Mitogen-Activated Protein Kinase–Activated Protein Kinase 2 in Neuroinflammation, Heat Shock Protein 27 Phosphorylation, and Cell Cycle: Role and Targeting

Fadi Maged Shokry Gurgis, William Ziaziaris, and Lenka Munoz

Department of Pharmacology, School of Medical Sciences, University of Sydney, New South Wales, Australia

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ABSTRACT

Mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MAPKAPK-2 or MK2) is a downstream substrate of the p38 MAPK responsible for the signaling events influencing inflammation, cell division and differentiation, apoptosis, and cell motility in response to a wide range of extracellular stimuli. After the failure of p38 MAPK inhibitors in clinical trials, MK2 was unveiled as a potential target to regulate inflammatory cytokines’ mRNA stability and translation. Recent work suggests that this mechanism may underlie the pathophysiology of brain disorders associated with inflammation. In addition, MK2 is a prominent kinase that phosphorylates heat shock protein 27 (Hsp27), an intensely investigated biomarker of cancer progression. This phosphorylation decreases the chaperone properties of Hsp27, making MK2 an endogenous inhibitor of Hsp27. MK2 is also one of the major players in the signal transduction pathways activated in response to DNA damage. Experimental evidence highlights the role of MK2 in G2/M and the mitotic spindle checkpoints, two mechanisms by which MK2 contributes to the maintenance of genomic stability. Thus, MK2 is considered a good molecular target to increase, in combination with chemotherapeutic agents, the sensitivity of treatment, especially in p53-mutated tumors. This review looks at the functions of MK2 in inflammation, Hsp27 regulation, and cell cycle checkpoint control with a focus on brain pathologies. Analysis of MK2 signaling in various disease models and a summary of the data on MK2 inhibitors suggest novel indications for MK2 inhibitors in addition to their mainstream use against peripheral inflammatory disorders.

Introduction

Mitogen-activated protein kinase (MAPK)–activated protein kinase 2 (MAPKAPK-2 or MK2) is a downstream substrate of the p38 MAPK known to transduce a range of extracellular signals that result in inflammatory response, cell division and differentiation, apoptosis, and cell motility. Because of its central role in inflammation, p38 MAPK has been extensively investigated over the past decades. However, the tremendous effort invested into the development of selective and efficacious p38 MAPK inhibitors has not delivered the much-needed small-molecule anti-inflammatory drug. As p38 MAPK regulates activity of more than 60 substrates (Trempolec et al., 2013), p38 MAPK inhibition is explicably accompanied with unwanted side effects and failure in clinical trials. MK2 is the main and essential downstream target of p38 MAPK, regulating biosynthesis of tumor necrosis factor α (TNFα) and other cytokines (Kotlyarov et al., 1999). Thus, MK2 emerged as a potential anti-inflammatory target, as convincingly documented by the increasing number of publications and reviews related to MK2 inflammation. In addition, MK2 governs not only peripheral inflammation but also neuroinflammation.
the inflammation of the brain (Culbert et al., 2006; Thomas et al., 2008b; Ghasemlou et al., 2010). As the prominent kinase phosphorylating heat shock protein 27 (Hsp27) (Stokoe et al., 1992), MK2 has become a promising target for cancer treatment. Finally, MK2 is activated after DNA damage (Manke et al., 2005; Reinhardt et al., 2007), resulting in cell cycle arrest such that cells have the capacity to repair their DNA and continue to proliferate. Because this mechanism underlies resistance to chemotherapy, MK2 inhibitors could as serve as anticancer agents to improve the efficacy of chemotherapy.

As these aspects of MK2 biology have not been recently summarized, we present here a review of the role of MK2 in neuroinflammation-associated brain disorders. We then discuss studies outlining the biologic role of MK2-dependent regulation of Hsp27 activity and cell cycle control. Finally, we conclude with the therapeutic potential of MK2 inhibitors, focusing on brain pathologies whenever possible.

### Structure and Function of MK2

MK2 was discovered as an extracellular signal-regulated kinase 1/2–activated protein kinase that had the capacity to phosphorylate and thus inactivate Hsp27 and its murine homolog Hsp25 (Stokoe et al., 1992). In following years, p38 MAPK was found to phosphorylate MK2 in response to stress stimuli. Further studies confirmed that MK2 activation depends on p38α/β MAPK-regulated phosphorylation of Thr222 in the activation loop, Ser272 in the catalytic domain, and Thr334 (Fig. 1A) located in the hinge region between the catalytic domain and C-terminal regulatory domain (Ben-Levy et al., 1995).

MK2 displays high homology (75% amino acid identity) to MK3, and their kinase domains are similar to other members of the calcium- and calmodulin-regulated kinases superfamily. MK2 expresses an N-terminal proline-rich region, which is responsible for interactions with Src homology 3 domains in vitro (Plath et al., 1994). The C-terminus contains a functional bipartite nuclear localization signal (NLS) sequence that maintains the location of MK2 predominantly in the nuclei of resting cells (Fig. 1). Conversely, the nuclear export sequence (NES) is located in the N terminus to the NLS domain and triggers nuclear export after MK2 activation (Fig. 1) (Ben-Levy et al., 1998; Engel et al., 1998).

The crystal structure of MK2 revealed that Thr334 phosphorylation serves as a switch for MK2 nuclear import and export. Indeed, phosphomimetic mutation of Thr334 enhances cytoplasmic localization of MK2, suggesting that MK2 contains a constitutively active NLS and a phosphorylation-regulated NES. In resting cells, p38 MAPK and MK2 form a complex in the nucleus (Fig. 1B) (Ben-Levy et al., 1998). Cellular stress causes the phosphorylation of p38 MAPK by upstream kinases, such as MAPK kinase 3. The activated p38 MAPK then phosphorylates MK2 at Thr222, Ser272, and/or Thr334. When activated at Thr334, both p38 MAPK and MK2 translocate to the cytoplasm while still physically bound together using the exportin 1–dependent mechanism, where both kinases activate their downstream substrates (Fig. 1B).

Phosphorylation at Thr222 within the activation loop is crucial for MK2-dependent activation of several target substrates, including enzymes, proteins that regulate cytoskeleton motility, mRNA-binding proteins, and regulators of the cell cycle (Deleault et al., 2008).

MK2-dependent phosphorylation of TTP at Ser52 and Ser178, two sites necessary for binding to 14-3-3 proteins (Chrestensen et al., 2004; Stoecklin et al., 2004). For example, MK2 phosphorylates TTP at Ser52 and Ser178, two sites necessary for binding to 14-3-3 proteins (Chrestensen et al., 2004; Stoecklin et al., 2004). This phosphorylation abolishes the TTP function as a TNFα mRNA suppressor by inhibiting ARE-mediated decay of TNFα mRNA and allowing efficient translation via subcellular translocation of the mRNA from P-bodies (Sandler and Stoecklin, 2008). TTP phosphorylation by MK2 also increases TTP protein expression via exclusion from proteasomal degradation and cytoplasmic retention (Deleault et al., 2008).

Furthermore, MK2-dependent phosphorylation of TTP changes its affinity for ARE binding. Phosphorylated TTP has shown 10-fold lower Kd for ARE mRNA, which led to the replacement of TTP from AREα by human antigen-R, a protein known to stabilize short-lived mRNAs (Tiedje et al., 2012). These complex mechanisms of posttranscriptional regulation of cytokines synthesis via MK2-dependent phosphorylation of RNA-binding proteins (RBPs) have been the topic of several excellent reviews (Cargnello and Roux, 2011; Gaestel, 2013).
The above-mentioned RBPs have been also reported to stabilize the short-lived mRNA coding for cyclooxygenase 2 (COX-2), IL-8, and vascular endothelial growth factor (Suswam et al., 2008; Miyata et al., 2013). As the function of the RBPs is modified by MK2, but at the same time the substrate spectrum of MK2 is significantly smaller than that of p38 MAPK, MK2 has emerged as an attractive anti-inflammatory target. Although our review covers predominantly MK2, it is important to briefly reference MK3 (McLaughlin et al., 1996; Sithanandam et al., 1996). The expression levels of this kinase...
are much lower than MK2 (Ronkina et al., 2007), but the high structural identity and nearly the same substrate spectrum with MK2 (Cheng et al., 2010) imply similar functional behavior in the biologic systems. Indeed, the C termini of MK3 contain NLS and NES signals rendering unphosphorylated MK3 in the nucleus and inducing translocation to the cytoplasm upon p38 MAPK-dependent phosphorylation. Furthermore, MK3 is involved in posttranscriptional regulation of the ARE-containing mRNAs described for MK2 (Fig. 2), indicating that MK3 could control cytokine biosynthesis in addition to MK2 (Ronkina et al., 2008). Indeed, MK2/3 double knockout mice showed a higher reduction of lipopolysaccharide (LPS)-induced TNFα production than MK2 knockout animals (Ronkina et al., 2007). Intriguingly, functional differences between MK2 and MK3 have been shown in a similar system. Ehlting et al. (2011) demonstrated that in LPS-stimulated macrophages MK2 regulates expression of interferon β by preventing MK3 from negative regulation of nuclear factor-κB and interferon regulatory factor 3 signaling.

The Role of MK2 in Neuroinflammation

Neurodegenerative conditions such as multiple sclerosis, Parkinson’s disease, and Alzheimer’s disease are associated with chronic neuroinflammation, which becomes problematic when unresolved. Furthermore, neuroinflammation worsens the progression and outcome of brain tumors, ischemic injury, and epileptic seizures. Accumulating evidence points at MK2 as involved in neuroinflammatory responses. MK2 is particularly enriched in the microglia, the resident macrophages of the brain, and has been found to influence neurotoxicity through MK2-dependent activation of these immune cells (Culbert et al., 2006). Microglia from MK2-deficient (MK2−/−) mice showed significant inhibition of cytokine release upon LPS and interferon-γ stimulation, which abolished neurotoxicity in coculture with neurons. Importantly, in the transgenic mouse model of Alzheimer’s disease, elevated activation and expression of MK2 correlated with amyloid-β deposition, microglial activation, and cytokine upregulation (Culbert et al., 2006).

Further supporting the role of MK2 in microglial inflammation, Bachstetter et al. (2011) demonstrated that antineuroinflammatory efficacy of the p38 MAPK inhibitor WM01-2-069A-SRM is associated with decreased phosphorylation state of MK2. Similarly, in primary neuron-glia cocultures, the dopaminergic neurons from MK2-deficient mice were significantly more resistant to LPS-induced neurotoxicity compared with cells from wild-type mice. This neuroprotection in MK2-deficient cultures was associated with reduced production of TNFα, IL-6, and nitric oxide. Furthermore, in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson’s disease, MK2-deficient mice showed reduced neuroinflammation and less degeneration of dopaminergic neurons in substantia nigra (Thomas et al., 2008b).

MK2 depletion also protected rodent brains from ischemic injury. MK2-deficient mice subjected to focal ischemia showed markedly reduced infarct size after transient and permanent ischemia as well as attenuation of neurologic deficits. Biochemical analysis revealed a decrease in IL-1β (but interestingly not TNFα) production (Wang et al., 2002). Similarly, the inflammatory response after spinal cord injury contributed to secondary tissue damage and functional loss. Microarray analysis of spinal cord tissues taken at the peak of the inflammatory response to spinal cord injury showed an increased expression of phosphorylated MK2 in microglia and also in neurons and astrocytes. Locomotor recovery was significantly improved in MK2-deficient animals and was associated with reduced neuronal and myelin loss, and decreased expression of proinflammatory cytokines and decreased protein nitrosylation (Ghasemlou et al., 2010).

Finally, MK2 has been reported to regulate the synaptic plasticity that underlies the ability to learn and remember. Synaptic plasticity is stimulated by membrane depolarization, and MK2 mRNA levels have been described to be inducible by depolarization or after kainic acid–induced seizures in the hippocampus (Vician et al., 2004). Depolarization by potassium chloride or increasing cellular cAMP by forskolin treatment also have led to elevated levels of MK2 expression. This induction of MK2 is characteristic of neuronal cells and is absent in fibroblasts, macrophages, and kidney cells. In vivo, induction of status epilepticus distinctly reduced neurodegeneration in MK2-deficient mice compared with wild-type mice (Thomas et al., 2008a). However, the downstream substrates and detailed molecular mechanism of MK2-regulated neuroinflammation and neurodegeneration remain to be elucidated.

MK2-Dependent Regulation of Actin Remodeling and Cell Migration

Hsp27 (also known as Hspb1) is a member of the human small heat shock protein family characterized by highly conserved α-crystalline domain. It is generally recognized to act as a molecular chaperone that can sequester damaged proteins and prevent their aggregation. This property of a molecular sponge is regulated via phosphorylation on three serine residues, namely, Ser15, Ser78, and Ser82. Several kinases have been identified to phosphorylate Hsp27 in vitro; however, in cell culture and in vivo experiments, the p38 MAPK–MK2 axis is the dominant pathway of Hsp27 phosphorylation (reviewed in Kostenko and Moens, 2009). This phosphorylation is a reversible process in response to a variety of stimuli, including mitogens, inflammatory cytokines IL-1β and TNFα, hydrogen peroxide, and other oxidants. Unphosphorylated Hsp27 forms large multimers presenting chaperone functions, whereas phosphorylation of Hsp27 results in complex dissociation and loss of chaperoning activity.

Hsp27-deficient mice are fertile without any obvious abnormalities (Huang et al., 2007; Crowe et al., 2013) and provide invaluable insight into the in vivo function of this chaperone. Hsp27 was found to not be essential for the embryonic development under physiologic conditions, but was important, together with other Hsp proteins, for tissue maintenance under stress conditions, where it provided cytoprotective and antiapoptotic effects (Huang et al., 2007). Furthermore, Hsp27-deficient fibroblast showed an increased expression of IL-6 but reduced entry into S-phase, and an increased expression of cyclin-dependent kinase inhibitors p27kip1 and p21waf1, resulting in decreased proliferation. There was also a significant impairment in wound healing in Hsp27-deficient mice associated with collagen deposition, increased inflammation, and augmented neutrophil infiltration into the wounds (Crowe et al., 2013).

Although hardly expressed in nontransformed cells, Hsp27 is abundantly expressed in cancer cells and has been investigated as a prognostic marker (Castro et al., 2012; Ischia
and So, 2013). Furthermore, it accumulates in the cancer cells during chemotherapy and radiation therapy and, thus, is strongly associated with resistance to anticancer therapy. The most prominent functions of Hsp27 are the control of apoptosis and cell migration through regulation of actin remodeling (Fig. 2B).

Actin remodeling requires actin filament assembly and disassembly as well as the interaction between actin filaments and myosin thick filaments. Hsp27 is an actin filament capping protein that normally inhibits actin polymerization in its non-phosphorylated form. MK2-mediated phosphorylation of Hsp27 releases this inhibition and promotes actin polymerization and cell migration (Fig. 2B). Disruption of balance between Hsp27 phosphorylation and dephosphorylation states leads to either insufficient actin filaments, which prevents forward movements, or an excessive amount of actin filaments, which also inhibits cell movement by impeding the release of focal adhesion. Therefore, coordinated regulation of the actin filament assembly through MK2-mediated Hsp27 phosphorylation is a critical component of actin filament dynamics that regulates cell migration. As such, MK2−/− mouse embryonic fibroblasts (MEFs) and smooth muscle cells showed reduced migration (Kotlyarov et al., 2002), whereas MK2−/− neutrophils showed a partial loss of directionality but higher migration speed (Hannigan et al., 2001).

Cell migration is one of the essential biologic processes in cancer metastasis. In the prostate cancer model, both MK2 and Hsp27 were necessary for transforming growth factor β-mediated cell invasion, as transient transfection of dominant-negative MK2 or mutant Hsp27 each blocked transforming growth factor β-induced increase in matrix metalloproteinase 2 activity and consequent cell invasion (Xu et al., 2006). Hsp27 attenuation reversed epithelial-to-mesenchymal transition, an important determinant of prostate cancer metastasis, and decreased cell migration, invasion and matrix metalloproteinase activity. Mechanistically, silencing Hsp27 was associated with decreased IL-6-dependent signal transducer and activator of transcription 3 phosphorylation, nuclear translocation, and signal transducer and activator of transcription 3 binding to the Twist promoter. Importantly, Hsp27 inhibition using antisense therapy decreased the number of circulating tumor cells in patients with metastatic castration-resistant prostate cancer in a phase I clinical trials (Shiota et al., 2013). Immunohistochemistry of samples from 553 prostate cancer patients revealed that expression of Hsp27 is a reliable predictive biomarker of aggressive prostate cancer with a poor clinical outcome (Cornford et al., 2000; Foster et al., 2009).

Similarly, MK2 has been defined as a downstream effector of p38 MAPK associated with matrix metalloproteinase 2/9 activation in bladder cancer invasion (Kumar et al., 2010) and silencing Hsp27 using small-interfering RNA (siRNA) knockdown decreased metastatic behavior, invasion, and migration in head and neck squamous cancer cells (HNSSC) (Zhu et al., 2010). The HNSSC study reported that the Hsp27 mRNA and protein levels are 22.4- and 25-fold higher, respectively, in a metastatic UM-SCC-22B cell line when compared with primary UM-SCC-22A cells. Furthermore, the Hsp27 gene was found to be overexpressed in a large fraction of the metastatic breast cancer patients. Depletion of Hsp27 in a human breast cancer cell line MDA-MB231/B02 reduced cell migration and invasion, and this attenuation of invasion correlated in vivo with a decreased ability of breast cancer cells to metastasize and grow in the skeleton (Gibert et al., 2012). In another comprehensive study, down-regulation of Hsp27 in MDA-MB-436-A breast cancer cells induced long-term dormancy in vivo (Straume et al., 2012). Only 4 of 30 Hsp27 knockdown xenograft tumors initiated growth after 70 days, which also correlated with regaining of Hsp27 protein expression. Importantly, no tumors escaped from dormancy without Hsp27 expression. Clinically, strong Hsp27 expression was associated with markers of aggressive tumors and decreased patient survival with breast cancer and melanoma.

The MK2-dependent cell migration involves not only the direct p38 MAPK-MK2-Hsp27 phosphorylation axis. Chang et al. (2011) showed that SUMOylation of MK2 at lysine339 affects actin filament reorganization and cell migration. Loss of the MK2 SUMOylation site increased MK2 kinase activity and prolonged Hsp27 phosphorylation, enhancing its effects on actin-filament-dependent events. Furthermore, MK2 phosphorylates LIM-kinase 1 (LIMK1), which in turn then phos- phorylates coflin, a protein that depolymerizes actin filaments. This activity of coflin is reversibly regulated by phosphorylation and dephosphorylation of Ser3 residue, with the phosphorylated form being inactive. LIMK1 is responsible for phosphorylation of this site and can thereby inactivate coflin and regulate stimulus-induced actin reorganization. The signaling pathway composed of p38 MAPK–MK2–LIMK1 has also been identified critical for vascular endothelial growth factor–induced stress fiber forma- tion, cell migration, and tubule formation (Kobayashi et al., 2006).

In addition to its role in actin remodeling, Hsp27 is required for COX-2 mRNA stabilization by the p38 MAPK–MK2 signaling cascade (Lasala et al., 2000) and also for IL-1β and TNFα-induced activation of transforming growth factor β-activated kinase 1–p38 MAPK–MK2 and expression of inflammatory mediators including COX-2, IL-6, and IL-8 (Alford et al., 2007). Interestingly, Hsp27 was found to affect upstream signaling cascades. Hsp27 deletion downregulated p38 MAPK–MK2 activation by IL-1β, resulting in COX-2 and IL-6 mRNA de-stabilization. This suggests that, at the molecular level, Hsp27 contributes to the expression of proinflammatory mediators via regulation of the p38 MAPK–MK2-mediated mRNA stabilization.

**MK2–Hsp27 Axis in the Brain**

In nonmalignant diseases, Hsp27 overexpression has a neuroprotective function. For example, it has been identified as an immediately secreted biomarker for ongoing ischemia that offers long-lasting neuroprotection via physical association with apoptosis-inducing kinase 1 (ASK1) and consequent inhibition of ASK1–MKK4–JNK (e-Jun N-terminal kinase) pathway. The inhibition of this kinase cascade protects against progression of ischemic neuronal death (Stetler et al., 2008). Mechanistically, Hsp27 requires protein kinase D (PKD)–mediated phosphorylation for its suppression of ASK1 cell death signaling (Stetler et al., 2012). Although several kinases have been shown to phosphorylate Hsp27 at Ser15, Ser78, and Ser82 in a context-dependent manner, only PKD inhibitor CID755673 (7-hydroxy-2,3,4,5-tetrahydro-[1]benzofuro[2,3-c] azepin-1-one) was able to block Hsp27 phosphorylation at Ser15 and Ser82 after oxygen and glucose deprivation (model of ischemia-like conditions in vitro). In further support, pharmacologic inhibition of PKD, short hairpin RNA knockdown, or overexpression of dominant negative PKD mutant significantly blocked the phospho-Hsp27-mediated neuroprotection against
neuronal injury. An intriguing discovery of this study is that phosphorylation of Hsp27, which leads to dissociation of the Hsp27 oligomers and usually presents mechanism of Hsp27 inactivation, had beneficial effects in the context of neuronal injury as it inhibited cell death via targeting mitochondrial signaling.

The role of MK2 in this process has not been evaluated, but given that MK2 phosphorylates Hsp27 at Ser15, it is plausible to speculate that MK2 activity would be required for phosphorylation-dependent neuroprotection. However, this contradicts the neuroprotective phenotype observed in MK2-deficient animals through attenuation of the neuroinflammatory response to the injury (discussed earlier). One explanation could be that the loss of MK2-dependent Hsp27 phosphorylation at Ser15 is counterbalanced by activated PKD, and thus the net effect of MK2 inhibition is neuroprotection. However, the exact role of MK2 in the neuroprotection remains to be elucidated.

Aberrant expression and phosphorylation of Hsp27 has been implicated in some types of brain tumors. In gliomas, brain tumors derived from glial cells, the expression of Hsp27 correlates with the degree of malignancy and highest Hsp27 expression was found in glioblastomas, the most aggressive grade IV gliomas (Zhang et al., 2003; Castro et al., 2012). In addition, a 4.7-fold increase in Hsp27 mRNA expression was found in hypoxic glioma regions when compared with normoxic region in the in vivo approach of gene expression analysis. This indicates that Hsp27 could regulate hypoxia influence on biologic function in solid tumors (Marotta et al., 2011). Similar to other cancers, MK2–Hsp27 pathway has been implicated to drive glioma cell migration. In particular, secreted protein acidic and rich in cysteine (SPARC), a matricellular protein that negatively regulates cell proliferation while promoting migration and invasion, has been shown to activate p38 MAPK–MK2 pathway, leading to phosphorylation (thus inactivation) of Hsp27. Pretreatment of SPARC-expressing glioma cells with Hsp27 siRNA prevented SPARC-induced migration and invasion (Golembieski et al., 2008). Furthermore, inhibition of Hsp27 phosphorylation alone or in combination with pAkt inhibitor (for phosphatase and tensin homolog-null glioblastoma tumors) inhibited SPARC-induced invasion of brain cancer cells (Schultz et al., 2012; Alam et al., 2013). Phorbol-12-myristate-13-acetate (PMA)–induced migration of A172 glioma cells also activated p38 MAPK and induced lamellipodia. Upon PMA stimulation, both unphosphorylated and phosphorylated Hsp27 were translocated to lamellipodia, and the cell migration was abolished with p38 MAPK or Hsp27 knockdown (Nomura et al., 2007). Biochemical phosphorylation studies have revealed that p38 MAPK links p38 MAPK to Hsp27 phosphorylation, it is likely that MK2 deletion or inhibition would result in an antiinflammatory phenotype.

Finally, the phosphorylation status of Hsp27 was shown to have a switching role in the IL-1β-induced IL-6 synthesis in C6 glioma cells. IL-1β–induced IL-6 release in C6 glioma cells was significantly enhanced when C6 glioma cells were transfected with unphosphorylated Hsp27 and markedly suppressed when transfected with phosphorylated Hsp27 (Tanabe et al., 2010). However, the fact that chaperoning activity of unphosphorylated Hsp27 is required for IL-6 production contradicts the anti–IL-6 efficacy of MK2 inhibitors that actually increases levels of unphosphorylated Hsp27. Thus, it can be hypothesized that anti–IL-6 efficacy of MK2 inhibitors does not involve Hsp27 but another mechanisms such as activation of RNA-binding proteins that regulate IL-6 mRNA stability and translation.
phosphorylates CDC25B on Ser249 and that MK2 is able to phosphorylate CDC25B on multiple sites, including Ser169, Ser323, Ser353, and Ser375. Moreover, UV irradiation caused G1/S and G2/M arrest in MK2-proficient U2OS cells but not in MK2-depleted cells. The abrogation of the cell cycle checkpoints in MK2-deficient cells forced cells to progress to mitosis with unrepaired DNA, which resulted in cell death as a consequence of mitotic catastrophe. Thus, inhibition of MK2 increased sensitivity to UV-induced DNA damage. In mechanistic detail, MK2 was shown to directly phosphorylate CDC25B on Ser323, resulting in its binding to 14-3-3 and inactivation. This phosphatase inactivation in turn resulted in cell cycle arrest (Manke et al., 2005).

The role of MK2 in checkpoint signaling was reinforced by Reinhardt et al. (2007), who demonstrated that p53-deficient cells rely on MK2 for cell cycle arrest and survival after DNA damage caused by cisplatin and doxorubicin. Furthermore, MK2 depletion in p53-deficient MEFs, but not in p53-wild-type cells, caused abrogation of CDC25A-mediated S-phase arrest after cisplatin treatment as well as CDC25B-mediated G2/M arrest after doxorubicin exposure, resulting in enhanced antiproliferative effects and sensitization to chemotherapeutics (Reinhardt et al., 2007).

The activation of MK2 after the drug-induced DNA damage required activity of upstream ATM and ATR kinases, which occurred independently of Chk1. Conversely, Chk1 activation was independent of MK2 activity. This indicates that the ATM/ATR–p38 MAPK–MK2 pathway functions in parallel with the ATR–Chk1 pathway in response to DNA damage by chemotherapeutics.

Fig. 3. Molecular mechanism of the DNA damage response network. The DNA damage response network involves several kinases to inactivate CDK/cyclin complexes and result in cell cycle arrest for DNA repair. The upstream ATM responds to double-strand DNA breaks (DSB) while ATR is activated by single-strand DNA breaks (SSB). Activation of ATM–Chk2 and ATR–Chk1 pathways result in the inhibitory phosphorylation of the CDC25 phosphatases. Chk1 and Chk2 target CDC25A for degradation while Chk1-mediated phosphorylation of CDC25B and CDC25C leads to 14-3-3 binding, nuclear exclusion of the phosphatases, and cell cycle arrest. CDC25A normally activates CDK2/cyclin complexes causing S-phase progression while CDC25B/C activates CDK1/cyclin complexes resulting in G2/M transition. By removing inhibitory phosphate groups from specific CDKs, CDC25 phosphatases promote cell cycle progression. In contrast, CDK4/cyclin and CDK6/cyclin complexes control the G1/S checkpoint and are both regulated by p53 and its downstream cyclin-dependent kinase inhibitor p21. ATM and Chk2 activate p53 by promoting its stability. In response to DNA damage, the activation of the p38 MAPK–MK2 pathway is mediated by thousand-and-one amino acid (TAO) kinases, which are activated by ATM and ATR through as-yet-uncharacterized mechanisms. p38 MAPK can be directly activated by UV independently of ATM and ATR. MK2 inhibits CDC25A, CDC25B, and CDC25C phosphatases in a similar fashion to Chk1 and Chk2, resulting in inactivation of CDK1/2-cyclin complexes and cell cycle arrest. Wee1 is another checkpoint kinase downstream of Chk1 that directly phosphorylates CDK1, resulting in G2/M arrest. Through these pathways, cell cycle arrest enables cells to overcome their DNA damage and enhances survival.
In a further study, Reinhardt et al. (2010) illustrated that in p53-deficient cells Chk1 mediates an early nuclear G2/M checkpoint whereas MK2 mediates a late cytokplasmic checkpoint. This was achieved by translocation of the activated p38 MAPK–MK2 complex from the nucleus to the cytoplasm where MK2 phosphorylated heterogeneous nuclear ribonucleoprotein A0 and poly(A)specific ribonuclease to ultimately stabilize Gadd45α mRNA. Gadd45α in turn maintained the MK2 activity in the cytoplasm, and hence the cytokplasmic sequestration of CDC25B/C preventing CDK1 activation and mitotic entry until DNA damage was repaired.

Another mode by which MK2 may interfere with the cell cycle is through modulating the activity of the tumor suppressor p53, a target of p38 MAPK essential for cell cycle regulation at G2/S and entry into apoptosis (Bulavin et al., 1999). MK2 was found to increase the degradation of p53 through the phosphorylation of human double minute-2, a p53-interacting ubiquitin ligase. It was therefore proposed that MK2 dampens the extent and duration of p53 activity and contributes to the fine-tuning of the DNA damage response. The consequence of MK2 depletion was increased p53 protein levels and improved sensitivity of MK2−/− MEF cells to UV-induced apoptosis compared with wild-type cells (Weber et al., 2005). In further support, a study by Hopker et al. (2012) demonstrated MK2 as a repressor of p53-driven apoptosis. MK2-dependent phosphorylation of apoptosis-antagonizing transcription factor (AATF) caused dissociation of p-AATF from cytoplasmic myosin-regulatory light chain 3 and subsequent nuclear translocation (Fig. 2C). In the nucleus, p-AATF binds to the PUMA, BAX, BAK promoter regions to repress p53-driven expression of these proapoptotic proteins. However, nuclear AATF did not restrict the expression of the cell-cycle regulating genes CDKN1A, Gadd45α, and PRRM. The net result was a selective repression of p53-driven apoptosis in response to adriamycin. Thus, inhibiting MK2 pathway could be explored in chemotherapy-sensitizing approaches to treat both p53-deficient and p53-proficient cancers.

However, two studies have called MK2 involvement in checkpoint control into question. First, Xiao et al. (2006) found that Chk1 knockdown, but not MK2 or Chk2, was sufficient to abrogate S-phase and G2/M arrest after chemotherapy-induced DNA damage in HeLa and H1299 cells. Intriguingly, simultaneous depletion of Chk1 and MK2 partially reversed the checkpoint abrogation observed with Chk1 knockdown alone. Although depletion of Chk1 increased the CDC25A levels, loss of MK2 destabilized CDC25A protein. Hence, it was suggested that MK2 prevents Chk1-induced degradation of CDC25A, which is required for the checkpoint abrogation and cell cycle progression. Second, Phong et al. (2010) highlighted that the p38 MAPK–MK2 pathway is not required for G2/M DNA damage checkpoint control but rather plays an important cytoprotective role through regulation of antiapoptotic and survival pathways to allow cells to recover from DNA damage. Cancer cells were still able to undergo a G2/M arrest in response to doxorubicin, methyl methanesulfonate, or UV-induced DNA damage despite p38 MAPK or MK2 inhibition, but this inhibition triggered dramatic apoptosis in a p53-independent manner associated with decreased levels of antiapoptotic Bcl-2 proteins.

In addition to its role in checkpoint control and mitotic entry, the p38 MAPK–MK2 pathway has been suggested to regulate mitotic progression as well, although there are fewer details. The discovery that p38 MAPK knockdown strongly inhibited HeLa cell proliferation, instigated an examination of the underlying mechanism (Fan et al., 2005). In the absence of p38α MAPK, the cells underwent a G2/M arrest and exhibited marked defects in bipolar spindle formation and chromosome alignment. Intriguingly, this essential function of p38 MAPK for proper mitotic progression does not require its kinase activity. Likewise, loss of the p38γ MAPK isoform in HeLa cells resulted in multipolar spindle formation and chromosome misalignment, which caused M phase arrest. As a result, the p38 MAPK-depleted cells died at mitotic arrest or soon after abnormal exit from the M phase via caspase-dependent apoptosis.

In addition, activated p38 MAPK was found to localize to kinetochores and spindle poles during mitosis, which is crucial for the normal kinetochore localization of polo-like kinase 1 (Plk1), which regulates centrosome maturation, sister chromatid segregation, and cytokinesis (Barr et al., 2004; Kukkonen-Macchi et al., 2011). The expression of a mutant Plk1 or depletion of Plk1 caused spindle pole defects, accumulation of cells at the M phase, and massive cell death (Guan et al., 2005; Reagan-Shaw and Ahmad, 2005; Bu et al., 2008). Tang et al. (2008) described how MK2 colocalizes with activated p38 MAPK and Plk1 at spindle poles where it phosphorylates Plk1 at Ser326 to promote normal mitotic progression (Fig. 2D). Importantly, the same study reported that MK2 acts as a Plk1 kinase and that MK2 knockdown resulted in mitotic arrest. In summary, MK2 not only could function as a checkpoint kinase that controls mitotic entry but also appears to be involved in mitotic progression.

A new, less investigated role for MK2 was demonstrated by Chen et al. (2000). This work showed that Ras activated the p38 MAPK pathway, including the upstream kinase MAPK kinase 2 and downstream kinases MK2 and MK5 (also known as PRAK). Each of these kinases, when activated by Ras, was able to inhibit Ras-induced proliferation in tumor cells harboring endogenous Ras mutations. Mechanistically, the negative feedback of MK2, which completely blocked Ras proliferative signaling was achieved via inhibition of Ras-induced c-Jun N-terminal kinase activation. A follow up study (Kobayashi et al., 2012) demonstrated that MK2 knockdown increases Ras transformation in MEFs and enhances tumorigenesis in vivo. However, an opposite role for MK2 was demonstrated in human colon cancer cells, where MK2 depletion decreased the tumor growth. It was suggested that the differing effects of MK2 are related to its effects on reactive oxygen species because in MEFs MK2 decreased levels of reactive oxygen species but increased its production in human colon cancer cells.

Finally, adding more to the puzzle of MK2-controlled cell survival, MK2 knockdown has been reported to protect cells from DNA-damage-induced cell death (Köppere et al., 2013). Mice deficient for MK2 displayed decreased apoptosis in the skin upon UV irradiation, accompanied with reduced histone H2AX phosphorylation. MK2 inhibition increased survival of gemcitabine-treated U2OS cells. In contrast, Chk1 knockdown or pharmacologic inhibition strongly enhanced H2AX phosphorylation in gemcitabine-treated cells, suggesting that loss of MK2 in tumors is likely to constitute resistance to Chk1 inhibition.

The Therapeutic Potential of Targeting MK2

Small-molecule MK2 inhibitors are “a tough nut to crack” (Schlapbach and Huppertz, 2009) because of several challenges associated with their development. First, MK2 crystal structures have revealed that the ATP pocket of MK2 is deep...
and narrow (Anderson et al., 2007; Hillig et al., 2007), allowing only small planar molecules to bind and restricting addition of side chains that define kinase selectivity. Second, the low biochemical efficiency (BE) index of MK2 inhibitors hinders development of MK2 inhibitors into therapeutics. BE is defined as the binding affinity/functional response ratio, which is equivalent to $K_i/EC_{50}$ (Swinney and Anthony, 2011). Properties influencing BE are assay relevance, molecular properties of the drug, target engagement, and the molecular mechanism of action; however, given that all aspects are addressed properly in the early stages of drug discovery, BE is a good indicator of clinical success.

Swinney (2004) showed that BE $> 0.4$ is a property of many approved medicines. In other words, for the majority of successful drugs, the cellular EC$_{50}$ values are no more than 2.5-fold higher than the biochemical binding or inhibition $K_i/IC_{50}$ values. However, cellular efficacy data for MK2 inhibitors in disease-relevant assays are limited in the public domain, and BE calculated from these published values are far below the 0.4 threshold (Anderson et al., 2009; Mourey et al., 2010; Kaptein et al., 2011; Oubrie et al., 2012).

It is believed that the low BE is caused by the high affinity of unphosphorylated (inactive) MK2 to ATP (Mourey et al., 2010). As many kinases have low ATP affinity in the inactive conformation as opposed to high ATP affinity in the active conformation, their respective inhibitors explore predominantly the inactive conformation, thus avoiding competition with the high intracellular ATP concentration. This advantage is not available for MK2 inhibitors, so higher inhibitor concentrations are required for cellular efficacy.

As good BE enables efficacy at lower drug concentrations and increases the therapeutic index, there is a low probability of clinical success for ATP-competitive MK2 inhibitors. Nevertheless, Mourey et al. (2010) demonstrated in vivo efficacy of a selective ATP-competitive MK2 inhibitor PF-3644022 [(10R)-10-methyl-3-(6-methylpyridin-3-yl)-9,10,11,12-tetrahydro-8H-[1,4]diazepino[5’,6’:4,5]thieno[3,2-f]quinolin-8-one] despite its biochemical inefficiency (BE = 0.03). PF-3644022 reduced TNF$\alpha$ production and paw swelling in acute and chronic models of inflammation. These data, supported by the normal phenotype of MK2 knockout mice (Kotlyarov et al., 1999), suggest that MK2 is a viable target that requires non-ATP-competitive or allosteric approach.

Non-ATP-competitive inhibitors have been reported by Merck (Qin et al., 2011; Huang et al., 2012), and it will be interesting to see how this class of compounds progresses through in vivo studies. Until then, we can only assume the outcomes of MK2 inhibition by analyzing the efficacy of p38 MAPK inhibitors that inhibit MK2 activation or by analyzing the efficacy of Chk1 or Wee1 inhibitors, as these kinases have similar functions in cell cycle progression. Along these lines, Watterson et al. (2013) have recently demonstrated that the antineuroinflammatory efficacy of blood-brain-barrier-permeable p38 MAPK inhibitors in the animal model of Alzheimer’s disease correlates with the inhibition of MK2 activity.

### p38 MAPK, Chk1, and Wee1 Inhibitors in Cancer: Implication for MK2 Inhibitors

By arresting cell cycle progression, cancer cells are capable of repairing the DNA damage caused by chemotherapeutic agents and escaping apoptosis. The implication that MK2 activity is necessary for G$_2$/M checkpoint arrest provides an exciting possibility for the use of MK2 inhibitors as chemosensitizers. Indeed, MK2 depletion improved the effectiveness of chemotherapeutics in p53-deficient but not in p53-proficient cells (Reinhardt et al., 2007). As most cancers are p53 mutated (Vogelstein et al., 2000), they can be selectively targeted with MK2 inhibition. This synthetic lethality is due to the fact that in p53-proficient cells the cell cycle checkpoints are well maintained through p53 and Chk1 responses (Lam et al., 2004). On the other hand, the loss of p53 results in cells being entirely dependent on intra-S and G$_2$/M checkpoints to maintain their genomic integrity in response to DNA damage. Consequently, the p38 MAPK–MK2 pathway becomes more crucial for cell survival after DNA damage. Thus, it can be hypothesized that inhibition of MK2 will deliver outcomes similar to inhibition of Chk1 or Wee1, which are both targets of clinical investigation (Patil et al., 2013). Importantly, MK2-depleted mice are viable (Kotlyarov et al., 1999; Hegen et al., 2006; Runkina et al., 2007) in contrast to Chk1 and p38 MAPK knockout mice (Liu et al., 2000; Takai et al., 2000), indicating that MK2 inhibition would successfully target cancer cells like Chk1 and p38 MAPK inhibitors but with fewer side effects.

In brain cancer, our knowledge of whether p38 MAPK–MK2 inhibition could improve chemotherapeutic sensitivity is limited to in vitro data using only pharmacologic inhibitors of p38 MAPK. Hirose et al. (2003) reported that U87 glioblastoma cells underwent a p38 MAPK-dependent G$_2$/M arrest in response to the DNA methylating agent temozolomide (TMZ). Pharmacologic blocking of p38 MAPK activity with SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole] or siRNA knockdown bypassed the G$_2$/M checkpoint, resulting in increased sensitivity to the cytotoxic action of TMZ.

Interestingly, p38 MAPK and Chk1 were found to work cooperatively to activate G$_2$/M arrest (Hirose et al., 2004; Llopis et al., 2012). Both pathways were shown to deactivate CDC25C in response to TMZ treatment, resulting in CDK1 inactivation and G$_2$/M arrest in U87 glioblastoma cells. Moreover, pharmacologic inhibition of both pathways did not lead to a greater bypass of TMZ-induced G$_2$/M arrest or enhanced cytotoxicity than inhibition of either pathway alone. However, Chk1 was found to be crucial for both the initiation and maintenance of the TMZ-induced G$_2$/M arrest, whereas p38 MAPK appeared to be important only for initiation.

Further supporting this approach to brain cancer therapy, inhibition of ATM/ATR (Eich et al., 2013), Chk1 (Hirose et al., 2001), or Wee1 (Mir et al., 2010) potentiated TMZ efficacy in brain cancer in vivo models. In addition, Wee1 kinase was demonstrated as a major regulator of the G2 checkpoint in glioblastoma (Mir et al., 2010) and Wee1 inhibitor MK-1775 [1-[6-(2-hydroxypropan-2-yl)pyridin-2-yl]-6-[4(4-methylpipеразин-1-yl)aniлин]-2-prop-2-enylpyrazоlо[3,4-d]pyrimидин-3-one] enhanced radiosensitivity in established glioma cell lines in vitro and in vivo without modulating response in normal human astrocytes (Sarcar et al., 2011).

As discussed earlier, the p38 MAPK-MK2 pathway appears to play a role in mitosis progression through Plk1 activation. Therefore, it is possible that inhibition of MK2 could deliver outcomes similar to Plk1 inhibition, such as defects in bipolar spindle formation and cytokinesis, growth inhibition, and induction of apoptosis. The anticancer efficacy of Plk1 inhibition, without significant toxicity in nontransformed cells,
has been demonstrated in esophageal, prostate, and brain cancer models (Liu and Erikson, 2003; Guan et al., 2005; Reagan-Shaw and Ahmad, 2005; Bu et al., 2008; Strehbhardt, 2010; Tandle et al., 2013).

Finally, based on the concept that many tumors exhibit high levels of replicative stress, the sole inhibition of Chk1 has been shown to effectively target melanoma cells with significant intrinsic DNA damage (Brooks et al., 2013). The cytotoxic effect of Chk1 inhibitors resulted from inhibition of Chk1 activity in the S phase driving premature exit from the S phase into an aberrant mitosis, resulting in either failure of cytokinesis or cell death by an apoptotic mechanism. Likewise, Ferraro et al. (2012) showed that inhibition of Chk1 effectively targeted lymphoma cells with MYC-driven intrinsic replicative stress but not normal B cells. Similarly, Wee1 inhibition by MK-1775 has been shown as a potent anticancer therapy approach independent of a genotoxic agent (Guerkin et al., 2013).

**Conclusion**

Various functions of MK2 have been outlined herein that provide opportunities to intervene in the treatment of brain diseases (Fig. 2). Current evidence suggests that MK2 contributes to neuroinflammation and plays a role in the pathophysiology of conditions such as Parkinson’s disease and Alzheimer’s disease. MK2 regulates the expression of various inflammatory cytokines and thus is emerging as a novel target for neuroinflammation-associated brain disorders. In addition, MK2-dependent regulation of Hsp27 has been shown to be crucial in actin remodeling and cell migration. Although it is more prominently expressed in cancer cells than normal cells, modulating Hsp27 activity appears to be an interesting strategy to reduce migration and metastasis of cancer. Indeed, inhibition of MK2 or Hsp27 reduced the migration and invasion of a number of cancers such as HNSCC and breast cancer. The role of MK2 in controlling cell cycle arrest in response to DNA damage similar to Chk1 could have positive implications for cancer therapy.

Improving chemotherapy effectiveness by inhibiting MK2 has already been demonstrated in in vitro studies. However, translation of these interesting in vitro data into the in vivo proof-of-principle stage is challenging. Many MK2 inhibitors have been developed, but they may require high intracellular ATP, and their BE is below the threshold required for therapeutic efficacy. When focusing on brain disorders, another challenge for MK2 inhibitors to overcome is blood-brain barrier permeability as well as the ability to avoid excessive efflux by the P-glycoprotein transporter. Finally, it is important to note that the efficacy of MK2 inhibitors will also depend on other factors such as target engagement in the disease pathophysiology and/or the (in)ability of the targeted cells to bypass MK2 inhibition.

**Authorship Contributions**

**Wrote or contributed to the writing of the manuscript: Gurgis, Ziaiaris, Munoz.**

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Wrote or contributed to the writing of the manuscript: Gurgis, Ziaiaris, Munoz.
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Address correspondence to: Dr. Lenka Munoz, Department of Pharmacology, School of Medical Sciences, University of Sydney, NSW 2006 Australia. E-mail: lenka.munoz@sydney.edu.au