NF-Kb, A Potential Therapeutic Target, In Cardiovascular Diseases

Weijia Cheng
Xinjiang Medical University
can Cui
Xinjiang Medical University
Gang Liu
Xinjiang Medical University
Chenji Ye
Xinjiang Medical University
Fang Shao
Xinjiang Medical University
Jay L. Mehta (mehtajl@uams.edu)
University of Arkansas for Medical Sciences https://orcid.org/0000-0003-0384-2097
Xianwei Wang
Xinjiang Medical University

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Abstract

Cardiovascular diseases (CVDs) are the leading cause of death globally. Atherosclerosis is the basis of major CVDs - myocardial ischemia, heart failure and stroke. Among numerous functional molecules, the transcription factor nuclear factor κB (NF-κB) has been linked to downstream target genes involved in atherosclerosis. The activation of NF-κB family and its downstream target genes in response to environmental and cellular stress, hypoxia and ischemia initiate different pathological events such as innate and adaptive immunity, and cell survival, differentiation and proliferation. Thus, NF-κB is a potential therapeutic target in the treatment of atherosclerosis and related CVDs. Several biologics and small molecules as well as peptide/proteins have been shown to regulate NF-κB dependent signaling pathways. In this review, we will focus on the function of NF-κB in CVDs and the role of NF-κB inhibitors in the treatment of CVDs.

1 Introduction

Cardiovascular diseases (CVDs) are the leading causes of death in the Western hemisphere and rapidly becoming so in the developing world. While the major risk factors leading to CVDs have been well addressed and targeted to prevent and treat CVDs, the precise steps and molecular mechanism leading to atherogenesis and its major manifestations are yet being described. Here we focus on nuclear-factor kappa beta (NF-κB) as an important transcription factors involved in several steps in the development of CVDs.

1.1 N F-κB family

Many genes contain a nearly palindromic DNA sequence with a consensus of 50-GGGRNWYYCC-30 (N, any base; R, purine; W, adenine or thymine; Y, pyrimidine), which is termed κB [1]. The elements in enhancers or promoters of these genes can be recognized by a class of proteins such as NF-κB that subsequently initiate the transcription of these genes. NF-κB in mammals is a family of five related proteins: RelAIp65, RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) (Fig. 1-part I) [2]. These subunits share N-terminal Rel homology domain (RHD) with the v-Rel oncogene and form dimers that can positively or negatively regulate gene expression. RHD is responsible for homo- and heterodimerization as well as for sequence-specific DNA binding to κB sites. NF-κB family contains two subfamilies: p65, RelB and c-Rel containing a C-terminal transcriptional transactivation domain (TAD) which confers the ability to promote gene expression; p50 and p52 lacking a TAD have a dual role (Fig. 1-part I). Homodimers of p50 and p52 are thought to repress transcription through competing with transcriptionally active dimers to bind to their DNA targets. While they are bound to p65, RelB or c-Rel as part of a heterodimer, p50 and p52 can promote transcription. RelB is unique in that it also contains an N-terminal leucine zipper (LZ) region that needs to work with its TAD to be fully functional. Since RelB has only been verified to form dimers with p50 and p52, there are only 12 dimers existing in cells. The C-terminal death domain (DD) in C-terminal half of p100 and p105 is N-terminal to the IKK phosphorylation site. The main function of DD motif is to promote protein-adapter interactions

between receptors and corresponding adapters related to NF-κB and activator protein-1 (AP1) pathways and cell apoptosis. The DD motif in p105 acts as a docking site for IKK facilitating serine 927 phosphorylation, while acts as a processing inhibition domain (PID) in p100 that limits the basic processing to p52 (Fig. 1-part I).

1.2 IκB family

In unstimulated cells, inactive NF-κB dimers are sequestered in the cytoplasm due to the binding of “inhibitor of kB” (IkB) family, the most important NF-κB-interacting proteins [3]. IκB family members contain five to seven tandem ankyrin repeats (AnkRs). AnkRs are 33 amino acid ankyrin-like protein-protein association domains that bind to nuclear localization signal (NLS) of NF-kB. Classic IκB proteins include IκBα, IκBβ and IκBε (Fig. 1-part II). In resting cells, they sequester NF-κB dimer to retain in the cytoplasm and then degrade it in an IKK-dependent manner after activation. Precursor IκB proteins include p105 and p100. The "precursor" IκB proteins p105 and p100 isolate the NF-κB subunit in the cytoplasm in an unstimulated state similar to classical IκB proteins. Proteasome-dependent limited proteolysis of p105 and p100 results in the release of NF-κB subunit p50 and p52, respectively (Fig. 1-part II). The release of p50 from p100 usually induces downstream activation of the atypical NF-κB pathway in an ubiquitin-dependent manner. The coordinative and post-transcriptional processing of p105 is thought to be primarily compositional and occurs in an ubiquitin-independent fashion. Additionally, there are four atypical IκB proteins: B-cell lymphoma 3 (BCL-3) and IκBζ, IκBNS and IκBη (Fig. 1-part II). Their expression is usually low and is induced by various stimulations including NF-κB. Unlike other members of the IκB family, they are localized in the nucleus, where they can inhibit target gene expression or promote specific gene expression.

1.3 IKK complex

NF-κB activation occurs in a canonical pathway (CP) and an alternative pathway involving post-translational modification of IκB inhibitors [4]. For the canonical pathway, degradation of IκB proteins is led by phosphorylation induced by a kinase complex called IκB kinase (IKK) [5]. IKK consists of IKKα, IKKβ and a regulatory scaffold subunit called NF-κB essential modulator (NEMO, also called IKKγ) (Fig. 1-part III). IKKα and IKKβ both include a helix-loop-helix (HLH) that functions in modulating IKK kinase activity and a LZ that allows of the kinase homo- or heterodimerization. IKKα contains a putative NLS which is possibly linked to its nuclear activity. NEMO tethers IKKα and IKKβ into a regulatory complex thereby causes ubiquitination of NEMO and phosphorylation of IKK that induce its kinase activity. The interaction domain between the kinase subunits and NEMO results in a small peptide at the C terminus of IKKα and IKKβ. The exact composition of the IKK complex in vivo remains a topic of great debate. Canonical IKK complexes contain NEMO while non-canonical IKK complex contains NEMO-independent IKKα homodimers. NEMO-containing canonical IKK complexes may consist of homodimers of either IKKα or IKKβ, or a heterodimer of the two. In non-stimulated cells, the HLH motif contacts the KD of IKKs forming an activation loop. Once cell is stimulated, the KD domain of IKKβ is phosphorylated, IKKs are recruited to the IKK complex through NEMO, then IKK complex is activated. Once activated, IKK complex

phosphorylate IκB in NF-κB/IκB complex, IκB is degraded by ubiquitination. C-terminal serine of IKK cluster undergoes autophosphorylation after sensing decreased IκB [6]. It is possible that the autophosphorylation of C-terminal serine sites alters the interaction between the activating HLH motif and KD, the kinase reaches a low activity state. This regulatory mechanism explains why IKK is usually activated instantaneously.

2. Nf-κb Dependent Signaling Pathway

So far, there are two main NF-κB activating pathways involved in multiple physiological and pathological processes in inflammation and immunity—the canonical and the non-canonical signaling pathway. The canonical signaling pathway is induced by a diversity of stimuli, especially pro-inflammatory factors including tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and lipopolysaccharide (LPS) [7–10]. These ligands usually bind to their own receptors, leading to the conformational change or oligomerization of themselves, then initiating a series of signaling cascades and finally releasing p65/p50 dimer into nuclear for target gene regulation [10]. Otherwise, the non-canonical pathway is primarily triggered by CD40 ligand (CD40L) or lymphotoxin β (LTbeta) that are specific members of the TNF family, resulting in activation and nuclear translocation of RELB/p52 dimer [11–13]. Importantly, as a crucial regulatory factor, the IKK complex exists in both two activating pathways, which predominantly affect NF-κB activity in an inducible manner. IKK complex consists of two catalytical subunits IKKα and IKKβ, as well as one regulatory subunit NEMO [14]. In the canonical activating pathway, IKKβ and NEMO are essential for IKK complex to phosphorylate IκBα, which is independent of IKKα's activity [15]. However, in the non-canonical activating pathway, IKKα is the only effector involved in phosphorylation-dependent activation of p100, which is further processed to p52, and this entire process is independent of both IKKβ and NEMO [16].

2.1 The canonical NF-κB pathway

The canonical NF-κB pathway has been typically defined in signal transduction emitting from tumor necrosis factor receptor (TNFR) or Toll-like receptor 4 (TLR4) [8, 13, 17]. TNF signals generally derive from two kinds of TNFR, TNFR1, and TNFR2 [18]. TNFR1 is traceable in almost all tissues and can be continuously expressed in physical and pathological situations, so it is thought to be the dominant type of TNFR mediated in the TNF-associated signaling cascade [10]. Upon binding of TNF-α to TNFR1 (Fig. 2), a tail in the cytoplasmic side of TNFR1 appears, which contains a death domain that provides a binding site to recruit TRADD and RIP1 [19]. Next, TRADD is essential for the recruitment of TRAF2/5 that recruits E3-type ubiquitin ligases cIAPs, leading to RIP1 conjugated with two linear lysine 63 (K63)-linked polyubiquitin chains [20]. Adapter protein TAB2/3 and its associated kinases TAK1 are thought to anchor together with one K63-linked polyubiquitin chain, which can phosphorylate and activate the IKK complex [21]. LUBAC, another E3 ligase, is required for the formation of methionine 1 (M1)-linked polyubiquitin chain connected with RIP1, which mediates the interaction with downstream NEMO of IKK complex and activates IKK complex in another way [22, 23]. The other canonical activating pathway is triggered by LPS as recognized by TLR4. Signals from TLR4 induce the recruitment of adaptor MyD88, followed by IRAK4

and TRAF6 [24, 25]. TRAF6 as an E3 ligase leads to autoubiquitination and then promotes the formation of the platform with TAB2/3 and TAK1, eventually leading to auto-phosphorylation of activation of TAK1 [26–28].

The key event in NF-κB activating pathway is the activation of the IKK complex, which begins with the phosphorylation in serine 176 and 177 of IKKα/IKKβ by TAK1 and the oligomerization with NEMO, leading to induce proximity, and trans-autophosphorylation and full activation of IKK complex [23, 29]. It is accepted that the IKKβ is the necessary subunit responsible for the activation of the IKK complex in a NEMO-dependent way. In unstimulated situations, the NF-κB dimer composed of p65 and p50 commonly interacts with negative regulator IκBα as a complex in an inactive and steady form in the cytoplasm. Upon activation of the IKK complex, the IκBα is rapidly phosphorylated by IKKβ, which leads to the K48-linked ubiquitination and subsequent proteasomal degradation by the 26S proteasome [30]. The removal of IκBα allows the release of p65/p50 dimer from the complex, and the unbound NF-κB dimer translocates into the nucleus and targets for specific gene regulation [31].

2.2 The non-canonical NF-κB pathway

The non-canonical NF-κB pathway is activated by several members of the TNFR family including CD40, lymphotoxin β receptor (LTβ-R), B-cell activating factor receptor (BAFF-R), and receptor activation of NF-κB (RANK) [11, 12, 32, 33]. Among those, however, CD40 is the most studied and the best-characterized receptor in non-canonical signaling (Fig. 2). Similar to the function of IKKβ in the canonical signaling pathway, IKKα servers as the major mechanism for activating NF-κB, which is independent of NEMO. Another important difference is that the activation of IKKα relies on the cumulation of NF-κB-inducing kinase (NIK) [34].

In a resting situation, NIK is expressed at low level that is incapable of activating downstream IKKα because of their rapid and constitutive proteasomal degradation [34]. The degradation of NIK is caused by the binding of linear K48 polyubiquitylation mediated by TRAF2/3-cIAPs E3 ligase complex [35]. TRAF3 regarding as adaptor protein doesn't have polyubiquitination activity, which recruits E3 ubiquitin ligases cIAPs via linker TRAF2, and ultimately cIAPs contribute to NIK ubiquitination [36]. Therefore, the accumulation of NIK is deeply dependent on the activity of the TRAF2/3-cIAPs complex that acts as a negative regulator playing an important role in the non-canonical NF-κB activating pathway. In stimulated conditions, however, CD40L induces oligomerization of CD40 upon binding, leading to the recruitment of TRAF2/3-cIAPs complex through the cytoplasmic motifs of CD40 [13]. Within the TRAF2/3-cIAPs complex, both TRAF2 and TRAF3 are linked with a linear polyubiquitination chain via cIAPs-mediated ubiquitination, resulting in the proteasomal degradation of TRAF2/3, which contributes to the accumulation and the activation of NIK [37, 38]. Subsequently, NIK phosphorates and activates IKKα, leading to p100 processing [39]. p100 as the precursor of p52 functions as an IκB-like molecule to preferentially inhibit RELB translocation. The processing of p100 results in the generation of p52 that dimerizes with RELB, translocating in the nucleus and then binding with DNA for target gene expression [35, 39]. Other than the canonical NF-κB pathway, RELB/p52 dimer is considered as the key nuclear transcriptional factor in the non-canonical pathway.

3. The Role Of Nf-κb In Various Cardiovascular Diseases

The activation of NF-κB is critical in the pathological processes of most CVDs. NF-κB mediates inflammation, cell survival, cell differentiation and cell proliferation which contribute to the pathogenesis of CVDs such as hypertension, atherosclerosis and related manifestations such as myocardial ischemia and infarction, cerebrovascular ischemia and strokes, heart failure and cardiac hypertrophy. NF-κB activation also regulates extracellular matrix formation by affecting the production of matrix metalloproteinases (MMPs) and collagens in a variety of cells in the cardiovascular system (Fig. 3). Upon induction of NF-κB, inflammatory cytokines, chemokines as well as adhesion molecules are generated from innate and adaptive immune cells, such as macrophages, neutrophils, dendritic cells, T cell and B cells. The subsequent inflammatory process leads to injury in tissues and organs of cardiovascular system (Fig. 3).

3.1 Atherosclerosis

Atherosclerosis is a chronic inflammatory disorder characterized by the accumulation of lipids particles in arterial walls with pathological risk to heart attack or stroke [40–42]. The activated NF-κB signaling is involved in all stages of atherosclerosis development [40].

The initiation of atherosclerosis is mediated by (endothelial cell) EC activation and engulfment of oxidized low-density lipoproteins (ox-LDLs). Upon stimulation by various stimuli, vascular ECs express cytokines, chemokines and cell adhesion molecules which facilitate the recruitment of circulated leukocytes and their migration to the subendothelial layer of arterial intima. The essential role of NF-κB-mediated signaling cascade has been shown in this process [43–47]. Various pro-atherogenic molecules, including cytokines like TNF-α, IL-1, bacterial and viral infections, ox-LDL, ROS and advanced glycation endproducts (AGEs), activate NF-kB [48–52]. NF-κB mediated downstream canonical and non- canonical signaling pathways are involved in different aspects of atherosclerotic process [43]. Subsequent induction of a large array of molecules in the ECs, such as ICAM-1, VCAM-1, P- and E-selectins, TNF-α, IL-1, IL-6, IL-8, MCP-1 and MMPs, promote the recruitment of innate and adaptive immune cells to the vessel wall [44, 53, 54]. It is noteworthy that conditional deletion of NEMO or transgenic expression of dominant-negative IκBα leads to inhibition of expression of adhesion molecules in vascular ECs and impaired monocyte/macrophage recruitment to atherosclerotic plaques in ApoE KO mice fed high-fat diet. Subsequently, there is a reduction in the severity of atherosclerosis [45].

Further, Ox-LDL mediated TLR activation in vascular ECs results in the upregulation of NF-κB induced expression of proinflammatory cytokines and adhesion molecules, triggering atherosclerotic progress of inner coat of the blood vessels [45, 55–57]. Importantly, the degree of atherosclerosis was shown to be inhibited by suppressing TLR2/4-MyD88 signaling with decreased expression of chemokine and macrophage recruitment [45, 55].

Fluid mechanical force is another factor that regulates NF-κB expression and activity in ECs. Enhanced NF-κB (p50 and p65) activation was showed in human aortic ECs under steady low shear stress than

under high shear stress [58] (Fig. 4). The increased NF-κB activity in blood flow stressed ECs and susceptibility to atherosclerosis suggest that disturbed blood flow induced NF-κB activation in vascular ECs might be involved in the early stages of atherogenesis by facilitating monocyte/macrophage recruitment and plaque formation [45, 59, 60]. In early state of atherogenesis when NF-κB is activated, ROS acts as second messenger in response to extracellular stimuli such as ox-LDL and Ang II (angiotensin II). Ox-LDL mediated ROS generation results in reduced NO production with subsequent further increase in NF-κB activation [61]. Ang II is the most important mediator of the RAAS (renin–angiotensin–aldosterone system) in state of high blood pressure. Ang II-ROS-NF-κB activation links hypertension to increased risk factor of atherosclerosis [62–65].

NF-κB is also involved in differentiation of monocytes into macrophages after their recruitment to the intima [41]. The macrophages express surface scavenger receptors, such as lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), SR-A and CD36 which facilitate binding and uptake the native and oxidized form of LDL as a protective reaction to eliminate the accumulation of these inflammatory components [66]. As a consequence, the damage caused by modified LDLs to vascular ECs and smooth muscle cells is reduced. CD36-mLDL interaction induces LDL absorption, oxidative stress, and the production of proinflammatory cytokines through NF-kB activation [67, 68]. The various forms of LDL phagocytosis results in overconsumption of cholesterol in the cytoplasm and this cellular event converts the macrophages into foam cells. foam cell formation and aggregation on the arterial vessels promotes the development of fatty streaks [40, 69]. Enrichment of lipids, foam cells and T-lymphocytes has been identified in early lesions. The NF-κB activation has also been characterized in smooth muscle cells, macrophages and T-cells in progressing atherosclerotic lesions [70]. NF-κB mediated M-CSF generation is involved in monocyte-to-macrophage differentiation [71]. NF-κB mediated MMP-9 expression contributes to the degradation of extracellular matrix, facilitating the migration of monocyte/macrophages into the target tissues [72]. Reduced macrophage infiltration and reduced vessel media damage and limited progression of atherosclerosis have been shown in MMP-9 and apoE dual deficiency mice [73]. Short periods of stimulation of monocytes with ox-LDL activates NF-κB and its target genes while longer-time stimulation reverse these responses [51]. Ox-LDL-NF-κB mediated chemokines production strengthen inflammatory effect by promoting the resident macrophages proliferation and migration of new monocytes into lesion sites [74]. In addition, ox-LDL-NF-κB mediated pro-inflammatory cytokines expression promotes the binding of LDL to ECs and smooth muscle cells (SMCs) and upregulates the expression of CD36, leading to further inflammation [74].

Atherosclerotic lesion development involves migration of SMCs from the vessel wall media into the intima from where proliferation of the SMCs and ECM formation occur. This event drives the early atherosclerotic lesion and fibrotic cap formation. NF-κB activation plays a critical role in this pathological process. Activated NF-kB p65 and p50 components have been identified in SMCs derived from human atherosclerotic lesions compared to SMCs from healthy tissues [50]. Also, several activated NF-κB components including p65, p50 and c-Rel have been found in cells isolated from human atherosclerotic tissue [44]. Vascular SMCs convert from health contractile phenotype to pathological fibroblast-like synthetic phenotype which is the major source of connective tissue in the lesion site. This switching

progress is an important step in atherosclerotic pathology [75, 76]. In vitro studies have shown that the production of TNF-α, IL-1, M-CSF, GMCSF, or MCP-1 is regulated by NF-κB in synthetic-state SMCs [70, 77–80]. These inflammatory molecules produced by SMCs, monocyte/macrophages, ECs, as well as lymphocytes induce the activation of NF-κB in an autocrine manner [70, 78]. NF-κB signaling in ECs may promote recruitment and activation of inflammatory cells, whereas NF-κB activity in SMCs leads to their proliferation [81].

In later stages of atherosclerosis, programmed lipid-laden cells death (apoptosis) is an important feature; it controls the stability of the lesion and the generation of the necrotic core. Apoptosis plays an essential role in atherogenesis and its progression in all stages of atherosclerosis [82]. Clearance of dead cells derived from foam cells apoptosis by the innate and adaptive immune system in early lesions suppresses macrophage burden and inhibits atherosclerosis progression. However, in the late stages of atherosclerosis, impaired ability to clear dead foam cells facilitates the growth of lipid core, leading to inflammation, necrosis, and reduced stability of plaque. These pathological events increase the risk of rupture of an unstable atherosclerotic lesion, which causes acute vascular diseases such as stroke and acute myocardial infarction/ ischemia [83–86].

In atherosclerosis, NF-κB pathway exhibits its dual effect in cell survival/apoptosis in the context of various activating stimuli [87, 88]. NF-κB promotes survival signal by inhibiting TNFRs mediated apoptosis signal while it contributes to apoptosis through regulation of ROS generation and activation of the JNK- MAPK signal transduction [87, 89–91]. Activation of NF-κB increases the expression of Fas ligand which induces cell apoptosis via its receptor CD95. IκB kinase (IKK) promotes NF-κB-induced anti-apoptotic signal by mediating phosphorylation and degradation of the NF-κB inhibitory IκBα proteins [41, 87], and inhibition of IKK-mediated NF-κB activation facilitates apoptosis in monocytes [87, 92]. Deviant NF-κB mediated inhibition of apoptosis might be involved in the initiation of atherosclerosis [87, 93–95].

In atherosclerotic plaques, the secretion of MMP-1, -3 and − 9, induces the destruction of the ECM and the loss of fibrous cap integrity, by reduction of collagen protein and might be important in plaque rupture; this release process is regulated by NF-κB [47, 96]. However, the types of MMPs might be different in various cell type and stimulus [72, 97, 98]. Following the inhibition of NF-κB, decreased MMP-1 production in response to of CD40L stimulation has been identified in healthy human macrophages. In addition, inhibition of NF-κB reduces MMP-1 and MMP-3 expression in Cholesterol-Feeding rabbits[99]. Ox-LDL stimulation in macrophages promote the expression MMP-9 with upregulated activation of NF-κB [72, 97].

3.2 Myocardial infarction

Rupture of the coronary atherosclerotic plaque results in localized clot formation and subsequently myocardial infarction. Clot may spontaneously resolve or may be therapeutically dissolved. Unfortunately, reperfusion also results in the phenomenon of reperfusion injury. NF-κB plays a critical role in tissue responses to ischemia and reperfusion including inflammation, regional cardiomyocyte death and myocardial infarction. TNFR and TLR-mediated NF-κB activation are induced following

ischemia/reperfusion. Moreover, hypoxia downregulates the activity of PHD1 (prolyl hydroxylase 1) and enhanced IKKβ expression as well as phosphorylation of IκBα for NF-κB activation following Ischemia [100, 101] (Fig. 4). Hypoxia also induces the expression of HIF1 (hypoxia-inducible factor 1) which also increases the expression of NF-κB subunits [102]. Negative regulator of the NF-κB including redox-sensitive enzymes is inhibited and NF-κB is induced in response to ROS generation. Myocardial ischemia/reperfusion induce NF-κB activation in several cell types including ECs and resident immune cells in the heart. The production of pro-inflammatory molecules, including the adhesion proteins ICAM-1 and P-selectin, in these cells facilitates the migration of leukocyte to the infarct area [103].

NF-κB activation (p50/p65) with elevated expression of ICAM-1, TNF-α and IL-1β has been characterized in human atrial tissue derived from patients following myocardial ischemia/reperfusion. In vivo studies have shown that inhibiting NF-κB by pharmacological inhibitors or decoy oligonucleotides suppresses myocardialinjury following ischemia [104–106]. Additionally, loss of NF-κB1 also leads to improved cardiac function and lower mortality after myocardial infarction [107].

Similar to its effect in cell survival/apoptosis, NF-κB also exerts protective function following myocardial ischemia/reperfusion. For example, in a mouse myocardial infarction model, cardiomyocytes are protected by the expression of cytoprotective genes such as c-IAP1 and Bcl-2 induced by NF-κB activation [108].

3.3 Heart failure

Long-term ischemia following myocardial infarction promotes associated with cardiomyocyte apoptosis resulting in cardiac failure [109]. Several clinical studies have found a strong link between NF-κB activation and heart failure. Failed human hearts contain activated forms of NF-κB [110, 111]. Improved cardiac function with decreased cardiac NF-κB activity was characterized in patients that received left ventricular assist devices [112]. Conditions like hypertension result in cardiac hypertrophy- which is associated with KF-kb activation; however, the rhe role of NF-κB component p50 in cardiac hypertrophy is controversial. One study showed that loss of NF-κB led to a reduced cardiac hypertrophy (Fig. 4), However, another study showed that p50 deficiency enhanced cardiac dysfunction following myocardial infarction. The mechanism of discrepancy between the results of these two studies is not clear. However, p50 deficiency mice display reduced cardiac hypertrophy in response to TNF-α and Ang II, suggesting that NF-κB might be a positive regulator of hypertrophy [107, 113, 114].

The *In vitro* investigations also showed that Ang II facilitated IκB degradation, as well as p65 nuclear translocation and transcriptional activity in cardiomyocytes [115, 116]. Cardiac-specific overexpression of a non-degradable form of IκBα in mice attenuates cardiac hypertrophy phenotype and facilitates heart failure in response to Ang II and isoproterenol infusion [117, 118]. Several other studies have showed the protective effect of NF-κB in mice. Conditional deletion of NEMO in heart blocks NF-κB Activation and the mice showed enlargement of left atrium and eccentric hypertrophy. Moreover, the heart function was also significantly impaired. In another investigation, cardiac-specific IKKβ-deficient mice exhibited normal heart morphology and function. However, heart failure including cardiac dilation and dysfunction was

observed in these mice under acute pressure overload [119, 120]. Channeling the mice with antioxidants reversed these phenotypes, indicating that NF-κB may perform its protective effect against cardiomyopathy by the induction of its downstream antioxidant genes.

3.4 Ischemic strokes

Atherosclerotic plaque rupture in the carotid artery increases the risk of ischemic strokes with subsequently tissue injury, breakdown of the blood–brain barrier, and hemorrhage. In this process, NF-κB activation has been demonstrated in neurons, ECs, microglia and astrocytes [121–124]. NF-κB is upregulated in the penumbra of human stroke patients and nuclear translocation of RelA has been identified in the brain samples from patients. Nuclear translocation of RelA has been observed in neurons derived from mouse models of both permanent and transient carotid occlusion [122]. In addition, significantly smaller infarct size was found in p50 deficiency mice of transient and permanent stroke models and selective deletion of NF-κB in neurons led to dramatically reduced infarct size [125]. Conditional knockout of RelA results in decreased brain infarct as compared to wild-type mice [126]. These data reveal the critical role of NF-κB in cerebral ischemia. The injurious effect of NF-κB in cerebral ischemia is mediated by its downstream target genes, such as TNF, IL-1 and, IL-6, inducible nitric oxide synthase, ICAM-1, and MMP-9 [127]. Although anti-apoptotic effect of NF-κB activation has been described in numerous studies, it seems to mainly contribute to prolonged cerebral ischemia in most investigations.

4. Inhibitors Of Nf-κb Dependent Signaling Pathway

The NF-κB pathway seems to serve as a link between inflammatory processes and CVDs, and may be an attractive target for development of drugs in ischemia-reperfusion injury treatment (Fig. 4).

NF-κB is an important regulator of inflammatory response which plays an important role in the pathogenesis of several CVDs. Thus, a variety of NF-κB inhibitors have been developed for treatment of CVDs. Table 1 summarizes the function and mechanisms of NF-κB inhibitors in CVDs. For example, Traf-stop inhibitor 6877002, a selective inhibitor of CD40-TRAF6 interaction, was shown to specifically inhibit the NF-κB pathway and maintain CD40-mediated immunity, thereby affecting the progression of atherosclerosis in mice [128].

Table 1
List of NF-κB inhibitors in CVDs

	Compounds	Cell lines/animal models/patients	Functions/mechanisms	Ref.
Natural inhibitors	Vinpocetine	Atherosclerosis in mice	↓NF-κB, atherosclerosis	129
	Parthenolide	Atherosclerosis in mice	↓NF-κB, atherosclerosis	130
	Epigallokatechingallat	THP-1 cells	↓NF-κB,Nrf-2/keap1	131
	Muscone	MI in mice	↓NF-κB, NLRP3, IL-1β, TNF-α and IL-6,↑cardiac function	137
	Ophiopogonin D	Human umbilical vein endothelial cells	↓NF-κB, TNF-α, IL-6	138
	Curcumin	MI in rats	↓NF-κB, myocardial injury,↑BCL-2, cardiac function	139
	Quercetin	Clinical study: CAD patients	↓NF-κB, IL-1β, TNF-α	140
	Fisetin	Isoprenaline-induced cardiac ischemic injury in rats	↓NF-κB, TNF-α, IL-6, myocardial injury	141
	Rutin	Carfilzomib-induced cardiotoxicity in rats	↓NF-κB ↑ IκB-α, ↓myocardial hypertrophy	146
	Chrysin	ISO-induced myocardial injury in rats	↓NF-κBp65, IκK-β, TNF-α, myocardial damage	142
		MI in rats	↓NF-κB, IκK-β, MMP-2, MMP-9, myocardial fibrosis	145
	Kaempferol	ISO-induced myocardial injury in rats	↓p38, JNK, NF-κBp65, TNF-α, IL-6, myocardial damage	143, 144
Chemical inhibitors	Traf-stop inhibitor 6877002	RAW cells and atherosclerosis in mice	↓NF-κB, CD40-TRAF6, atherosclerosis	128
	Pyrrolidine dithiocarbamate	THP-1 cells, MI in rat	↓NF-κB, lipid accumulation, myocardial hypertrophy, myocardial remodeling	147, 148, 149
	Oxypropoxybenzoic acid	MACO in rats	↓NF-κB, brain infarct size	152

	Compounds	Cell lines/animal models/patients	Functions/mechanisms	Ref.
	4-methylcyclopentadecanone	MACO in rat	↓NF-κB, brain infarct size	153
Proteins	Ulinastatin	MACO in rat	↓NF-κB, brain infarct size	154
	Neuregulin 1	MACO in rat	↓NF-κB, brain infarct size	155, 156
	s-nitroso glutathione	MACO in rat	↓NF-κB, brain infarct size	157
	progranulin	MACO/reperfusion in rats	↓NF-κB, MMP-9, TNF-α	158
Clinical medicants or inhibitors	Atorvastatin	THP-1 cells	↓ TLR4/MyD88/NF-κB	132
	Pioglitazone	Hyperlipidemia in rats	↓ NF-κB ↑ IκB-α, ↓ atherosclerosis	133
	Benidipine	Human aortic endothelial cells, hypercholesterolemic rabbits	↓Ca^{2+}, NF-κB, LOX-1, atherosclerosis	134, 135
	Dilazep	Human endothelial cells	↓NF-κB, MCP-1	136
	Naloxone	MACO in rat	↓NIK/IKKα/IKBα, ischemic brain injury	150
	Pitavastatin	Transient cerebral ischemia in gerbils	↓NF-κB, neuronal cell death	151

Different plant extracts exhibit their anti-atherosclerosis effect which may be related to inhibition of NF-κB activation. For example, exact of two herbs, Vinpocetine and Parthenolide, reduce the severity of atherosclerosis in mice through the regulation of NF-kB [129, 130] (Fig. 4). Flavonoid is a large class of polyphenolic compound extracted from a large variety of natural origins. These compounds are effective in the treatment of various CVDs. Epigallokatechingallat (EGCG), a typical flavonoids which is isolated from green tea, exerts its anti-atherosclerosis effect through activation of Nrf-2/keap1 pathway which inhibits the function of NF-κB in foam cells [131].

Some available drugs exert their anti- atherosclerosis effect, perhaps by inhibiting NF-κB. Atorvastatin, a lipid-lowering drug, exerts an anti-inflammatory effect by inhibiting NLRP3 inflammasome through suppressing TLR4/MyD88/NF-κB pathway [132]. Pioglitazone, a hypoglycemic drug, downregulates lipid levels as well as p65 expression and thereby slow the progression of atherosclerosis [133]. Benidipine, a dihydropyridine-Ca^{2+} channel blocker, may exert anti-atherosclerosis effect by its anti-inflammatory and anti-NF-κB effect [134, 135]. Dilazep, an antiplatelet drug with antioxidative activity, may potentially

prevent atherosclerosis in diabetes mellitus through inhibition of NF-κB mediated MCP-1 expression following the stimulation of glycoxidized-LDL [136].

Several other NF-κB inhibitors have shown cardio-protective effect in pathologic states. Muscone, the main active component of musk, has been shown to reduce inflammatory response and improve cardiac function in mice after myocardial infarction by inhibiting the activation of NF-κB and NLRP3 inflammasome [137]. Ophiopogonin D is a natural glycoside isolated from the tuber of Ophiopogonin. Studies have shown that it can inhibit NF-κB, reduce cell inflammation and protect vascular endothelium from injurious stimuli [138]. Several flavonoids also show protection against impaired cardiac function. Curcumin, a natural polyphenol, was found to antagonize cardiomyocyte apoptosis and inflammatory infiltration after myocardial infarction by inhibiting the expression of NF-KB in cardiomyocytes [139]. IL-1β and TNF-α concentration as well as NF-κB activity are inhibited in patients with stable coronary artery disease (CAD) after the treatment of Quercetin [140]. Fisetin, Chrysin and Kaempferol, which are bioactive flavonoids widely found in fruits, vegetables and flowers, also suppresses the myocardial injury through inhibiting the activity of NF-κB in isoproterol-induced myocardial injury model [141–144]. In a rat myocardial infarction model, chrysin administration results in the decreased expression of MMP-2 and MMP-9 through the downregulated IκKβ phosphorylation and NFκB expression, protecting the heart from cardiac injury[145]. Another flavonoid rutin, inhibits myocardial hypertrophy by increasing the expression of IκB-α and decreasing the expression of NFκB in carfilzomib-induced cardiotoxicity rat model administration[146]. NF-κB inhibitor pyrrolidine dithiocarbamate inhibits ox-LDL-induced lipid accumulation and improve myocardial hypertrophy and myocardial remodeling after acute myocardial infarction by inhibiting the activation of NF-κB [147–149].

NF-κB inhibitors also play an important role in blocking the occurrence and development of ischemic stroke. The inhibitors are divided into two subgroups, small-molecules and proteins (Fig. 4). Among the small-molecules, after the stimulation by naloxone, an clinically opioid receptor antagonist, nerves are protected from ischemic brain injury via inhibition of NIK/IKKα/IKBα pathway [150]. Pitavastatin prevents neuronal cell death after cerebral ischemia by inhibiting upregulations of NF-κB [151]. Oxypropoxybenzoic acid, a pyruvate and salicylate ester, has been shown to protect nerves after cerebral ischemia by inhibiting the canonical NF-KB pathway [152]. In another study, 4-methylcyclopentadecanone (4-MCPC) treatment was found to significantly reduce infarct size in a rat model of middle cerebral artery occlusion (MACO). This effect is thought to be achieved by inhibiting the activation of NF-κB [153].

In addition, a naturally occurring protein, ulinastatin, reduces infarct size in the middle cerebral artery occlusion rat model and protects neurological function by inhibiting NF-κB [154]. Neuregulin 1, a member of the epidermal growth factor family, was found to protect neural function and reduce infarct size in a rat model of MACO [155]. This effect may be related to the inhibition of NF-KB activity [156]. s-nitroso glutathione, also a natural product, was found to reduce infarct size in the MACO model and to reduce NF-κB activation [157]. Progranulin is a glycoprotein growth factor that inhibits the activation of inflammatory cells and protects nerves from cellular damage [158].

5. Conclusion

NF-κB is a therapeutic target to treat several CVDs. Although several studies have shown adverse effect of NF-κB signaling in a number of CVDs, some investigations have shown an opposite effect in the pathogenesis of CVDs. For example, NF-κB activity in ECs and macrophages may have opposite effect. The effect of NF-κB activation might be cell type-dependent. Thus, the cell-type specific effects of NF-κB in CVDs still need to be determined, and this may facilitate the development of specific inhibitors of NF-κB in CVDs.

Declarations

Author declaration

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Authors are encouraged to include a statement that specifies the contribution of every author to the research and preparation of the manuscript.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Drs. Weijia Cheng, Can Cui, Gang Liu, Chenji Ye and Fang Shao. The first draft of the manuscript was written by Dr Weijia Cheng and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Data availability

This paper is an invited review. Data was obtained from multiple sources, including Medline search and authors' own work.

References

1. Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-κB: A Blossoming of Relevance to Human Pathobiology. Cell. 2017;168(1–2):37–57.

2. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol. 2009;27:693–733.
3. Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. Nat Rev Immunol. 2008;8(11):837–48.
4. Prescott JA, Mitchell JP, Cook SJ. Inhibitory feedback control of NF-κB signalling in health and disease. Biochem J. 2021;478(13):2619–64.
5. Israël A. The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harb Perspect Biol. 2010;2(3):a000158.
6. Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene. 1999;18(49):6867–74.
7. Yu H, et al. Targeting NF-kappaB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther. 2020;5(1):209.
8. Takeuchi O, Akira S. Pattern Recognit receptors Inflamm Cell. 2010;140(6):805–20.
9. Gong T, et al. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol. 2020;20(2):95–112.
10. Hayden MS, Ghosh S. Regulation of NF-kappaB by TNF family cytokines. Semin Immunol. 2014;26(3):253–66.
11. Coope HJ, et al. CD40 regulates the processing of NF-kappaB2 p100 to p52. EMBO J. 2002;21(20):5375–85.
12. Dejardin E, et al. The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity. 2002;17(4):525–35.
13. Tang T, et al. Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint. Pharmacol Ther. 2021;219:107709.
14. Israel A. The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harb Perspect Biol. 2010;2(3):a000158.
15. Rao P, et al. IkappaBbeta acts to inhibit and activate gene expression during the inflammatory response. Nature. 2010;466(7310):1115–9.
16. Sun SC. Non-canonical NF-kappaB signaling pathway. Cell Res. 2011;21(1):71–85.
17. Cohen P, Strickson S. The role of hybrid ubiquitin chains in the MyD88 and other innate immune signalling pathways. Cell Death Differ. 2017;24(7):1153–9.
18. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood. 2012;119(3):651–65.
19. Hsu H, et al. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity. 1996;4(4):387–96.
20. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell. 1995;81(4):495–504.

21. Xia ZP, et al. Direct activation of protein kinases by unanchored polyubiquitin chains. Nature. 2009;461(7260):114–9.
22. Rahighi S, et al. Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. Cell. 2009;136(6):1098–109.
23. Chen ZJ. Ubiquitination in signaling to and activation of IKK. Immunol Rev. 2012;246(1):95–106.
24. Fitzgerald KA, et al. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J Exp Med. 2003;198(7):1043–55.
25. Wesche H, et al. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity. 1997;7(6):837–47.
26. Lamothe B, et al. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. J Biol Chem. 2007;282(6):4102–12.
27. Deng L, et al. Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell. 2000;103(2):351–61.
28. Jiang Z, et al. Interleukin-1 (IL-1) receptor-associated kinase-dependent IL-1-induced signaling complexes phosphorylate TAK1 and TAB2 at the plasma membrane and activate TAK1 in the cytosol. Mol Cell Biol. 2002;22(20):7158–67.
29. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84.
30. Wertz IE, Dixit VM. Signaling to NF-kappaB: regulation by ubiquitination. Cold Spring Harb Perspect Biol. 2010;2(3):a003350.
31. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008;132(3):344–62.
32. Claudio E, et al. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. Nat Immunol. 2002;3(10):958–65.
33. Novack DV, et al. The IkappaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis. J Exp Med. 2003;198(5):771–81.
34. Senftleben U, et al. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science. 2001;293(5534):1495–9.
35. Liao G, et al. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. J Biol Chem. 2004;279(25):26243–50.
36. de Jong SJ, et al. Noncanonical NF-kappaB activation by the oncoprotein Tio occurs through a nonconserved TRAF3-binding motif. Sci Signal. 2013;6(272):ra27.
37. Zarnegar BJ, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. Nat Immunol. 2008;9(12):1371–8.
38. Vallabhapurapu S, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. Nat Immunol.

2008;9(12):1364–70.

39. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. Mol Cell. 2001;7(2):401–9.
40. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473(7347):317–25.
41. Libby P, *Inflammation in atherosclerosis Nature 420: 868–874.* Find this article online, 2002. **68**.
42. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. Lab Invest. 2005;85(1):9–23.
43. Yu X-H, Zheng X-L, Tang C-K. *Nuclear factor-κB activation as a pathological mechanism of lipid metabolism and atherosclerosis*. Advances in clinical chemistry, 2015. 70: p. 1–30.
44. Monaco C, et al., *Canonical pathway of nuclear factor κB activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis.* Proceedings of the National Academy of Sciences, 2004. **101**(15): p. 5634–5639.
45. Gareus R, et al. Endothelial cell-specific NF-κB inhibition protects mice from atherosclerosis. Cell Metabol. 2008;8(5):372–83.
46. Kempe S. K., NF-kappaB controls the global pro-inflammatory response inendothelial cells: evidence for theregulation of apro-atherogenic program. NucleicAcidsResearch, 2005. 33: p. 5308–5319.
47. Monaco C, Paleolog E. Nuclear factor κB: a potential therapeutic target in atherosclerosis and thrombosis. Cardiovascular Res. 2004;61(4):671–82.
48. Ea C-K, et al. Activation of IKK by TNFα requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol Cell. 2006;22(2):245–57.
49. Molestina RE, et al. Requirement for NF-κB in transcriptional activation of monocyte chemotactic protein 1 by Chlamydia pneumoniae in human endothelial cells. Infect Immun. 2000;68(7):4282–8.
50. Bourcier T, Sukhova G, Libby P. The nuclear factor κ-B signaling pathway participates in dysregulation of vascular smooth muscle cells in vitroand in human atherosclerosis. J Biol Chem. 1997;272(25):15817–24.
51. Cominacini L, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-κB through an increased production of intracellular reactive oxygen species. J Biol Chem. 2000;275(17):12633–8.
52. Rodríguez-Ayala E, et al. Enhanced RAGE-mediated NFκB stimulation in inflamed hemodialysis patients. Atherosclerosis. 2005;180(2):333–40.
53. De Winther MP, et al. Nuclear factor κB signaling in atherogenesis. Arterioscler Thromb Vasc Biol. 2005;25(5):904–14.
54. Lamon BD, Hajjar DP. Inflammation at the molecular interface of atherogenesis: an anthropological journey. Am J Pathol. 2008;173(5):1253–64.
55. Björkbacka H, et al. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med. 2004;10(4):416–21.

56. Mullick AE, et al. Increased endothelial expression of Toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events. J Exp Med. 2008;205(2):373–83.
57. Michelsen KS, et al., *Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E.* Proceedings of the National Academy of Sciences, 2004. **101**(29): p. 10679–10684.
58. Mohan S, Mohan N, Sprague EA. Differential activation of NF-kappa B in human aortic endothelial cells conditioned to specific flow environments. Am J Physiology-Cell Physiol. 1997;273(2):C572–8.
59. Hajra L, et al., *The NF-κB signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation.* Proceedings of the National Academy of Sciences, 2000. **97**(16): p. 9052–9057.
60. Orr AW, et al. The subendothelial extracellular matrix modulates NF-κB activation by flow: a potential role in atherosclerosis. J Cell Biol. 2005;169(1):191–202.
61. Yao Y, et al. Klotho ameliorates oxidized low density lipoprotein (ox-LDL)-induced oxidative stress via regulating LOX-1 and PI3K/Akt/eNOS pathways. Lipids Health Dis. 2017;16(1):1–10.
62. Han C, et al. Angiotensin II induces C-reactive protein expression through ERK1/2 and JNK signaling in human aortic endothelial cells. Atherosclerosis. 2010;212(1):206–12.
63. Savoia C, Schiffrin EL. Vascular inflammation in hypertension and diabetes: molecular mechanisms and therapeutic interventions. Clin Sci. 2007;112(7):375–84.
64. Hernandez-Presa M, et al. Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. Circulation. 1997;95(6):1532–41.
65. Tham DM, et al. Angiotensin II is associated with activation of NF-κB-mediated genes and downregulation of PPARs. Physiol Genom. 2002;11(1):21–30.
66. Kzhyshkowska J, Neyen C, Gordon S. Role of macrophage scavenger receptors in atherosclerosis. Immunobiology. 2012;217(5):492–502.
67. Sukhorukov VN, et al. Lipid metabolism in macrophages: focus on atherosclerosis. Biomedicines. 2020;8(8):262.
68. Park YM. CD36, a scavenger receptor implicated in atherosclerosis. Exp Mol Med. 2014;46(6):e99–9.
69. Lind L. Circulating markers of inflammation and atherosclerosis. Atherosclerosis. 2003;169(2):203–14.
70. Brand K, et al. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. J Clin Investig. 1996;97(7):1715–22.
71. Brach MA, et al. Regulation of M-CSF expression by M-CSF: Role of protein kinase C and transcription factor NFkB. Pathobiology. 1991;59(4):284–8.
72. Bond M, et al. Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: an absolute requirement for transcription factor NF-κB. FEBS Lett. 1998;435(1):29–34.

73. Luttun A, et al. Loss of matrix metalloproteinase-9 or matrix metalloproteinase-12 protects apolipoprotein E–deficient mice against atherosclerotic media destruction but differentially affects plaque growth. Circulation. 2004;109(11):1408–14.
74. Kiyan Y, et al. oxLDL induces inflammatory responses in vascular smooth muscle cells via urokinase receptor association with CD36 and TLR4. J Mol Cell Cardiol. 2014;66:72–82.
75. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362(6423):801–9.
76. Amento EP, et al. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler thrombosis: J vascular biology. 1991;11(5):1223–30.
77. Barath P, et al. Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by in situ hybridization. Am J Pathol. 1990;137(3):503.
78. Hiscott J, et al. Characterization of a functional NF-kappa B site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop. Mol Cell Biol. 1993;13(10):6231–40.
79. Rosenfeld M, et al. Macrophage colony-stimulating factor mRNA and protein in atherosclerotic lesions of rabbits and humans. Am J Pathol. 1992;140(2):291.
80. Clinton S, et al. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. Am J Pathol. 1992;140(2):301.
81. Grassia G, et al., The IκB Kinase Inhibitor Nuclear Factor-κB Essential Modulator–Binding Domain Peptide for Inhibition of Injury-Induced Neointimal Formation. Arteriosclerosis, Thromb Vascular Biology, 2010. 30(12): 2458–66.
82. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell. 2011;145(3):341–55.
83. Tabas I. *Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency.* Arteriosclerosis, thrombosis, and vascular biology, 2005. 25(11): p. 2255–2264.
84. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol. 2010;10(1):36–46.
85. Littlewood TD, Bennett MR. Apoptotic cell death in atherosclerosis. Curr Opin Lipidol. 2003;14(5):469–75.
86. Yao S, et al., *D4F alleviates macrophage-derived foam cell apoptosis by inhibiting endoplasmic reticulum stress-CHOP pathway*. J Lipid Res, 2015.
87. Pamukcu B, Lip GY, Shantsila E. The nuclear factor–kappa B pathway in atherosclerosis: a potential therapeutic target for atherothrombotic vascular disease. Thromb Res. 2011;128(2):117–23.
88. Kasibhatla S, et al. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-κB and AP-1. Mol Cell. 1998;1(4):543–51.
89. Bubici C, et al. NF-κB and JNK: an intricate affair. Cell Cycle. 2004;3(12):1524–9.

90. Luo J-L, Kamata H, Karin M. IKK/NF-κB signaling: balancing life and death–a new approach to cancer therapy. J Clin Investig. 2005;115(10):2625–32.
91. Papa S, et al. The NF-κB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. Cell Death & Differentiation. 2006;13(5):712–29.
92. Ottonello L, et al. Delayed apoptosis of human monocytes exposed to immune complexes is reversed by oxaprozin: role of the Akt/IκB kinase/nuclear factor κB pathway. Br J Pharmacol. 2009;157(2):294–306.
93. Kumar A, et al. Nuclear factor-κB: its role in health and disease. J Mol Med. 2004;82(7):434–48.
94. Shoelson S, Lee J, Yuan M. Inflammation and the IKK β/I κ B/NF-κ B axis in obesity-and diet-induced insulin resistance. Int J Obes. 2003;27(3):S49–52.
95. Dandona P, et al. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation. 2005;111(11):1448–54.
96. Galis ZS, et al. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Investig. 1994;94(6):2493–503.
97. Bond M, et al. Inhibition of transcription factor NF-κB reduces matrix metalloproteinase-1,-3 and-9 production by vascular smooth muscle cells. Cardiovascular Res. 2001;50(3):556–65.
98. Feldmann M, et al. Is NF-κB a useful therapeutic target in rheumatoid arthritis? Ann Rheum Dis. 2002;61(suppl 2):ii13–8.
99. Chase AJ, et al., *Role of nuclear factor-κB activation in metalloproteinase-1,-3, and-9 secretion by human macrophages in vitro and rabbit foam cells produced in vivo.* Arteriosclerosis, thrombosis, and vascular biology, 2002. 22(5): p. 765–771.
100. Cummins EP, et al., *Prolyl hydroxylase-1 negatively regulates IκB kinase-β, giving insight into hypoxia-induced NFκB activity.* Proceedings of the National Academy of Sciences, 2006. **103**(48): p. 18154–18159.
101. Koong AC, et al. Hypoxic activation of nuclear factor-κB is mediated by a Ras and Raf signaling pathway and does not involve MAP kinase (ERK1 or ERK2). Cancer Res. 1994;54(20):5273–9.
102. Walmsley SR, et al. Hypoxia-induced neutrophil survival is mediated by HIF-1α–dependent NF-κB activity. J Exp Med. 2005;201(1):105–15.
103. Weyrich AS, et al. Time course of coronary vascular endothelial adhesion molecule expression during reperfusion of the ischemic feline myocardium. J Leukoc Biol. 1995;57(1):45–55.
104. Zingarelli B, et al. Sesquiterpene lactone parthenolide, an inhibitor of IκB kinase complex and nuclear factor-κB, exerts beneficial effects in myocardial reperfusion injury. Shock. 2002;17(2):127–34.
105. Guo J, et al. Ischaemia/reperfusion induced cardiac stem cell homing to the injured myocardium by stimulating stem cell factor expression via NF-κB pathway. Int J Exp Pathol. 2009;90(3):355–64.
106. Morishita R, et al. In vivo transfection of cis element “decoy” against nuclear factor-κ B binding site prevents myocardial infarction. Nat Med. 1997;3(8):894–9.

107. Frantz S, et al. Absence of NF-κB subunit p50 improves heart failure after myocardial infarction. FASEB J. 2006;20(11):1918–20.
108. Misra A, et al. Nuclear factor-κB protects the adult cardiac myocyte against ischemia-induced apoptosis in a murine model of acute myocardial infarction. Circulation. 2003;108(25):3075–8.
109. Dorn GW. The fuzzy logic of physiological cardiac hypertrophy. Hypertension. 2007;49(5):962–70.
110. Saito T, Giaid A. *Cyclooxygenase-2 and nuclear factor-kappaB in myocardium of end stage human heart failure.* Congestive heart failure (Greenwich, Conn.), 1999. 5(5): p. 222–227.
111. Frantz S, et al. Sustained activation of nuclear factor kappa B and activator protein 1 in chronic heart failure. Cardiovascular Res. 2003;57(3):749–56.
112. Grabellus F, et al. Reversible activation of nuclear factor-κB in human end-stage heart failure after left ventricular mechanical support. Cardiovascular Res. 2002;53(1):124–30.
113. Kawamura N, et al. Blockade of NF-κB improves cardiac function and survival without affecting inflammation in TNF-α-induced cardiomyopathy. Cardiovascular Res. 2005;66(3):520–9.
114. Kawano S, et al. Blockade of NF-κB improves cardiac function and survival after myocardial infarction. Am J Physiol Heart Circ Physiol. 2006;291(3):H1337–44.
115. Purcell NH, et al., *Activation of NF-κB is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes.* Proceedings of the National Academy of Sciences, 2001. **98**(12): p. 6668–6673.
116. Hirotani S, et al. Involvement of nuclear factor-κB and apoptosis signal-regulating kinase 1 in G-protein–coupled receptor agonist–induced cardiomyocyte hypertrophy. Circulation. 2002;105(4):509–15.
117. Freund C, et al. Requirement of nuclear factor-κB in angiotensin II–and isoproterenol-induced cardiac hypertrophy in vivo. Circulation. 2005;111(18):2319–25.
118. Zelarayan L, et al. NF-κB activation is required for adaptive cardiac hypertrophy. Cardiovascular Res. 2009;84(3):416–24.
119. Kratsios P, et al. Antioxidant amelioration of dilated cardiomyopathy caused by conditional deletion of NEMO/IKKγ in cardiomyocytes. Circul Res. 2010;106(1):133–44.
120. Hikoso S, et al. The IκB kinase β/nuclear factor κB signaling pathway protects the heart from hemodynamic stress mediated by the regulation of manganese superoxide dismutase expression. Circul Res. 2009;105(1):70–9.
121. Huang C-Y, et al. SOD1 down-regulates NF-κB and c-Myc expression in mice after transient focal cerebral ischemia. J Cereb Blood Flow Metabolism. 2001;21(2):163–73.
122. Nurmi A, et al. Nuclear factor-κB contributes to infarction after permanent focal ischemia. Stroke. 2004;35(4):987–91.
123. Kaushal V, Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. J Neurosci. 2008;28(9):2221–30.

124. Zhang X, et al. Tissue-type plasminogen activator and the low-density lipoprotein receptor-related protein mediate cerebral ischemia-induced nuclear factor-κB pathway activation. Am J Pathol. 2007;171(4):1281–90.
125. Schneider A, et al. NF-κB is activated and promotes cell death in focal cerebral ischemia. Nat Med. 1999;5(5):554–9.
126. Inta I, et al. Bim and Noxa are candidates to mediate the deleterious effect of the NF-κB subunit RelA in cerebral ischemia. J Neurosci. 2006;26(50):12896–903.
127. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol. 2007;184(1–2):53–68.
128. Targeting CD*40-Induced TRAF6 Signaling in Macrophages Reduces Atherosclerosis*. Journal of the American College of Cardiology, 2018. **71**(5): p. 527–542.
129. Zhuang J, et al. Inhibitory effects of vinpocetine on the progression of atherosclerosis are mediated by Akt/NF-κB dependent mechanisms in apoE-/-mice. PLoS ONE. 2013;8(12):e82509.
130. Lopez-Franco O, et al. W09-P-006 The NF-KB inhibitor parthenolide modulates the inflammatory responses in experimental atherosclerosis. Atherosclerosis (Supplements)(Component). 2005;1(6):41.
131. Jiang J, et al. Epigallocatechin-3-gallate prevents TNF-α-induced NF-κB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells. Int J Mol Med. 2012;29(5):946–56.
132. Kong F, et al. Atorvastatin suppresses NLRP3 inflammasome activation via TLR4/MyD88/NF-κB signaling in PMA-stimulated THP-1 monocytes. 82: Biomedicine & Pharmacotherapy; 2016. pp. 167–72.
133. Rong LI, Cai H, Zhao LJ. Effect of pioglitazone on activity of NF-KB and expression of aorta apoptosis proteins in rats with hyperlipidemia. Chin J Geriatric Heart Brain Vessel Dis. 2012;39(9):865–70.
134. Matsubara M, Hasegawa K. Effects of benidipine, a dihydropyridine-Ca2 + channel blocker, on expression of cytokine-induced adhesion molecules and chemoattractants in human aortic endothelial cells. Eur J Pharmacol. 2004;498(1–3):303–14.
135. Takayama M, et al. Comparison of the antiatherosclerotic effects of dihydropyridine calcium channel blocker and HMG-CoA reductase inhibitor on hypercholesterolemic rabbits. Vascul Pharmacol. 2007;46(4):302–8.
136. Sonoki K, et al. Dilazep and fenofibric acid inhibit MCP-1 mRNA expression in glycoxidized LDL-stimulated human endothelial cells. Eur J Pharmacol. 2003;475(1–3):139–47.
137. Du Y, et al., *Muscone improves cardiac function in mice after myocardial infarction by alleviating cardiac macrophage-mediated chronic inflammation through inhibition of NF-κB and NLRP3 inflammasome*. American Journal of Translational Research, 2018. 10(12).
138. Huang X, et al., *Ophiopogonin D and EETs ameliorate Ang II-induced inflammatory responses via activating PPARα in HUVECs*. Biochem Biophys Res Commun, 2017: p. 123.

139. Lv F, et al. Effects of curcumin on the apoptosis of cardiomyocytes and the expression of NF-κB, PPAR-γ and Bcl-2 in rats with myocardial infarction injury. Experimental & Therapeutic Medicine; 2016.
140. Chekalina N, et al. Quercetin reduces the transcriptional activity of NF-kB in stable coronary artery disease. Indian Heart J. 2018;70(5):593–7.
141. Garg S, et al. Fisetin attenuates isoproterenol-induced cardiac ischemic injury in vivo by suppressing RAGE/NF-κB mediated oxidative stress, apoptosis and inflammation. Phytomedicine. 2019;56:147–55.
142. Rani N, et al. Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting AGE-RAGE mediated oxidative stress and inflammation. Chemico-Biol Interact. 2016;250:59–67.
143. Suchal K, et al., *Kaempferol attenuates myocardial ischemic injury via inhibition of MAPK signaling pathway in experimental model of myocardial ischemia-reperfusion injury.* Oxidative Medicine and Cellular Longevity, 2016. **2016**.
144. Suchal K, et al. Kampeferol protects against oxidative stress and apoptotic damage in experimental model of isoproterenol-induced cardiac toxicity in rats. Phytomedicine. 2016;23(12):1401–8.
145. Yang M, et al. Chrysin attenuates interstitial fibrosis and improves cardiac function in a rat model of acute myocardial infarction. J Mol Histol. 2018;49(6):555–65.
146. Imam F, et al. Rutin attenuates carfilzomib-induced cardiotoxicity through inhibition of NF-κB, hypertrophic gene expression and oxidative stress. Cardiovasc Toxicol. 2017;17(1):58–66.
147. Zhao G, Tang S, Guoping T. Effect of PDTC on Lipid Accumulation and Cholesterol Efflux in THP-1 Macrophages Induced by ox-LDL. Medical Science Journal of Central South China; 2013.
148. Ming HE, et al. The effects of pyrrolidine dithiocarbamate on myocadial hypertrophy after myocardial infarction in rats. Journal of Cardiovascular and Pulmonary Diseases; 2012.
149. Jin JL, et al., Improvement of Left Ventricular Remodelling by Inhibition of NF-κB in a Rat Model of Myocardial Infarction. Heart Lung & Circulation, 2016. 25(10).
150. *Naloxone attenuates ischemic brain injury in rats through suppressing the NIK/IKKα/NF-κB and neuronal apoptotic pathways*. Acta Pharmacologica Sinica.
151. Tounai H, et al. Immunohistochemical study on distribution of NF-κB and p53 in gerbil hippocampus after transient cerebral ischemia: effect of pitavastatin. Metab Brain Dis. 2007;22(1):89–104.
152. Lee HK, et al. Anti-inflammatory effects of OBA-09, a salicylic acid/pyruvate ester, in the postischemic brain. Brain Res. 2013;1528(Complete):68–79.
153. Jin Y, et al., *Anti-inflammatory effect of 4-methylcyclopentadecanonein rats submitted to ischemic stroke*. Fundamental and Clinical Pharmacology, 2018. 32(3).
154. Cho YS, et al., *Ulinastatin inhibits cerebral ischemia-induced apoptosis in the hippocampus of gerbils*. Molecular Medicine Reports, 2015. 12(2).
155. Wang S, et al. Evaluation of neuregulin-1's neuroprotection against ischemic injury in rats using diffusion tensor imaging. Magn Reson Imaging. 2018;53:63–70.

156. Simmons LJ, et al., *Regulation of inflammatory responses by neuregulin-1 in brain ischemia and microglial cells in vitro involves the NF-kappa B pathway*. Journal of Neuroinflammation, 2016. 13(1).

157. Khan M, et al. S-Nitrosoglutathione reduces inflammation and protects brain against focal cerebral ischemia in a rat model of experimental stroke. J Cereb Blood Flow Metabolism Official J Int Soc Cereb Blood Flow Metabolism. 2005;25(2):177.

158. Egashira Y, et al., *The growth factor progranulin attenuates neuronal injury induced by cerebral ischemia-reperfusion through the suppression of neutrophil recruitment*. Journal of Neuroinflammation, 2013. 10.

Figures

Figure 1

N-terminus of the NF-κB family members contains a conserved 300 amino acid: Rel homology domain (RHD) and NLS. RHD mediates DNA binding, dimerisation, IκBα interaction. The members in the family

are divided into two groups: C-terminal transactivation domain (TAD) contained p65, c-Rel and RelB; p50 and p52 lacking a TAD. TAD is responsible for the transcriptional activity of dimers. p50 and p52 act as transcriptional repressors in their homodimeric form because of lacking of a TAD. p50 and p52 are derived from the limited proteasomal processing of precursors p105 and p100, respectively. p100 and p105 also share C-terminal ankyrin repeats (ANKR) which can bind to other NF-κB subunits thus inhibit their nuclear translocation and a death domain (DD) which can mediate protein interactions with adaptor proteins. The nine IκBs contain five to seven tandem ankyrin repeats (AnkR), which mediate binding to NF-kB dimers which formally defines the family. The classical IκB proteins are IκBα, IκBβ and IκBε. Their N-terminal regions contain two serine residues (P) which permit the ubiquitination (Ub) and the C-terminal PEST domains of IκBα and IκBβ are involved in constitutive turnover. The precursor IκB proteins p100 and p105, which can be processed to form the NF-κB family members p52 and p50, respectively, or can be degraded. p105 and p100 contains C-terminal ankyrin repeat domains which enable them to sequester NF-κB subunits in the cytosol. The atypical IκB proteins (BCL-3, IκBζ, IκBNS and IκBη) have typically low expression and can be induced by various stimuli, including NF-κB activation. BCL3 has similar serine residues as classical IκBs at N-terminus. IKK complex include three components IKKa, IKKb and the non-enzymatic subunit NEMO.Both IKKα and IKKβ consist of a kinase domain at the N-terminus, a LZ, a helix loop helix (HLH) motif and a NEMO-binding domain (NBD) at the C-terminus. NEMO has two coiled coil domains (CC1 and CC2), a LZ and a zinc finger (ZF) domain.

Figure 2

Canonical and non-canonical NF-κB activating pathway. Canonical NF-κB pathway is induced by multiple signals from receptors including TNFR1, IL-1R, and TLR4. It involves activation of the IKK complex by TAK-1 mediated by several intermediate adaptor molecules and E3 ligases, such as TRADD, Myd88, TRAF, cIAPs, and LUBAC. The phosphorylation and the degradation of IκBα are mediated by IKKβ activation, leading to the nuclear translocation of unbound p65/P50 heterodimer. The non-canonical NF-κB pathway is triggered by CD40 or LTβR upon binding with their ligand, which leads to the upregulation and the aggregation of NIK via TRAF2/3-cIAPs complex-dependent way. NIK-mediated IKKα activation promotes the processing of p100 and liberates RELB/p52 heterodimer to the nucleus.

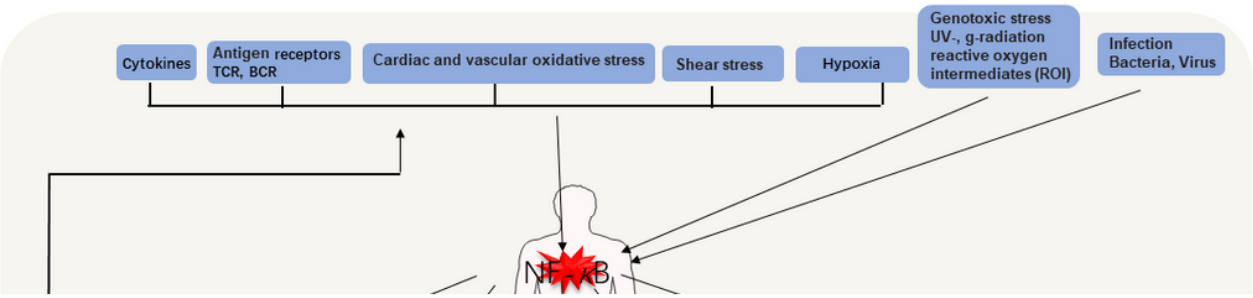

Figure 3

NF-κB is an inducible regulator transcription factor of immune and inflammatory responses, to environmental and cellular stress. NF-κB target genes are involved in inflammation and development and progression of CVDs. After its activation, the transcription of inflammatory cytokines, chemokines and adhesion molecules increase and cell maturation, proliferation, apoptosis, morphogenesis and differentiation occur.

Figure 4

NF-κB signaling plays a pathogenic role in various CVDs including Atherosclerosis, myocardial infarction and reperfusion injury, heart failure and Hypertrophy, ischemia stroke.; NF-κB inhibitors perform it effect on different therapeutic target in NF-κB dependent signaling. For example, Vinpocetine suppresses atherosclerotic development in apoE$^{-/-}$ mice through blocking IKKα/β, IκBα phosphorylation and subsequently NF-κB activity.