in DFG motif "out" or inactive conformation (IC50= 149 nM) Also inhibits MAP4K2 (IC50= 21.7 nM	Tumor suppression (breast cancer, colorectal cancer), cerebral injury	[95, 109, 138, 139]
	Takinib		322.36	Amino-benzimidazole	DMSO: 2 mg/mL	Competitive inhibitor of TAK1. Binds to DFG "in" conformation of ATP binding site of TAK1 (IC50= 9.5 nM) Prolongs the rate limiting step of TAK1 activation i.e., prolongs time for TAK1 autophosphorylation Also a panel of at least 11 other kinases	Tumor suppression (melanoma), arthritis	[40, 94]
	LYTAK1	N/A	N/A	N/A	N/A	Only known orally active TAK1 inhibitor Blocks TAK1 phosphorylation at Thr184/187	Tumor suppression (pancreatic cancer, colorectal cancer), proliferative vitreoretinopathy	[102, 140–142]

Table 1 (continued)

	Inhibitor	Structure	M.W	Origin	Solubility	Comments	Pre-clinical research	References
Uncommon chemical inhibitors	Fisetin		286.24	Natural flavonol	DMSO: ≥ 50 mg/mL	Under research Attenuates TAK1 and TAB1 interaction	Tumor suppression, sepsis-induced multiple organ dysfunction	[143, 144]
	γ-tocotrienol		410.63	Lipid-soluble isomers of the essential micronutrient vitamin E	Neat	Under research	Tumor suppression	[145]
	Tanshinone IIA		294.34	Root of *Salvia miltiorrhiza Bunge*	Methanol: 5 mg/mL	Under research	Inflammatory modulation, atherosclerosis	[146]
Chinese Natural inhibitors	Rubiaceae-type cyclopeptides (RAs)	N/A	N/A	A type of plant cyclopeptides from *Rubia*	N/A	Binds to ATP binding pocket to interrupt TAK1–TAB2 interaction	Inflammatory modulation, tumor suppression, angiogenesis inhibition	[147]
	Sesamin	N/A	N/A	A lipid-soluble lignan from sesame (*Sesamum indicum*)	N/A	Under research	Tumor suppression, prevention of heart failure	[148]
	Pinitol (3-O-methyl-chiro-inositol)	N/A	N/A	A nature component from traditional Ayurvedic medicine (talisapatra)	N/A	Under research	Inflammatory modulation, prevention of diabetic complication	[149]
	Gambogic acid	N/A	N/A	A xanthone derived from the resin of the *Garcinia hanburyi*	N/A	Under research	Tumor suppression	[150]
	Celastrol	N/A	N/A	A quinone-methide triterpene derived from the medicinal plant *Tripterygium wilfordii*	N/A	Under research	Tumor suppression, gastric cancer	[151, 152]

diseases, such as cerebral ischemia and subarachnoid hemorrhage, studies have shown that inhibiting TAK1 can downregulate p38 MAPK-JNK and NF-κB-related inflammatory pathways, thereby reducing cerebral inflammation and brain damage [91–93]. Similarly, treatment with (5Z)-7-oxozeaenol can also reduce the production of inflammatory cytokines and the formation of abnormal blood vessels in the cavum articulare by suppressing synovial fibroblast activation [138] and attenuating neointimal formation in wire-injured femoral arteries of mice [49]. Although (5Z)-7-oxozeaenol is widely used to study the biological functions of TAK1 in diseases, it also effectively inhibits a panel of at least 50 other kinases and forms a covalent bond with reactive cysteines in the activation loop of its targets, producing several undesired side effects. Such nonspecific binding creates off-target effects, which likely limits its potential use in clinical settings [86, 94].

NG25

Other small molecules that target TAK1 have also been investigated. Tan et al. found that NG25 is a potent dual inhibitor that targets TAK1 and MAP4K2 kinases, with weak inhibition of 11 other kinases [95]. Wang et al. also reported that targeting TAK1 with NG25 can partially block doxorubicin (Dox)-induced p38 MAPK phosphorylation and IκBα degradation and enhance Dox-induced cytotoxic effects and apoptosis in breast cancer cells by targeting TAK1 [96]. Wang et al. further confirmed that injection of NG25 prior to insult significantly inhibited TAK1/JNK activity and dramatically attenuated acute hypoxic and ischemic cerebral injury and abnormal angiogenesis by regulating cell survival and behavior in perinatal rats [97]. Therefore, NG25 may also be a potential candidate drug that can be applied to target TAK1 by inhibiting TAK1-related inflammation and angiogenesis.

Takinib

A recently developed compound named takinib has proven to bind more specifically to TAK1 than (5Z)-7-oxozeaenol. Totzke et al. found that takinib is more selective than other TAK1 inhibitors since it targets germinal center kinase (GCK), an important kinase that participates in both the determination of cell fate and the regulation of cell functions, with a 45-fold lower potency than TAK1 [94]. Takinib is an aminobenzimidazole-based competitive inhibitor of TAK1 that was previously identified as a Src kinase family inhibitor. However, the initial kinome profiling study showed that takinib only weakly inhibited Src and Yes1 [98]. In contrast, takinib shows significant inhibitory activity against six other kinases, including TAK1, IRAK4, IRAK1, GCK, CDC-like kinase 2 (CLK2), and misshapen like kinase 1 (MINK1); of these targets, TAK1 is most potently inhibited by takinib [99]. Compared to (5Z)-7-oxozeaenol, takinib does not inhibit any members of the MAP2K or MAP3K family and shows no efficacy on TAK1-related MAP3K5/apoptosis signal-regulating kinase 1 (ASK1). Additionally, p38 MAPK is completely insensitive to takinib [94]. Due to its higher specificity for TAK1 and its capability to phosphorylate IKK, MAPK 8/9 and c-Jun upon TNF-α stimulation, takinib induces apoptosis upon TNFα stimulation in cell models of breast cancer and rheumatoid arthritis [94]. Furthermore, takinib treatment was found to inhibit proinflammatory cytokines in a mouse model of type II collagen-induced arthritis and in NRAS-mutated melanoma cells through TAK1 inhibition [100], suggesting that it may be useful in progressive malignant diseases and inflammatory diseases.

LYTAK1

Other orally active TAK1 inhibitors, such as LYTAK1, have been described; LYTAK1 attenuates the chemoresistance of pancreatic cancer by inhibiting TAK1 but has cytotoxic activity in vitro [101]. LYTAK1 was reported to significantly suppress LPS-induced TAK1-NFκB and MAPK (ERK, JNK and p38 MAPK) activation in vitro and in vivo [102]. Oral administration of LYTAK1 can significantly inhibit the growth of colorectal cancer cell xenografts in nude mice [103]. Moreover, LYTAK1 attenuates proliferation and epithelial-mesenchymal transition in retinal pigment epithelial cells through the TAK1-mediated Smad and ERK/AKT signaling pathways, which may be useful for the management of proliferative vitreoretinopathy [104, 105].

Other TAK1 inhibitors

Some uncommon TAK1 inhibitors, including fisetin, γ-tocotrienol and tanshinone IIA, are currently being developed [106–108]. In addition, there are some new discoveries of plant extracts that may be useful for inhibiting TAK1. Rubiaceae-type cyclopeptides, a type of plant cyclopeptide from Rubia, can inhibit the NF-κB signaling pathway by disrupting the TAK1–TAB2 interaction and targeting TAK1, ultimately suppressing the inflammatory response and angiogenesis [109]. Other molecules, such as sesamin, pinitol, gambogic acid and celastrol, can inhibit the NF-κB signaling pathway and related genes involved in apoptosis (cIAP-1/2, Bcl-2, Bcl-xL, XIAP, survivin, and TRAF1), proliferation (cyclin D1, c-Myc, COX2), metastasis (ICAM-1 and MMP-9), and angiogenesis (VEGF) by targeting TAK1, thus enhancing apoptosis and attenuating proliferation, invasion and angiogenesis in cancer [110–113]. However, even though there are a number of studies on the development of

different kinds of TAK1 inhibitors and the characterization of their specific mechanisms, there is still a lack of evidence regarding their clinical effects. In this case, more preclinical studies are needed to determine whether there is potential to develop TAK1 inhibitors as antiangiogenic therapies for various diseases.

Genetic approaches for TAK1 gene targeting

Although the aforementioned TAK1 inhibitors can inhibit the activation of TAK1 at the protein level, their off-target effects may need to be considered. Even though more selective TAK1 inhibitors have been reported, most of them can also target a wide range of kinases other than TAK1 [85, 94, 95]. Therefore, the side effects induced by many TAK1 inhibitors remain largely unclear. Thus, pharmaceutical inhibitors of TAK1 may not be ideal candidates for specifically inhibiting TAK1. As a result, emerging approaches such as microRNA (miRNA)-based targeting strategies and clustered regularly interspaced short palindromic repeat (CRISPR)-based gene editing have been increasingly applied in research.

TAK1 regulation by miRNA

miRNAs are small noncoding RNA molecules (22 to 25 nucleotides long) found in all eukaryotes and some viruses. miRNA silence gene expression at the posttranscriptional level through base pairing with complementary sequences at the 3′ untranslated region (3′-UTR) of mRNA [114–116]. The role of various miRNAs in TAK1 inhibition has been extensively studied. Jiang et al. revealed that when overexpressed, miR-892b can attenuate NF-κB signaling by directly targeting and suppressing TAK1 in breast cancer, resulting in significantly decreased tumor growth, metastatic capacity and angiogenesis [117]. Likewise, miR-26b can also inhibit the expression of TAK1 and TAB3 by binding to their 3′-UTRs, thus blocking the activation of NF-κB signaling and sensitizing cells to apoptosis [118].

TAK1 silencing by miR-143 has been shown in pancreatic ductal adenocarcinoma cells and hepatocytes. miR-143 can directly target TAK1 and inactivate MAPKs/NF-κB signaling, therefore inhibiting cell proliferation, cell migration, inflammation and fibrosis, which are important cellular activities related to angiogenesis [119]. TAK1 can also be targeted by miR-10a in endothelial cells; miR-10a is expressed at lower levels in the atherosusceptible regions of the inner aortic arch and aortorenal branches than in other regions. Interestingly, the *TAK1* gene contains a highly conserved miR-10a binding site in the 3′-UTR by which miR-10a can negatively regulate TAK1 expression. Such regulation by miR-10a directly mediates TAK1/NF-κB signaling cascades and contributes to the regulation of proinflammatory endothelial phenotypes in atherosusceptible regions in vivo [120]. It is worth noting that a single miRNA can target multiple mRNAs, suggesting that miRNAs that regulate TAK1 may target other genes, causing off-target effects.

CRISPR/Cas-mediated gene modification

Clustered regularly interspaced short palindromic repeats (CRISPR) is a repetitive DNA sequence in the genome of prokaryotic organisms that is derived from DNA fragments of bacteriophages that have previously infected prokaryotes. It can detect and destroy DNA from similar bacteriophages during subsequent infections, generating a unique immune response to protect against foreign invasion [121]. CRISPR-associated protein (Cas) is an enzyme that uses guide RNA to recognize and cleave target strands of DNA that are complementary to the guide RNA [122]. As CRISPR/Cas-based gene editing technology has become more established, it is being widely used to knock out genes completely and permanently by targeting gene loci, thus achieving stable and persistent gene editing. These engineered nucleases generate a double-strand DNA break at the targeted genome locus. The break activates repair through error-prone nonhomologous end joining (NHEJ) or homology-directed repair (HDR). In the absence of a template, NHEJ is activated, resulting in insertions and/or deletions that disrupt the target loci. In the presence of a donor template with homology to the targeted locus, the HDR pathway is initiated, allowing for precise mutations to be made [123].

Although CRISPR/Cas-based gene editing has not been used extensively as a therapeutic measure for the treatment of pathological angiogenesis, it has been increasingly used in studies to understand the role of TAK1 in various disease contexts. In a study of the role of TAK1 in pneumoconiosis, CRISPR technology was used to generate TAK1 knockout in vivo via lentiviral vectors expressing CRISPR/Cas9 components. Li et al. confirmed that TAK1 knockout in mice significantly reduced fibrotic nodule formation in the lung tissues after silica exposure [124]. Morioka et al. also showed that the endothelial-specific deletion of TAK1 by CRISPR/Cas9 editing caused increased cell death and vessel regression at embryonic day 10.5 (E10.5), eventually leading to embryo death, which made it difficult to breed endothelial-specific TAK1 knockout mice [11]. CRISPR/Cas-based gene editing has been increasingly studied in the context of manipulating the expression of specific genes in pathological angiogenesis. Huang et al. used AAV1-mediated CRISPR/Cas9 editing to target the genomic VEGFR2 locus, resulting in abrogation of angiogenesis in a mouse model of oxygen-induced retinopathy

and laser-induced choroidal neovascularization [125]. Moreover, depletion of ONECUT homeobox 2, a highly expressed gene in ovarian cancer tissues, by CRISPR/Cas9 editing remarkably suppressed the expression of several proangiogenic growth factors, such as VEGFA, HGF, and HIF-1α, and the activation of Akt/ERK pathways, thus attenuating ovarian cancer progression [126]. With the great advantages of CRISPR/Cas-based gene editing, research has rapidly moved to clinical study. In fact, the latest clinical study using CRISPR/Cas9 editing to design immune cells with enhanced abilities to seek and attack tumors has shown promise in treating some cancers without causing any significant side effects [127]. It is worth noting that the long-term efficacy and safety of CRISPR/Cas-based therapy remains unclear. Nevertheless, the rapid developments in modified CRISPR technology have validated its efficacy and safety, providing a new path for the clinical study of gene editing to treat pathological angiogenesis.

Potential adverse effects on TAK1 inhibition

Given the pleiotropic nature of TAK1 gene, we can observe diverse roles of TAK1 in multiple physiological activities such as inflammation, immune responses, neural and vascular development. However, this also brings additional risks of undesired side effects when targeted to inhibit its kinase activity. So far, such undesired side effects of TAK1 inhibition either by gene knockout or pharmaceutical inhibitors have not been clinically studied. Nevertheless, a number of studies have suggested that such adverse effects have been observed in various in vitro and in vivo models. For instance, a conditional TAK1 knockout in parenchymal cells of mice liver caused hepatocyte dysplasia and liver carcinogenesis with spontaneous hepatocyte apoptosis and cholangiocytes fibrosis [128, 129]. Moreover, a study showed that 5Z-7-oxozeaenol can attenuate inflammation and fibrosis in experimental rats with silica-induced pneumoconiosis. However, cytotoxicity in primary lung fibroblasts of healthy rats was detected, suggesting that 5Z-7-oxozeaenol may be toxic during the treatment of pneumoconiosis [130]. Similar cytotoxic effects of 5Z-7-oxozeaenol were observed on SK-N-AS and IMR-32 cells at a relatively high dose during the treatment of neuroblastoma [131]. In retinal pigment epithelial cells, TAK1 inhibition led to accelerated cellular senescence, decreased cell proliferation and increased senescence-associated β-galactosidase expression [132]. Selective TAK1 inhibitor such as Takinib has also demonstrated a significant amount of synoviocyte death at 48 h when used for the treatment of arthritis in type II collagen-induced arthritis mice [133]. These findings unarguably suggest that more work is needed on comprehending potential adverse effects of TAK1 inhibition. Regardless, TAK1 is still an immensely attractive molecular target for small molecule interventions against diseases, including cancer and inflammation- and angiogenesis-related diseases.

Conclusions and future perspectives

TAK1 is an important mediator of multiple signaling pathways that is involved in a variety of pathophysiological processes, including inflammation and the responses to hypoxia and oxidative stress. Increasing evidence indicates that these TAK1-mediated processes clearly participate in angiogenesis-related disorders, such as tumor angiogenesis and retinal neovascularization. Pharmacological inhibitors and genetic approaches for targeting TAK1 have been widely studied in various cancers, such as breast, colon and cervical cancers. Inhibition of TAK1 and its downstream signaling are also effective strategies for inducing the apoptosis of cancer cells and enhancing the chemotherapeutic efficacy of TAK1 inhibitors by regulating the inflammatory and angiogenic processes in tumors. However, precisely how TAK1 is involved in regulating angiogenesis and related diseases and the crosstalk between TAK1 and downstream signaling pathways under different conditions remain to be clarified. Nevertheless, TAK1 is a potential therapeutic target that needs to be further studied to provide an alternative to current treatment for pathological angiogenesis.

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Declarations

Conflict of interest The authors have declared that no competing interest exists.

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