Trends in Biochemical Sciences



Opinion

MK2-TNF-Signaling Comes Full Circle

Manoj B. Menon¹ and Matthias Gaestel^{1,*}

MK2 (p38^{MAPK}-activated protein kinase 2) is essential for tumor necrosis factor (TNF) biosynthesis, mainly operating by post-transcriptional regulation. Deletion of the gene encoding MK2 strongly reduced serum TNF and protected against endotoxic shock, demonstrating the positive role of p38^{MAPK}/MK2 in TNF signaling at the level of ligand expression. Recent evidence indicates that MK2 directly phosphorylates the TNF receptor interactor RIPK1 and suppresses its activity, thereby limiting TNF-mediated apoptosis and necroptosis – pointing to a more complex, double-edged role of MK2 in TNF signaling. In addition, novel MK2 substrates have emerged in the DNA damage response, autophagy, and obesity, making MK2 a multifunctional kinase at the crossroads of stress response and cell death. We therefore propose a more general role of p38^{MAPK}/MK2 signaling in the timely coordinated onset and resolution of inflammation and beyond.

The Canonical Role of p38^{MAPK}/MK2 in TNF Signaling

TNF is a major cytokine of systemic inflammation that is secreted mainly by macrophages, is recognized by TNF receptor type 1 (TNFR1) that is present on most cells and tissues, and stimulates inflammatory gene expression as well as cell death. In parallel to the classical mitogen-activated kinase (MAPK) cascade, there are two further stress-activated kinase cascades that contain c-Jun N-terminal kinases (JNKs) and p38^{MAPKs} as their central elements, comprising three (JNK1, 2, 3) and four (p38^{MAPK} α - δ) gene products and their splice variants, respectively. An essential role for the stress- and lipopolysaccharide (LPS, see Glossary)activated protein kinase p38^{MAPK} in TNF biosynthesis and inflammation became obvious more than 20 years ago when $p38^{MAPK} \alpha$ and β were identified as targets for a novel class of cytokine-suppressive anti-inflammatory drugs [1]. Soon after it became clear that the two downstream kinases phosphorylated and activated by $p38^{MAPK\alpha}$ and β , MK2 (also known as MAPKAPK2) [2] and its less-expressed 'sister' kinase MK3 (MAPKAPK3) [3], are prominently involved. While there are many substrates for MK2/3, only the mRNA-binding protein tristetraprolin (TTP/ZFP36) [4,5] significantly contributes to the regulation of TNF production. TTP regulates TNF biosynthesis post-transcriptionally by binding to an **adenylate-uridylate** (AU)-rich element (ARE) in the 3'-untranslated region (3'-UTR) of TNF mRNA. This binding destabilizes TNF mRNA and suppresses its translation (reviewed in [6]). Phosphorylation of TTP by MK2 neutralizes these functions [7,8] and, conversely, phosphorylation site mutation (TTP-S52A, S178A) renders TTP constitutively active, leading to increased mRNA binding [9] and a strong reduction of TNF biosynthesis and inflammation in vivo [10].

Although genetic studies have established a role for the MK2–TTP axis in TNF production, understanding the underlying molecular mechanism of phosphorylation-dependent TTP action required further insight into TTP binding to the ARE of TNF mRNA and the kinetics of inflammation. In fact, different mRNA-binding proteins compete for binding to the TNF-ARE. TTP can be effectively replaced at the ARE by its major antagonist HuR, but only when the affinity of TTP is reduced as a result of MK2-mediated phosphorylation [11]. In contrast to

Highlights

MK2 regulates biosynthesis of TNF and other cytokines at the posttranscriptional level by phosphorylating and inactivating the mRNAdestabilizing and translation-inhibiting protein tristetraprolin (TTP).

Phosphorylation of the TNF receptorinteracting protein kinase RIPK1 by MK2 inhibits autophosphorylation of RIPK1 and its integration into cytoplasmic cytotoxic complexes, and thus suppresses TNF-dependent apoptosis and necroptosis.

The p38/MK2 pathway negatively regulates SMAC mimetic (SM)induced RIPK1 kinase-dependent TNF production in myeloid cells. Thus, p38/MK2 coinhibition emerges as a therapeutic strategy to circumvent SM resistance in leukemia, aiding in autocrine TNF-induced apoptosis.

Novel roles for MK2 are emerging in cancer, inflammation, and autophagy with the identification of novel substrates, such as TRIM29, BECN1, and CEP131, and their distinct loss-of-function phenotypes.

¹Institute of Cell Biochemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

*Correspondence: gaestel.matthias@mh-hannover.de (M. Gaestel).

Trends in Biochemical Sciences



TTP, HuR stabilizes TNF mRNA and may stimulate its translation, thereby promoting inflammation. In a later stage of resolution of inflammation, protein phosphatase 2A (PP2A) dephosphorylates enzymes of the p38^{MAPK}/MK2 pathway as well as TTP itself to promote the degradation of cytokine mRNAs [12]. In addition, MK2 stimulates TTP transcription by phosphorylating the transcription factor SRF, completing an additional feedback control loop from MK2 to the biosynthesis of active TTP [13]. The phosphorylation-driven exchange between TTP and HuR at mRNA AREs seems to be a general mechanism that regulates not only TNF but also other cytokines and further proteins relevant to inflammation [14,15].

MK2-stimulated translation of pre-pro-TNF is a prerequisite for its processing, maturation, and trimerization in the endoplasmic reticulum (ER)/*trans*-Golgi, and its final TACE-mediated shedding as a bioactive ligand for TNF receptors (reviewed in [16]). Therefore, facilitation of ligand biosynthesis was considered to be the canonical role of MK2 in TNF signaling. This role was underlined by low serum levels of TNF in LPS-stimulated *Mapkapk2^{-/-}* (*MK2* knockout, KO) mice and the increased resistance of these animals to experimental LPS/D-gal endotoxic challenge [2] as well as increased susceptibility to *Listeria* infection [17] – two models where endogenously produced serum TNF is the mediator of pathology. Unexpectedly, a recent study observed increased sensitivity of *MK2* KO mice to injections of low doses of exogenous TNF [18], indicating a novel additional role of MK2 downstream from TNFR1. Indeed, various recent studies have now identified MK2 as a modulator of TNFR1 signaling by phosphorylating a central component, the TNF receptor-interacting protein kinase 1 (RIPK1) [19–21]. The parallel action of MK2 in ligand generation and downstream receptor signaling opens new perspectives for our understanding of the regulation of TNF signaling.

A Novel Role for MK2 in TNF Receptor Downstream Signaling

An early indication that MK2 functions downstream from TNFR1 was the discovery of MK2 as a mediator of TNF-induced apoptosis [22]. However, in this study MK2-dependent IL-1 production was found to be a contributing factor rather than a direct participant in the TNF response. Interestingly, another study, which reported RIPK1-RIPK3 interaction and phosphorylation in TNF-induced programmed necrosis, identified MK2 as one of 10 pro-necroptotic genes from a panel of 691 kinase-related genes. In this study siRNAs against MK2 protected FADD-deficient Jurkat cells from TNF-induced necroptosis [23]. MK2 also phosphorylates cleaved caspase 3 (CASP3) and facilitates its nuclear translocation, promoting apoptosis in a model of LPS-induced vascular permeability [24]. In contrast to these findings, several recent reports have established MK2 as a RIPK1 kinase that clearly suppresses cell death signaling downstream from TNFR1 and the Toll-like receptors (TLRs) [19-21]. This new role would now explain why MK2 deletion sensitizes mice to low doses of intravenous TNF [18,20]. Mechanistically, RIPK1 is recruited to TNFR1 and TLR3/4 in response to ligand binding where it forms a signaling hub for further cell fate decisions (Figure 1). Non-degradative ubiquitination of RIPK1 at the receptor recruits and activates components of the NF-kB and MAPK pathways, leading to a proinflammatory/pro-survival response comprising upregulation of cytokines, inhibitor of apoptosis proteins (IAPs), and other pro-survival transcripts. A pro-death response involves the recruitment of deubiquitinated RIPK1 into cytosolic complexes containing CASP8. In addition, RIPK1 can be recruited to a RIPK3 containing pro-necrotic complex. Recent evidence also indicates a role for kinase activity-dependent ubiquitination of RIPK1 in the stabilization of these cytosolic complexes [25,26]. There is significant plasticity in the responses to ligand stimulation, and the outcome is fine-tuned by coordinated post-translational modifications and interactions of RIPK1 (reviewed in [27]) (Figure 1B,C). Although the pro-survival functions of RIPK1 are kinase activity-independent, apoptotic and necroptotic responses involve RIPK1 autophosphorylation and require RIPK1 activity. In addition to its role in NF-κB activation, IKK

Glossary

Adenylate-uridylate (AU)-rich elements (AREs): sequence

elements in the 3'-untranslated regions (3'-UTRs) of many tightly regulated transcripts including mRNAs of cytokines and cell-cycle regulators. They are rich in adenine and uridine bases, often harbor repeats of a core AUUUA sequence, and usually target mRNAs for rapid mRNA decay or suppress translation by interacting with an array of AREbinding proteins.

Cellular FLICE (FADD-like IL-1βconverting enzyme)-inhibitory protein (cFLIP): also known as caspase (CASP) 8 and FADD-like apoptosis regulator (CFLAR), cFLIP is an anti-apoptotic protein that binds to and inhibits CASP8 and CASP10. Multiple splice forms of cFLIP exist with altered functions, and their expression levels significantly affect the cytotoxic responses to death receptor ligation and chemotherapy.

Inhibitor of apoptosis proteins (IAPs): a family of related proteins which act as endogenous inhibitors of apoptotic death by suppressing caspase activation. They are characterized by the presence of one or more baculoviral IAP repeat (BIR) domains. The best-characterized members are cIAP1, cIAP2, and XIAP, which additionally possess a poly-ubiquitin-binding UBA (Ubassociated) domain and a RING (really interesting new gene) domain that identified them as ubiquitin E3 ligases. This ligase activity of cIAPs is involved in the regulation of NF-KB signaling. In addition, cIAP1/2mediated ubiquitination of RIPK1 is crucial for preventing the assembly of RIPK1-containing cell deathpromoting complexes.

Lipopolysacharide (LPS): also known as endotoxin, LPS is an integral component of the cell wall of Gram-negative bacteria such as *E. coli*. It is one of the bestcharacterized pathogen-associated molecular pattern (PAMP) molecules which are recognized by the innate immune system as a means to detect bacterial infection. The cellular receptor for LPS consists of Toll-like receptor 4 (TLR4) and the coreceptor CD14 (cluster of differentiation 14). Binding of LPS to the receptor

ARTICLE IN PRESS

Trends in Biochemical Sciences

CelPress



Trends in Biochemical Sciences

Figure 1. RIP Kinases and RIPK1-Harboring Protein Complexes in Cell Death and Survival. (A) Schematic representation of the primary structure of RIPKs. The RIP kinase family includes RIPK1-RIPK5 and the less-related RIPK6/ 7 (known as leucine-rich repeat kinases-1/2, LRRK1/2) (reviewed in [64]). RIPK1–5 induce NF-κB/MAPK activation upon overexpression, and RIPK2 is involved in NOD1/2 (nucleotide-binding oligomerization domain-containing proteins 1/2)dependent bacterial peptidoglycan sensing and RIPK1/3-mediated TNFR1/TLR-driven cell death signaling. The death domain unique to RIPK1 aids in death receptor recruitment, while the RIP-homeotypic interaction motif (RHIM) mediates RIPK1-RIPK3 association and the recruitment of other RHIM-containing adapters including TRIF. (B,C) RIPK1-containing pro-survival (B) and pro-cell death (C) protein complexes. (B) RIPK1 is a cellular switch that balances cell death decisions by its regulated integration into diverse multiprotein complexes. Upon ligand binding, RIPK1 is recruited to a membraneassociated receptor complex, is ubiquitinated, and scaffolds a pro-survival complex that is essential for NFkB and MAPK activation (complex I), independently of its kinase activity. In response to DNA damage, ataxia telangiectasia mutated protein kinase (ATM) activation leads to the formation of a cytosolic complex [58] which provides NE_KB and MAPK survival signals similarly to complex I. (C) When RIPK1 is dissociated from complex I, it is deubiquitinated and upon further activation nucleates a pro-apoptotic complex IIb. Cytosolic ubiquitinated RIPK1 can also assemble into a complex IIb-like structure after deubiquitination by depletion of IAPs or genotoxic stress. Upon prolonged DNA damage, the ATM-induced pro-survival response can also induce complex IIb by autocrine TNF production. After depletion of cellular FLICE (FADDlike IL-1β-converting enzyme)-inhibitory protein (cFLIP) and CASP8 dimerization, the premature ripoptosome forms the active complex IIb which executes apoptosis. In the absence of CASP8 activity, active RIPK1 recruits RIPK3 to form a necrosome, leading to RIPK3 and MLKL phosphorylation and oligomerization, and subsequent necroptosis. RIPK1 activity-dependent RIPK1 ubiquitination is important for necrosome formation. A RIPK1-independent pathway for apoptosis also exists downstream from TNFR1, and is mediated by complex IIa consisting of TRADD, FADD, and CASP8.

complex on myeloid cells initiates a strong proinflammatory response. Ripoptosome: a cytosolic deathpromoting complex that is nucleated in response to genotoxic stress or IAP depletion. Its nucleation is dependent on RIPK1 activity, but is independent of death receptor stimulation. Cytosolic RIPK1 exist in a dormant state, being basally ubiquitinated by cIAP1/2. Genotoxic stress or depletion of IAPs by SMAC mimetics (SMs) leads to the deubiquitination, dimerization, and activation of RIPK1, which recruits FADD and CASP8 as core components to form the ripoptosome. cFLIP is part of a premature ripoptosome, and suppresses its cytotoxic potential by preventing CASP8 homodimerization and cleavage. The ripoptosome resembles complex llb, which is seeded by death receptor signaling and can similarly induce RIPK1 activity-dependent apoptosis or RIPK3-dependent necrosis.

Tristetraprolin (TTP): also known as ZFP36, TTP is a zinc-finger nonenzymatic RNA-binding protein and a substrate for MK2. It is a feedback regulator of inflammatory signaling and mediator of post-transcriptional gene regulation. Non-phosphorylated TTP binds to AREs in the 3'-UTRs of mRNAs and subjects them to rapid degradation or translational suppression by recruiting further factors.

Trends in Biochemical Sciences



was demonstrated to phosphorylate receptor-associated and ubiquitinated RIPK1 and to provide a direct pro-survival signal [28]. MK2/3-mediated phosphorylation of RIPK1 at the intermediate domain residues S321/S336 similarly results in cytoprotective effects. However, unlike IKK, MK2 is not part of the receptor-associated complexes but phosphorylates the abundant cytosolic pool of RIPK1, suppressing its autophosphorylation and recruitment into cytosolic death-inducing complexes [19–21]. Thus, IKK and MK2, although both are activated downstream from RIPK1, provide two spatially distinct checkpoints to the TNF-induced death response by phosphorylating distinct residues in the RIPK1 intermediate domain, thereby suppressing RIPK1-dependent apoptosis/necroptosis [29]. The relative contributions of the two pathways vary depending on the cytotoxic stimulus [20,21]. While IKK displays a dominant role in cell survival upon TNF treatment, combinatorial treatments using TNF together with SMAC mimetics (SMs) or the CASP8 inhibitor zVAD demonstrate a more prominent role for MK2 in cell survival.

LPS as a proinflammatory stimulus is an IKK/MAPK activator and the major inducer of TNF during bacterial infection. LPS can be converted to a pro-death stimulus when IKKs or caspases are inhibited. Also in this case, the MK2-RIPK1 axis is able to suppress the cytotoxic response [21]. Interestingly, the Gram-negative bacterial pathogens of the genus Yersinia counteract this process to induce apoptosis of infected macrophages. By their type III secretion system they inject Yersinia outer-membrane proteins (YOPs) to inactivate the IKK- and MK2dependent checkpoints and trigger LPS-induced RIPK1-dependent apoptosis of host macrophages. While cell death induced under these conditions does not correlate with autocrine TNF production, cell-extrinsic TNF may contribute to Yersinia-induced apoptosis in vivo [21,30,31]. Although infection-induced macrophage death is a bacterial strategy, it is also beneficial for effector-triggered immunity, increasing host survival and the control of bacterial burden [31]. Thus, one might hypothesize that MK2 KO mice should better survive Yersinia infection, but it is difficult to predict the dominant function of MK2 in vivo. In the course of infection, MK2 could act as part of a feed-forward loop that supports enhanced TNF production and immune cell survival. The outcome of several studies on the effect of MK2 deficiency in inflammation needs to be revisited based on this understanding. MK2 KO mice showed enhanced sensitivity to the intracellular Gram-positive pathogen Listeria, wherein the increased bacterial load in the KO mice was explained by compromised TNF production and phagocytosis [17]. A recent study has shown a role for RIPK1-dependent necroptosis of liver-resident macrophages in the resolution of Listeria infection [26], suggesting that MK2 could contribute to the postulated feed-forward loop here as well. In a model of inflammation-induced skin carcinogenesis, comparative analysis of $Tnf^{-/-}$ and MK2 KO mice revealed that, despite similar suppression of inflammation in both genotypes, the MK2 KO mice developed fewer skin tumors with more apoptotic cells in the epidermis [32]. This phenotype could well be attributed to RIPK1dependent cell death. Interestingly, the Crohn's disease-like intestinal pathology in the Tnf Δ^{ARE} mouse is exacerbated in the absence of MK2 [33]. Because TNF production is independent of MK2 in this model, the intestinal inflammation and mortality in the *Tnf* Δ^{ARE} *MK2* KO mice could result from altered TNF-downstream signaling. Indeed, MK2 completes a full circle with respect to TNF signaling with crucial roles upstream and downstream of the TNFR1 (Figure 2, Key Figure). Therefore, a knock-in model of the non-phosphorylatable RIPK1 mutant (mRIPK1 S321A/S336A) will be necessary to conclusively differentiate roles of MK2 downstream and upstream of TNF in the various models of inflammation.

TLR-induced TNF production is the best-established role of p38^{MAPK}/MK2 signaling and has been in the focus of extensive research in the past two decades. It was completely unexpected that p38^{MAPK}/MK2 inhibition enhances SM-induced TNF production in macrophages [34,35].

Trends in Biochemical Sciences



Key Figure

MK2-TNF Signaling Comes Full Circle



Trends in Biochemical Sciences

Figure 2. TLR ligation leads to the activation of NFκB-dependent transcription of the *TNF* gene and parallel p38/MK2 activation. TNF mRNA is constitutively degraded and translationally suppressed by the non-phosphorylated RNA-binding protein TTP. MK2-mediated TTP phosphorylation facilitates replacement of TTP at the ARE by HuR, which facilitates TNF translation (canonical MK2 function). The translated pre-pro-TNF is processed and secreted as active trimerized TNF. TNF binding recruits RIPK1 to TNFR1, and ubiquitinated RIPK1 further nucleates pro-survival complexes at the membrane which induce NFκB and p38/MK2 signals. IKK phosphorylates RIPK1 bound to TNFR1. MK2-mediated phosphorylation of RIPK1-S321/336 in the cytosol prevents autophosphorylation/activation of RIPK1 and its integration into cytosolic apoptosis/necroptosis-promoting complexes, completing the full circle of MK2–TNF signaling. While some cells including the immune cells of the myeloid lineage secrete TNF, driving autocrine signaling, other cells (e.g., fibroblasts) respond to TNF in a paracrine manner, eliciting pro-survival or cytotoxic responses. Symbols and abbreviations: –, inhibition; +, activation; P, phosphorylation; PAMP, pathogen-associated molecular pattern; SMAC mim, SMAC mimetic.

SM-induced death involves induction of TNF production and subsequent autocrine signaling (Box 1). This depends on RIPK1 activity-dependent JNK/ERK MAPK activation and is negatively regulated by the p38^{MAPK}/MK2 pathway [35,36]. In addition, non-canonical NF- κ B activation could also contribute to SM-induced TNF production independently of RIPK1 kinase activity [36–38]. Although there is a possible rewiring of MAPK circuits upon SM treatment, the question remains of whether this is also relevant to other physiological models of inflammation. Recent studies have demonstrated a cell death-independent role for RIPK1 activity in cytokine

ARTICLE IN PRESS

Trends in Biochemical Sciences

CellPress REVIEWS

Box 1. SMAC Mimetics: Anti-IAPs by Design

SMAC (second mitochondria-derived activator of caspases) or DIABLO (direct IAP binding protein with low pl) is a protein which inhibits IAPs to facilitate caspase activation. During apoptosis SMAC is released from the mitochondria and proteolytically cleaved to expose a tetrapeptide motif which helps in binding to the BIR domains of XIAP, cIAP1, and cIAP2. While this interaction compromises XIAP function by disrupting its interaction with caspases, cIAP1 and cIAP2 undergo auto-ubiquitination and degradation upon SMAC binding, which unleashes the cell death response by activating caspases. The availability of high-resolution structural information regarding the interaction between a SMAC-derived peptide and IAPs has enabled the design and development of peptidomimetics which display properties of endogenous SMAC and bind to IAPs with high affinity and specificity. These cell-permeable small molecules, termed SMAC mimetics (SMs), induce RIPK1 kinase activity-dependent TNF-production and robust autocrine TNF-dependent apoptotic cell death in various cancer cells. Further cancer cells could be targeted by combining SMs with death receptor ligands or agonists. IAPs are involved in basal ubiquitination and inactivation of RIPK1-dependent cytotoxic responses in cancer cells. Depletion of IAPs by a SM therefore leads to an increased level of non-ubiquitinated RIPK1, enhances ripoptosome assembly, and promotes subsequent apoptosis-independent death receptor activation. This accounts for the synergistic effect of these compounds in combination with genotoxic chemotherapeutics such as etoposide. Several SM compounds are currently undergoing clinical trials as anticancer drugs for single and combinatorial therapy, with promising results with respect to tolerance and efficacy (reviewed in [65]).

production, specifically when inflammatory stimuli such as TNF or LPS are combined with caspase inhibition [39,40]. Is it possible that the negative role for MK2 in TNF production is only present in cases of RIPK1-dependent proinflammatory responses? Further research combining the *MK2* KO and catalytically dead RIPK1^{K45A} mutant mice models will be necessary to answer this question.

Novel Functions of MK2 beyond TNF

The past decade has witnessed the rise of MK2 as a DNA damage checkpoint kinase akin to Chk1 and Chk2. Interestingly, the p38^{MAPK}/MK2 pathway is only crucial for genotoxic stressinduced cell-cycle arrest in cancer cells lacking the functional tumor suppressor p53, and is dispensable in p53-proficient cells [41-44]. MK2-mediated phosphorylation of the cell cycleassociated phosphatases CDC25B/C/A, as well as post-transcriptional control of cell-cycle regulators through the MK2 substrate RNA-binding protein hnRNPA0 and poly-A ribonuclease PARN, were shown to be involved in this control [41-44]. Although MK2/p53 co-deficiency enhanced tumorigenesis in a mouse model of lung cancer, the double-deficient tumors were more susceptible to DNA-damaging chemotherapy, suggesting a complex interplay of MK2 and p53 in tumor initiation and survival in vivo [45]. Apart from this, MK2-mediated phosphorylation of TRIM29, a p53 binding protein, seems to mediate resistance to radiation therapy in pancreatic cancer [46]. In the context of DNA damage and repair, MK2 also participates in replication fork stalling because since an siRNA screen identified MK2 as a crucial mediator of replication stress-induced accumulation of phosphohistone 2AX, a marker of DNA damage [47]. Moreover, MK2 depletion or inhibition also rescued cells from the DNA replication block and cytotoxicity induced by nucleoside analogs. This protective effect required translesion polymerases including Pol η (POLH), which is *in vitro* phosphorylated by MK2. It is interesting to note that, although MK2 inhibition potentiates the cytotoxicity induced by DNA crosslinking agents such as cisplatin, it protects cells from the toxicity of nucleoside analogs which only interfere with DNA replication [41]. UV irradiation-induced apoptosis was also significantly suppressed in MK2 KO skin [47], but the relative contributions of diverse MK2-dependent pathways in this phenotype have yet to be determined.

The footprint of MK2 signaling has spread far and wide in the past few years with the discovery of novel substrates and functions (Table 1). An interesting recent development was identification of its crosstalk with the autophagic pathway. Beclin (BECN) 1 is a central regulator of autophagy interacting with the pro-apoptotic component Bcl-2. An *in vitro* screen for the kinase

ARTICLE IN PRESS

Trends in Biochemical Sciences



Target	Phospho-sites	Evidence	Function	Refs
RIPK1	S320, S335	Direct MK2 substrate <i>in vitro</i> ; pharmacological (MK2 inhibitor PF3644022) and genetic proof (<i>MK2</i> KO and <i>MK2/3</i> double KO, DKO) for MK2/3-mediated phosphorylation	TNF/TLR-driven cell death signaling	[19–21]
hnRNPA0	S84	<i>In vitro</i> phosphorylation; pharmacological (p38 inhibitor: SB203580) and genetic (<i>MK2</i> KO) evidence; residual SB203580-sensitive phosphorylation detectable in <i>MK2</i> KO cells	Post-transcriptional control of cell cycle regulators and cytokine mRNAs	[43,66]
TRIM29	S550, S552	Direct MK2 substrate in vitro; pharmacological evidence (SB302580 and undisclosed MK2 inhibitor)	Resistance to ionizing radiation	[46]
BECN1	S90	Direct MK2/3 substrate; <i>in vitro</i> and genetic proof (<i>MK2/3</i> DKO)	Initiation of autophagy	[48]
CEP131	S47, S78, S731	Direct MK2 substrate <i>in vitro</i> ; pharmacological evidence (SB203580 and PF3644022)	UV-induced centriolar satellite remodeling	[67]
Dazl	S65	In vitro substrate of MK2; pharmacological evidence (SB203580 and MK2 inh-III)	Post-transcriptional control of spermatogenesis	[68]
POLH	S380*	*Predicted site and only an <i>in vitro</i> substrate, not investigated in cells	Translesion DNA synthesis	[47]
UBE2J1	S184	<i>In vitro</i> phosphorylation; pharmacological (p38 inhibitors: SB202190, BIRB796; MK2 inhibitor: PHA781089) and genetic (<i>MK2/3</i> DKO) evidence; ER stress-induced phosphorylation not abrogated in <i>MK2/3</i> DKO	Ubiquitin-conjugating enzyme with a role in ER protein quality control	[56]

Table 1. New Targets for Emerging Novel Non-Canonical Functions of MK2/3

responsible for BECN1-S90 phosphorylation first identified MK3 in a library of 190 different kinases [48]. Further studies established that both MK2 and MK3 are positive regulators of starvation-induced autophagy by mediating BECN1-S90 phosphorylation and promoting autophagosome formation. A complex picture is emerging here, wherein JNK1/2 seem to facilitate MK2-mediated BECN1 phosphorylation by phosphorylating Bcl-2 and preventing it from competitively inhibiting MK2–BECN1 binding. In light of contradictory reports on the role of p38^{MAPK} in the regulation of autophagy [49], this MK2 function brings us one step closer to delineating the pathway of autophagy signaling.

In vivo studies using mouse models have provided insights into MK2 functions which would have been difficult to ascertain in *in vitro* systems. MK2 inactivation was recently shown to reduce insulin-resistance in obese mice [50]. By contrast, high-fat diet-induced insulin resistance was enhanced in MK2-deficient mice, indicating that MK2 plays distinct roles in the induction and maintenance of insulin resistance [51]. Additional roles for MK2 emerging from animal studies include the control of synaptic plasticity [52], diabetes-induced cardiac remodeling [53], and inflammation-induced colon carcinogenesis [54]. In addition, further interesting MK2 functions await mechanistic investigations. On the one hand, MK2 was found to regulate TLR-driven PKB-S473 phosphorylation, but the direct substrates of MK2 involved in this process remain unknown [55]. On the other hand, UBE2J1, an ER-associated ubiquitin-conjugating enzyme, was identified as a substrate of MK2 with a role in TNF production [56], but how this protein participates in cytokine production was not investigated further. In every aspect of stress and cytokine signaling there is seemingly a tug of war between different MK2 substrates to fine-tune the appropriate cellular response.

ARTICLE IN PRESS

Trends in Biochemical Sciences



MK2 as a Pharmaceutical Target in Inflammation, Cancer, and Neurodegeneration?

Its canonical function in cytokine biosynthesis has made the p38^{MAPK}/MK2/TTP axis a potential target for anti-inflammatory therapy for many years [16]. The recent finding that MK2-mediated RIPK1 phosphorylation supports the survival of TNF receptor-harboring cells, such as macro-phages, provides a novel mechanism for further proinflammatory action of MK2. Therefore, inhibition of MK2 in anti-inflammatory therapy could both inhibit cytokine biosynthesis at the cellular level and reduce the number of cytokine-producing cells, leading to resolution of inflammation.

MK2 targeting in combinatorial anticancer therapy is gaining prominence. A clear rationale for a direct link between the p38^{MAPK}/MK2/RIPK1 axis and cancer treatment is the finding that RIPK1 is an essential part of the **ripoptosome**, a high molecular weight protein complex which is assembled in response to genotoxic stress and to depletion of inhibitor of apoptosis proteins (IAPs) [57], and that MK2-phosphorylation of RIPK1 suppresses the recruitment of RIPK1 to cell death complexes such as the ripoptosome [19–21]. In response to DNA damage, a cytosolic complex consisting of ATM, NEMO, RIPK1, and TAK1 was shown to induce pro-survival p38/MK2 and NF-κB signaling, while extensive DNA damage can result in ATM/RIPK1-dependent autocrine TNF production and subsequent cell death [58,59]. Therefore, one may assume that inhibition of MK2 will improve the efficacy of chemotherapeutic agents that induce genotoxic stress [60]. In line with this, p38^{MAPK}/MK2 co-inhibition was recently reported as an effective approach to overcoming SM resistance in a subset of myeloid leukemia [35]. MK2 inhibition seems to facilitate cell killing in this system by both enhancing RIPK1-dependent TNF production and promoting the assembly of death-inducing ripoptosome-like complexes.

Recently, a combinatorial treatment with the mitotic checkpoint kinase Chk1 inhibitor PF477736 and the MK2 inhibitor PF3644022 was demonstrated to have a strong synergistic effect in killing KRAS- or BRAF-mutated tumor cells [61]. This synergy may be explained by the finding that MK2 is a third mitotic checkpoint kinase, as proposed for p53-deficient tumors [41], but this could also arise from increased recruitment of RIPK1 to the ripoptosome and its increased cell death-promoting activity in p53-containing cells.

Deficiency for the tumor-suppressor p53 is a characteristic of cellular resistance to genotoxic anticancer therapies, and hence therapeutic options for overcoming this resistance are highly desirable. The analysis of the role of MK2 depletion in a non-small cell lung cancer model *in vivo* revealed that loss of MK2 is synthetically lethal in combination with p53 mutations, and sensitizes p53-deficient tumor cells to the genotoxic action of cisplatin [45]. This might also be facilitated by increased recruitment of RIPK1 to the ripoptosome in the absence of S321/S336 phosphorylation. Hence, inhibition of MK2 together with DNA-damaging chemotherapy could be of advantage in the treatment of p53-deficient tumors.

In an established mouse model of Alzheimer's disease (AD), inhibition of microglial RIPK1 activity reduced amyloid burden and promoted degradation of extracellular β -amyloid, making RIPK1 a potential target for therapeutic approaches for AD [62]. Because MK2 also inhibits RIPK1 activity, one may speculate whether activation of MK2 might also target AD. However, the strong contribution of MK2 to microglial cytokine production, which contributes to neuro-inflammatory pathology in a mouse model of β -amyloid deposition [63], seems to exclude this possibility. However, it will be interesting to investigate whether coinhibition of MK2 and RIPK1 will have beneficial additive effects as a result of reduced inflammation and enhanced amyloid clearance.

Trends in Biochemical Sciences



Concluding Remarks

The recent addition of RIPK1 to the still growing panel of MK2 substrates has elucidated some of the unexplained effects of MK2, but has also added new questions for future research (see Outstanding Questions). How do multiple post-translational modifications of RIPK1 orchestrate pro-survival and pro-death events in TNF and TLR signaling? Understanding this post-translational modification code, which defines the spatial and temporal dynamics of RIPK1 function, will be an essential step forward in gaining a comprehensive view of MK2 functions in inflammation, cell death, and cancer.

Acknowledgment

We thank Dr Achim Gossler, Hannover, for critical reading of the manuscript.

References

- 1. Lee, J.C. *et al.* (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372, 739–746
- Kotlyarov, A. et al. (1999) MAPKAP kinase 2 is essential for LPSinduced TNF-alpha biosynthesis. Nat. Cell Biol. 1, 94–97
- Ronkina, N. et al. (2007) The mitogen-activated protein kinase (MAPK)-activated protein kinases MK2 and MK3 cooperate in stimulation of tumor necrosis factor biosynthesis and stabilization of p38 MAPK. Mol. Cell. Biol. 27, 170–181
- Carballo, E. (1998) Feedback inhibition of macrophage tumor necrosis factor-α production by tristetraprolin. Science 281, 1001–1005
- Neininger, A. et al. (2002) MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J. Biol. Chem. 277, 3065–3068
- Anderson, P. (2008) Post-transcriptional control of cytokine production. Nat. Immunol. 9, 353–359
- Chrestensen, C.A. et al. (2004) MAPKAP kinase 2 phosphorylates tristetraprolin on *in vivo* sites including Ser178, a site required for 14-3-3 binding. J. Biol. Chem. 279, 10176–10184
- Mahtani, K.R. et al. (2001) Mitogen-activated protein kinase p38 controls the expression and posttranslational modification of tristetraprolin, a regulator of tumor necrosis factor alpha mRNA stability. *Mol. Cell. Biol.* 21, 6461–6469
- Hitti, E. et al. (2006) Mitogen-activated protein kinase-activated protein kinase 2 regulates tumor necrosis factor mRNA stability and translation mainly by altering tristetraprolin expression, stability, and binding to adenine/uridine-rich element. *Mol. Cell. Biol.* 26, 2399–2407
- Ross, E.A. *et al.* (2015) Dominant suppression of inflammation via targeted mutation of the mRNA destabilizing protein tristetraprolin. *J. Immunol.* 195, 265–276
- Tiedje, C. *et al.* (2012) The p38/MK2-driven exchange between tristetraprolin and HuR regulates AU-rich element-dependent translation. *PLoS Genet.* 8, e1002977
- Sun, L. et al. (2007) Tristetraprolin (TTP)-14-3-3 complex formation protects TTP from dephosphorylation by protein phosphatase 2a and stabilizes tumor necrosis factor-alpha mRNA. J. Biol. Chem. 282, 3766–3777
- Ronkina, N. et al. (2011) Stress induced gene expression: a direct role for MAPKAP kinases in transcriptional activation of immediate early genes. *Nucleic Acids Res.* 39, 2503–2518
- Tiedje, C. et al. (2016) The RNA-binding protein TTP is a global post-transcriptional regulator of feedback control in inflammation. Nucleic Acids Res. 44, 7418–7440
- Sedlyarov, V. *et al.* (2016) Tristetraprolin binding site atlas in the macrophage transcriptome reveals a switch for inflammation resolution. *Mol. Syst. Biol.* 12, 868–868
- Gaestel, M. et al. (2009) Targeting innate immunity protein kinase signalling in inflammation. Nat. Rev. Drug Discov. 8, 480–499

- Lehner, M.D. et al. (2002) Mitogen-activated protein kinase-activated protein kinase 2-deficient mice show increased susceptibility to Listeria monocytogenes infection. J. Immunol. 168, 4667– 4673
- Vandendriessche, B. et al. (2014) MAPK-activated protein kinase 2-deficiency causes hyperacute tumor necrosis factor-induced inflammatory shock. *BMC Physiol.* 14, 5
- Jaco, I. *et al.* (2017) MK2 phosphorylates RIPK1 to prevent TNFinduced cell death. *Mol. Cell* 66, 698–710.e5
- Dondelinger, Y. et al. (2017) MK2 phosphorylation of RIPK1 regulates TNF-mediated cell death. Nat. Cell Biol. 19, 1237–1247
- Menon, M.B. et al. (2017) p38(MAPK)/MK2-dependent phosphorylation controls cytotoxic RIPK1 signalling in inflammation and infection. Nat. Cell Biol. 19, 1248–1259
- Janes, K.A. et al. (2008) Cytokine-induced signaling networks prioritize dynamic range over signal strength. Cell 135, 343–354
- Cho, Y.S. *et al.* (2009) Phosphorylation-driven assembly of the RIP1–RIP3 complex regulates programmed necrosis and virusinduced inflammation. *Cell* 137, 1112–1123
- Damarla, M. et al. (2013) MK2 mediates apoptosis during lung vascular permeability by regulating movement of cleaved caspase 3. Am. J. Respir. Cell Mol. Biol. 50, 932–941
- de Almagro, M.C. et al. (2016) Coordinated ubiquitination and phosphorylation of RIP1 regulates necroptotic cell death. Cell Death Differ. 24, 26–37
- 26. Wang, H. et al. (2017) PELI1 functions as a dual modulator of necroptosis and apoptosis by regulating ubiquitination of RIPK1 and mRNA levels of c-FLIP. *Proc. Natl. Acad. Sci. U. S. A.* 114, 11944–11949
- Liu, X. et al. (2016) Post-translational modifications as key regulators of TNF-induced necroptosis. Cell Death Dis. 7, e2293
- Dondelinger, Y. et al. (2015) NF-κB-independent role of IKKα/ IKKβ in preventing RIPK1 kinase-dependent apoptotic and necroptotic cell death during TNF signaling. Mol. Cell 60, 63–76
- Oberst, A. (2017) MK2 balances inflammation and cell death. Nat. Cell Biol. 19, 1150–1152
- Peterson, L.W. et al. (2016) Cell-extrinsic TNF collaborates with TRIF signaling to promote Yersinia-induced apoptosis. J. Immunol. 197, 4110–4117
- Peterson, L.W. *et al.* (2017) RIPK1-dependent apoptosis bypasses pathogen blockade of innate signaling to promote immune defense. *J. Exp. Med.* jem.20170347
- Johansen, C. et al. (2009) MK2 regulates the early stages of skin tumor promotion. Carcinogenesis 30, 2100–2108
- Kontoyiannis, D. *et al.* (2002) Genetic dissection of the cellular pathways and signaling mechanisms in modeled tumor necrosis factor-induced Crohn's-like inflammatory bowel disease. *J. Exp. Med.* 196, 1563–1574
- 34. Girnius, N. and Davis, R.J. (2016) TNFα-mediated cytotoxic responses to IAP inhibition are limited by the p38a MAPK pathway. *Cancer Cell* 29, 131–133

Outstanding Questions

Molecular mechanism: understanding the post-translational modification code governing RIPK1 functions in apoptosis and necroptosis.

RIPK1 autophosphorylation versus substrate phosphorylation: a search for direct substrates of RIPK1.

How does MK2 inhibit SM-induced TNF production?

Is the MK2–RIPK1–axis also involved in RIPK1-dependent cytokine production in inflammation?

Differentiating the roles of MK2 upstream and downstream of TNFR in inflammation: will RIPK1-S321A/ S336A knock-in mice give us the answer?

Trends in Biochemical Sciences



- Lalaoui, N. *et al.* (2016) Targeting p38 or MK2 enhances the antileukemic activity of Smac-mimetics. *Cancer Cell* 29, 145–158
- 36. Christofferson, D.E. *et al.* (2012) A novel role for RIP1 kinase in mediating TNFα production. *Cell Death Dis.* 3, e320
- Vince, J.E. et al. (2007) IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 131, 682–693
- Varfolomeev, E. et al. (2007) IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* 131, 669–681
- Shutinoski, B. et al. (2016) K45A mutation of RIPK1 results in poor necroptosis and cytokine signaling in macrophages, which impacts inflammatory responses in vivo. Cell Death Differ. 23, 1628–1637
- Najjar, M. et al. (2016) RIPK1 and RIPK3 kinases promote celldeath-independent inflammation by Toll-like receptor 4. Immunity 45, 46–59
- Manke, I.A. et al. (2005) MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. Mol. Cell 17, 37–48
- 42. Reinhardt, H.C. et al. (2007) p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. Cancer Cell 11, 175–189
- Reinhardt, H.C. et al. (2010) DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization. *Mol. Cell* 40, 34–49
- Cannell, I.G. *et al.* (2015) A pleiotropic RNA-binding protein controls distinct cell cycle checkpoints to drive resistance of p53-defective tumors to chemotherapy. *Cancer Cell* 28, 623–637
- 45. Morandell, S. et al. (2013) A reversible gene-targeting strategy identifies synthetic lethal interactions between MK2 and p53 in the DNA damage response *in vivo. Cell Rep.* 5, 868–877
- Wang, L. et al. (2014) ATDC/TRIM29 phosphorylation by ATM/ MAPKAP kinase 2 mediates radioresistance in pancreatic cancer cells. Cancer Res. 74, 1778–1788
- Köpper, F. et al. (2013) Damage-induced DNA replication stalling relies on MAPK-activated protein kinase 2 activity. Proc. Natl. Acad. Sci. U. S. A. 110, 16856–16861
- Wei, Y. et al. (2015) The stress-responsive kinases MAPKAPK2/ MAPKAPK3 activate starvation-induced autophagy through Beclin 1 phosphorylation. eLife 4, 1584
- Webber, J.L. and Tooze, S.A. (2010) Coordinated regulation of autophagy by p38alpha MAPK through mAtg9 and p38IP. *EMBO* J. 29, 27–40
- Ozcan, L. *et al.* (2015) Treatment of obese insulin-resistant mice with an allosteric MAPKAPK2/3 inhibitor lowers blood glucose and improves insulin sensitivity. *Diabetes* 64, 3396–3405
- de Boer, J.F. et al. (2014) Mitogen-activated protein kinase-activated protein kinase 2 deficiency reduces insulin sensitivity in high-fat diet-fed mice. PLoS One 9, e106300
- Eales, K.L. et al. (2014) The MK2/3 cascade regulates AMPAR trafficking and cognitive flexibility. Nat. Commun. 5, 1–16

- Ruiz, M. et al. (2016) MK2 deletion in mice prevents diabetesinduced perturbations in lipid metabolism and cardiac dysfunction. *Diabetes* 65, 381–392
- Ray, A.L. et al. (2015) Blockade of MK2 is protective in inflammation-associated colorectal cancer development. Int. J. Cancer 138, 770–775
- McGuire, V.A. et al. (2013) Cross talk between the Akt and p38α pathways in macrophages downstream of Toll-like receptor signaling. Mol. Cell. Biol. 33, 4152–4165
- 56. Menon, M.B. et al. (2013) Endoplasmic reticulum-associated ubiquitin-conjugating enzyme Ube2/1 is a novel substrate of MK2 (MAPKAP kinase-2) involved in MK2-mediated TNFα production. *Biochem. J.* 456, 163–172
- Tenev, T. *et al.* (2011) The ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. *Mol. Cell* 43, 432–448
- Yang, Y. et al. (2011) A cytosolic ATM/NEMO/RIP1 complex recruits TAK1 to mediate the NF-kappaB and p38 mitogen-activated protein kinase (MAPK)/MAPK-activated protein 2 responses to DNA damage. Mol. Cell. Biol. 31, 2774–2786
- Biton, S. and Ashkenazi, A. (2011) NEMO and RIP1 control cell fate in response to extensive DNA damage via TNF-α feedforward signaling. *Cell* 145, 92–103
- Menon, M.B. et al. (2017) To die or not to die: regulatory feedback phosphorylation circuits determine receptor-interacting protein kinase -1 (RIPK1) function. Mol. Cell. Oncol. Published online November 27, 2017. http://dx.doi.org/10.1080/ 23723556.2017.1396389
- Dietlein, F. et al. (2015) A synergistic interaction between Chk1 and MK2 inhibitors in KRAS-mutant cancer. Cell 162, 146–159
- Ofengeim, D. et al. (2017) RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 114, E8788–E8797
- 63. Culbert, A.A. *et al.* (2006) MAPK-activated protein kinase 2 deficiency in microglia inhibits pro-inflammatory mediator release and resultant neurotoxicity. Relevance to neuroinflammation in a transgenic mouse model of Alzheimer disease. *J. Biol. Chem.* 281, 23658–23667
- Humphries, F. et al. (2015) RIP kinases: key decision makers in cell death and innate immunity. *Cell Death Differ*. 22, 225–236
 Bai I. et al. (2014) Small-molecule SMAC mimetics as new
- Bai, L. *et al.* (2014) Small-molecule SMAC mimetics as new cancer therapeutics. *Pharmacol. Ther.* 144, 82–95
- Rousseau, S. *et al.* (2002) Inhibition of SAPK2a/p38 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. *EMBO J.* 21, 6505–6514
- Tollenaere, M.A.X. et al. (2015) p38- and MK2-dependent signalling promotes stress-induced centriolar satellite remodelling via14-3-3-dependent sequestration of CEP131/AZI1. Nat. Commun. 6, 1–12
- Williams, P.A. et al. (2016) Phosphorylation of the RNA-binding protein Dazl by MAPKAP kinase 2 regulates spermatogenesis. *Mol. Biol. Cell* 27, 2341–2350