MAPK-activated protein kinase 2 (MK2) in neuroinflammation, Hsp27 phosphorylation and cell cycle: Role and Targeting

Fadi Maged Shokry Gurgis, William Ziaziaris, Lenka Munoz

Department of Pharmacology, School of Medical Sciences, University of Sydney, NSW 2006, Australia (FMSG, WZ, LM)
Abbreviations: AATF, apoptosis-antagonizing transcription factor; ARE, AU-rich element; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; BE, biochemical efficiency; CAMK, calcium/calmodulin-dependent kinase; CDC25, cell division cycle phosphatase; CDK, cyclin-dependent kinase; COX2, cyclooxygenase 2; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; hnRNP, heterogeneous nuclear ribonucleoprotein; HNSCC, head and neck squamous cancer cells; HuR, human antigen R; Hsp, heat shock protein; Chk1/2, checkpoint kinase 1/2; IL, interleukin; IRF3, interferon regulatory factor 3; JNK, c-Jun N-terminal kinase; LIMK1, LIM-kinase 1; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinases; MK2/3, MAPK-activated protein kinase 2/3; MKK3, MAPK kinase 3; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRLC3, myosin-regulatory light chain 3; NES, nuclear export sequence; NFκB, nuclear factor κB; NLS, nuclear localization signal; PABP1, polyA-binding protein 1; PARN, poly(A)specific ribonuclease; PKD, protein kinase D; Plk1, polo-like kinase 1; PMA, phorbol-12-myristate-13-acetate; PTEN, phosphatase and tensin homolog; RBP, RNA binding protein; SCI, spinal cord injury; SPARC, secreted protein acidic and rich in cysteine; STAT3, signal transducer and activator of transcription 3; TAK1, transforming growth factor β-activated kinase 1; TAO, thousand-and-one amino acid; TGFβ, transforming growth factor β; TMZ, temozolomide; TNFa, tumour necrosis factor α; TTP, tristetraproline; VEGF, vascular endothelial growth factor.
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 as well as cell motility in response to a wide range of extracellular stimuli. Because of the failure of p38 MAPK inhibitors in clinical trials, MK2 unveiled as a potential target to regulate inflammatory cytokines mRNA stability and translation. Recent work suggests that this mechanism may underlie pathophysiology of brain disorders associated with inflammation. In addition, MK2 is a prominent kinase that phosphorylates heat shock protein 27, an intensively investigated biomarker of cancer progression. This phosphorylation decreases its chaperone properties, 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 focus on brain pathologies. Analysis of MK2 signaling in various disease models and 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 (MAPKAP-2 or MK2) is a downstream substrate of the p38 MAPK known to transduce a range of extracellular signals resulting in inflammatory response, cell division and differentiation, apoptosis as well as cell motility. Because of its central role in inflammation, p38 MAPK has been extensively investigated over the past decades. However, 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 down-stream 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, which is convincingly documented by increasing number of MK2-inflammation related publications and reviews. In addition, MK2 governs not only peripheral inflammation, but also the inflammation of the brain (termed neuroinflammation, (Culbert et al., 2006; Ghasemlou et al., 2010; Thomas et al., 2008b). MK2 is the prominent kinase phosphorylating heat shock protein 27 (Hsp27, Stokoe et al., 1992) that has turned as a promising target for cancer treatment. Finally, MK2 is activated following DNA damage (Manke et al., 2005; Reinhardt et al., 2007), resulting in cell cycle arrest so 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 anti-cancer agents that improve efficacy of chemotherapy. As these aspects of MK2 biology have not been recently summarized, we present here a review on the role of MK2 in neuroinflammation-associated brain disorders. We will then discuss studies outlining the biological role of MK2-dependent regulation of Hsp27 activity and cell cycle control.
Finally, we will conclude with therapeutic potential of MK2 inhibitors, and where possible focus on brain pathologies.

**Structure and function of MK2**

MK2 was discovered as an ERK1/2-activated protein kinase that had the capacity to phosphorylate and thus inactivate Hsp27 and its murine homologue 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 (Figure 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 (CAMK) 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 localisation signal (NLS) sequence that maintains the location of MK2 predominantly in the nuclei of resting cells (Figure 1). Conversely, the nuclear export sequence (NES) is located in the N-terminal to the NLS domain and triggers nuclear export following MK2 activation (Figure 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 enhanced cytoplasmic localization of MK2, suggesting that MK2 contain a constitutively active NLS and a phosphorylation-regulated NES. In resting cells, p38 MAPK and MK2 form a complex in the nucleus (Figure 1B) ((Ben-Levy et al., 1998). Cellular stress causes the
phosphorylation of p38 MAPK by upstream kinases such as MKK3. 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 CRM1-dependent mechanism, where both kinases activate their downstream substrates (Figure 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 cell cycle and apoptosis (Figure 2, reviewed in (Gaestel, 2006; Gaestel, 2013)).

The most extensively studied function of MK2 is the regulation of inflammation at the post-transcriptional level. The crucial role of MK2 in TNFα and interleukin-6 (IL-6) synthesis has been observed by phenotype analysis of MK2 knockout mice, which showed significantly reduced cytokines serum levels (Kotlyarov et al., 1999). Further studies have shown that MK2 modifies the stability and translation of TNFα and IL-6 mRNA through activation of AU-rich element-binding (ARE) proteins, such as tristetraproline (TTP), heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0), polyA-binding protein-1 (PABP1) and human antigen-R (HuR) (Figure 2A). For example, MK2 phosphorylated 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 abolished TTP function as TNFα 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 increased TTP protein expression via exclusion from proteasomal degradation and cytoplasmic retention (Deleault et al., 2008). Furthermore, MK2-dependent phosphorylation of TTP changed its affinity for ARE binding. Phosphorylated TTP had shown 10-fold lower $K_a$ for ARE mRNA, and this led to the replacement of TTP from AREs by HuR, a protein known to stabilize short-lived mRNAs
(Tiedje et al., 2012). These complex mechanisms of post-transcriptional regulation of cytokines synthesis via MK2-dependent phosphorylation of RNA binding proteins (RBPs) have been reviewed in several excellent reviews (Cargnello and Roux, 2011; Gaestel, 2013). The above-mentioned RBPs have been also reported to stabilize short-life mRNAs coding for cyclooxygenase 2 (COX-2), IL-8 and vascular endothelial growth factor (VEGF) (Miyata et al., 2013; Suswam et al., 2008). 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 this 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), however the high structural identity and nearly the same substrate spectrum with MK2 (Cheng et al., 2010) imply similar functional behavior in the biological systems. Indeed, the C termini of MK3 contains NLS and NES signals rendering unphosphorylated MK3 in the nucleus and inducing translocation to the cytoplasm upon p38 MAPK-dependent phosphorylation. Furthermore, MK3 was involved in post-transcriptional regulation of ARE-containing mRNAs described for MK2 (Figure 2), indicating that MK3 could control cytokines biosynthesis in addition to MK2 (Ronkina et al., 2008). Indeed, MK2/3 double knockout mice showed 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 demonstrated that in LPS-stimulated macrophages, MK2 regulates expression of interferon β by preventing MK3 from negative regulation of NFkB and IRF3 signaling (Ehlting et al., 2011).
The role of MK2 in neuroinflammation

Neurodegenerative conditions such as multiple sclerosis, Parkinson’s and Alzheimer’s diseases 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 the MK2 to be involved in the neuroinflammatory responses. MK2 is particularly enriched in 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 (IFN-γ) stimulation, which abolished neurotoxicity in co-culture 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 up-regulation (Culbert et al., 2006). Further supporting the role of MK2 in microglial inflammation, Bachstetter et al demonstrated that anti-neuroinflammatory efficacy of the p38 MAPK inhibitor MW01-2-069A-SRM is associated with decreased phosphorylation state of MK2 (Bachstetter et al., 2011). Similarly, in primary neuron-glia co-cultures, the dopaminergic neurons from MK2-deficient mice were significantly more resistant to LPS-induced neurotoxicity compared to 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 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 neurological deficits. Biochemical analysis
revealed decrease in IL-1β, but interestingly not TNFα production (Wang et al., 2002). Similarly, the inflammatory response after spinal cord injury (SCI) contributes to secondary tissue damage and functional loss. Microarray analysis of spinal cord tissues taken at the peak of the inflammatory response to SCI showed 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, decreased expression of pro-inflammatory cytokines and protein nitrosylation (Ghasemlou et al., 2010).

Finally, MK2 has been reported to regulate synaptic plasticity that underlies the ability to learn and remember. Synaptic plasticity is stimulated by membrane depolarization and MK2 mRNA levels were described to be inducible by depolarization or after kainic acid induced seizures in the hippocampus (Vician et al., 2004). Depolarisation by potassium chloride or increasing cellular cAMP by forskolin treatment also led to elevated levels of MK2 expression. This induction of MK2 was characteristic of neuronal cells, and was absent in fibroblasts, macrophages and kidney cells. In vivo, induction of status epilepticus distinctly reduced neurodegeneration in MK2-deficient mice compared to wild-type mice (Thomas et al., 2008a). However, the down-stream 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 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 
\textit{in vitro}, however in cell culture and \textit{in vivo} experiments the p38 MAPK-MK2 axis is the 
dominant kinase of Hsp27 phosphorylation (reviewed in (Kostenko and Moens, 2009)). This 
phosphorylation is a reversible process in response to variety of stimuli including mitogens, 
inflammatory cytokines IL-1$\beta$ and TNF\textalpha, 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 (Crowe et al., 
2013; Huang et al., 2007) and provide invaluable insight into the \textit{in vivo} function of this 
chaperone. Hsp27 was found not essential for the embryonic development under 
physiological conditions, by was important, together with other Hsp proteins for tissue 
maintenance under stress conditions, where it provided cytoprotective and anti-apoptotic 
effects (Huang et al., 2007). Furthermore, Hsp27-deficient fibroblast showed increased 
expression of IL-6, but reduced entry into S-phase and increased expression of cyclin-
dependent kinase inhibitors p27\textsuperscript{kip1} and p21\textsuperscript{waf1}, 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). While hardly expressed in non-transformed 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 chemo- and radiation therapy and thus it 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 (Figure 2B).
Actin remodeling requires the 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 (Figure 2B). Disruption of balance between Hsp27 phosphorylation and dephosphorylation states leads to either insufficient actin filaments, which prevents forward movements, or excessive amount of actin filaments that also inhibits cell movement by impeding the release of focal adhesion. Therefore, coordinated regulation of 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), while MK2−/− neutrophils showed a partial loss of directionality but higher migration speed (Hannigan et al., 2001).

Cell migration is one of the essential biological processes in cancer metastasis. In prostate cancer model, both MK2 and Hsp27 were necessary for transforming growth factor β (TGFβ)-mediated cell invasion as transient transfection of dominant-negative MK2 or mutant Hsp27 each blocked TGFβ-induced increase in matrix metalloproteinase 2 (MMP-2) activity and consequent cell invasion (Xu et al., 2006). Hsp27 attenuation reversed epithelial-to-mesenchymal transition (EMT), 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 STAT3 phosphorylation, nuclear translocation and STAT3 binding to the Twist promoter. Importantly, Hsp27 inhibition using antisense therapy decreased 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 poor clinical outcome (Cornford et al., 2000; Foster et al., 2009).

Similarly, MK2 has been defined as a down-stream effector of p38 MAPK associated with MMP-2/9 activation in bladder cancer invasion (Kumar et al., 2010) and silencing Hsp27 using siRNA knockdown decreased metastatic behavior, invasion and migration in head and neck squamous cancer cells (HNSCC) (Zhu et al., 2010). The HNSCC study reported that the Hsp27 mRNA and protein levels are 22.4- and 25-fold higher, respectively, in metastatic UM-SCC-22B cell line when compared to primary UM-SCC-22A cells. Furthermore, Hsp27 gene was found to be overexpressed in a large fraction of the metastatic breast cancer patients. Depletion of Hsp27 in 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 MDAMB-436-A breast cancer cells induced long-term dormancy in vivo (Straume et al., 2012). Only 4 of 30 Hsp27 knockdown xenograft tumor initiated growth after 70 days, which also correlated with a regain of Hsp27 protein expression. Importantly, no tumors escaped from dormancy without Hsp27 expression. Clinically, strong Hsp27 expression was associated with markers of aggressive tumor 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 showed that SUMOylation of MK2 at lysine339 affects actin filament reorganization and cell migration (Chang et al., 2011). 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) that in turn then phosphorylates coflin, a protein that depolymerizes
actin filaments. This activity of cofilin 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 cofilin and regulate stimulus-induced actin reorganization. The signaling pathway composed of p38 MAPK - MK2 - LIMK1 has also been identified critical for VEGF-induced stress fiber formation, cell migration and tubule formation (Kobayashi et al., 2006).

In addition to its role in actin remodeling, Hsp27 is required for COX2 mRNA stabilization by the p38 MAPK – MK2 signaling cascade (Lasa et al., 2000) and also for IL-1β and TNF-α-induced activation of TAK1 - p38 MAPK - MK2 and expression of inflammatory mediators including COX2, IL-6 and IL-8 (Alford et al., 2007) Interestingly, Hsp27 was found to affect upstream signaling cascades. Hsp27 deletion down-regulated p38 MAPK - MK2 activation by IL-1β, resulting in COX-2 and IL-6 mRNA destabilization. This suggests that, at molecular level, Hsp27 contributes to the expression of pro-inflammatory mediators via regulation of the p38 MAPK - MK2-mediated mRNA stabilization.

MK2 - Hsp27 axis in the brain

In non-malignant diseases, Hsp27 overexpression has 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 ASK1 and consequent inhibition of ASK1 - MKK4 - JNK pathway. The inhibition of this kinase cascade protected 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 was able to block Hsp27 phosphorylation at Ser15 and Ser82 following oxygen
and glucose deprivation (model of ischemia-like conditions in vitro). In further support, pharmacological inhibition of PKD, shRNA knockdown or overexpression of dominant negative PKD mutant significantly blocked the phospho-Hsp27-mediated neuroprotection against neuronal injury. Intriguing discovery of this study is that phosphorylation of Hsp27, that 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 phospho-Hsp27-dependent neuroprotection. However, this is contradicting the neuroprotective phenotype observed in MK2 deficient animals through attenuation of the neuroinflammatory response to the injury (discussed above). 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 (Castro et al., 2012; Zhang et al., 2003). In addition, 4.7-fold increase in Hsp27 mRNA expression was found in hypoxic glioma regions, when compared to normoxic region in the in vivo approach of gene expression analysis. This indicates that Hsp27 could regulate hypoxia influence on biological 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. Pre-treatment 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 PTEN-null glioblastoma tumors) inhibited SPARC-induced invasion of brain cancer cells (Alam et al., 2013; Schultz et al., 2012). 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 knock-down (Nomura et al., 2007). As MK2 links p38 MAPK to Hsp27 phosphorylation, it is likely that MK2 deletion or inhibition would result in anti-migratory phenotype.

Finally, 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-IL6 efficacy of MK2 inhibitors that actually increase levels of unphosphorylated Hsp27. Thus, it can be hypothesized that anti-IL6 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.
The involvement of MK2 pathway in cell cycle regulation

Eukaryotic cells are in constant attack by internal or external sources causing base and DNA damage (Hoeijmakers, 2001). In order to repair damaged DNA and ensure that the genomic integrity is maintained during replication, cells undergo three cell cycle checkpoints, namely G1/S, intra-S and G2/M, before entering mitosis (Harper and Elledge, 2007; Kastan and Bartek, 2004). These checkpoints involve complex signaling mechanisms that provide an opportunity for the cells not only to repair their damaged DNA but also to exclude ones with extensive genotoxic insult (Abraham, 2001; Fridman and Lowe, 2003; Kastan and Bartek, 2004; Sancar et al., 2004; Shiloh, 2003; Vousden and Lu, 2002). This process ensures that cells replicate their DNA successfully and that the integrity is sustained in daughter cells.

Phosphatidylinositol-3-OH kinase-like kinases ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) are two major signaling networks that are activated in response to DNA damage (Figure 3) (Bakkenist and Kastan, 2003; Falck et al., 2005; Jazayeri et al., 2006). By activating checkpoint kinases 1 and 2 (Chk1/2) (Ahn et al., 2000; Culbert et al., 2006; Griffin et al., 2006; Kumagai et al., 2004; Liu et al., 2000; Schwarz et al., 2003) respectively, ATR and ATM control the G1/S, intra-S and G2/M cell cycle arrest for DNA repair (Berg et al., 2005; Chaturvedi et al., 1999; Chehab et al., 2000; Matsuoka et al., 1998; Sanchez et al., 1997). In addition, p38 MAPK-MK2 pathway, downstream of ATM and ATR, is activated in response to DNA damage caused by chemotherapy-induced G2/M arrest (Manke et al., 2005; Reinhardt et al., 2007). Mechanistic studies have further elucidated that a family of phosphatases, namely CDC25A, CDC25B and CDC25C function to activate specific CDK/cyclin complexes that in turn allow the progression of the cell cycle (Donzelli and Draetta, 2003; Hoffmann et al., 1994; Lammer et al., 1998; Peng et al., 1997). The checkpoint effector kinases, Chk1, Chk2 and MK2 have been described to inactivate CDC25 phosphatases causing CDK/cyclin inactivation and cell
cycle arrest (Chen et al., 2003; Falck et al., 2001; Furnari et al., 1997; Manke et al., 2005; Reinhardt and Yaffe, 2009).

To date, most studies have focused solely on p38 MAPK in cell cycle control and investigation of down-stream targets in this process is missing. p38 MAPK has been found to be activated in response to DNA damage or high osmotic shock causing G1/S and G2/M arrest (Bulavin et al., 2001; Dmitrieva et al., 2002; Hirose et al., 2003; Mikhailov et al., 2004; Pedraza-Alva et al., 2006). The p38 MAPK controls G1/S checkpoint by directly activating tumor suppressor p53 (Kishi et al., 2001; Takekawa et al., 2000) or stabilizing p21 mRNA (Lafarga et al., 2009). G2/M phase arrest is achieved by p38 MAPK phosphorylation-dependent inactivation of CDC25B/C (Ancriile et al., 2007; Bulavin et al., 2001; Hirose et al., 2003; Mikhailov et al., 2005). Biochemical phosphorylation studies revealed that p38 MAPK phosphorylated CDC25B at Ser323 and Ser375, whereas CDC25C is phosphorylated at the highly homologous Ser216 residue. Phosphorylation of these sites was required for binding to 14-3-3 proteins and degradation (Bulavin et al., 2001). Lemaire et al extended these phosphorylation patterns of CDC25s inactivation by showing that p38 MAPK also phosphorylates CDC25B on Ser249 and that MK2 is able to phosphorylate CDC25B on multiple sites, including Ser169, Ser323, Ser353 and Ser375 (Lemaire et al., 2006). Moreover, UV irradiation caused G1/S and G2/M arrest in MK2 proficient U2OS cells, but not in the MK2 depleted ones. 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 has been reinforced by Reinhardt et al. (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 the CDC25A mediated S phase arrest after cisplatin treatment as well as the CDC25B mediated G2/M arrest following doxorubicin exposure, resulting in enhanced anti-proliferative effects and sensitization to the chemotherapeutics (Reinhardt et al., 2007). The activation of MK2 after the drug induced DNA damage required activity of upstream ATM and ATR kinases, however 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 ATR - Chk1 pathway in response to DNA damage by chemotherapeutics. In a further study, Reinhardt et al. (Reinhardt et al., 2010) illustrated that in p53-deficient cells Chk1 mediates an early nuclear G2/M checkpoint, whereas MK2-mediated a late cytoplasmic checkpoint. This was achieved by translocation of the activated p38 MAPK - MK2 complex from the nucleus to the cytoplasm where MK2 phosphorylated hnRNP A0 and PARN to ultimately stabilize Gadd45α mRNA. Gadd45α in turn maintained the MK2 activity in the cytoplasm and hence cytoplasmic 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 G1/S and entry into apoptosis (Bulavin et al., 1999). MK2 was found to increase the degradation of p53 through phosphorylation of HDM2, 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 to wild type cells (Weber et al., 2005). In further support, a study published in 2012 demonstrated MK2 as a repressor of p53-driven apoptosis (Hopker et al., 2012). MK2-dependent phosphorylation of apoptosis-antagonising transcription factor (AATF) caused dissociation of p-AATF from cytoplasmic myosin-regulatory light chain 3 (MRLC3) and subsequent nuclear translocation (Figure 2C). In the nucleus, p-AATF binds to the PUMA, BAX, BAK promoter regions to repress p53-driven expression of these pro-apoptotic proteins. However, nuclear AATF did not restrict the expression of the cell-cycle regulating genes CDKN1A, Gadd45α, and RPRM. 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. Firstly, Xiao et al. 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. While depletion of Chk1 increased 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 (Xiao et al., 2006). Secondly, Phong et al. highlighted that p38 MAPK - MK2 pathway is not required for G2/M DNA damage checkpoint control but rather plays an important cytoprotective role through regulation of anti-apoptotic and survival pathways to allow cells to recover from DNA damage (Phong et al., 2010). 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 anti-apoptotic Bcl-2 proteins.

In addition to its role in checkpoint control and mitotic entry, p38 MAPK - MK2 pathway has been suggested to regulate mitotic progression as well, although in less details. The discovery that p38 MAPK knockdown strongly inhibited HeLa cell proliferation, instigated examination of the underlying mechanism (Fan et al., 2005). In the absence of p38α MAPK, 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 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 the mitotic arrest or soon after abnormal exit from M phase via caspase-dependent apoptosis. In addition, activated p38 MAPK was found to localize on kinetochores and spindle poles during mitosis, which is crucial for the normal kinetochore localization of polo-like kinase 1 (Plk1) that 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 M phase and massive cell death (Bu et al., 2008; Guan et al., 2005; Reagan-Shaw and Ahmad, 2005). Tang et al described that MK2 co-localizes with activated p38 MAPK and Plk1 at spindle poles where it phosphorylates Plk1 at Ser326 to promote normal mitotic progression (Figure 2D) (Tang et al., 2008). Importantly, the same study reported that MK2 acts as a Plk1 kinase and that MK2 knockdown resulted in mitotic arrest. In summary, MK2 could function not only as a checkpoint kinase that controls mitotic entry, but appears to be involved in mitotic progression.
A new, less investigated, role for MK2 was demonstrated by Chen et al. (Chen et al., 2000). This work showed that Ras activated the p38 MAPK pathway, including the upstream kinase MKK3 and down-stream 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 JNK activation. A follow-up study (Kobayashi et al., 2012) demonstrated that MK2 knockdown increases Ras transformation in MEFs and enhances tumorigenesis in vivo. However, 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 (ROS), as in MEFs MK2 decreased levels of ROS, 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 (Köpper et al., 2013). Mice deficient for MK2 displayed decreased apoptosis in the skin upon UV irradiation, accompanied with reduced H2AX phosphorylation. MK2 inhibition increased survival of gemcitabine-treated U2OS cells. In contrast, Chk1 knockdown or pharmacological 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 “tough nut to crack” (Schlapbach and Huppertz, 2009) because of several challenges associated with their development. Firstly, MK2 crystal structures 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. Secondly, low biochemical efficiency (BE) index of MK2 inhibitors hinders development of MK2 inhibitors into therapeutics. BE is defined as 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 et al showed that BE > 0.4 is a property of many approved medicines (Swinney, 2004). In other words, for the majority of successful drugs, the cellular EC$_{50}$ values are no more than 2.5-fold higher than biochemical binding or inhibition $K_i/IC_{50}$ values. However, cellular efficacy data of 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; Kaptein et al., 2011; Mourey et al., 2010; Oubrie et al., 2012). It is believed that the low BE is caused by high affinity of un-phosphorylated (inactive) MK2 to ATP (Mourey et al., 2010). As many kinases have low ATP affinity in the inactive conformation, as opposed to the 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 and therefore higher inhibitor concentrations are required for cellular efficacy. As good BE enables efficacy at lower drug concentrations and increases the therapeutic index, there is low probability of clinical success for ATP-competitive MK2 inhibitors. Nevertheless, Mourey et al demonstrated in vivo efficacy of a selective ATP-competitive MK2 inhibitor PF-3644022 despite its biochemical inefficiency (BE = 0.03). PF-3644022 reduced TNFα production and paw swelling in acute and chronic models of inflammation (Mourey et al., 2010). This data, supported by the normal phenotype of MK2 knockout mice (Kotlyarov et al., 1999), suggests that MK2 is a viable target that requires
non-ATP competitive or allosteric approach. Non-ATP competitive inhibitors have been reported by Merck (Huang et al., 2012; Qin et al., 2011) and it will be interesting to see how this class of compounds will progress through in vivo studies. Until then, we can only assume the outcomes of MK2 inhibition by analyzing efficacy of p38 MAPK inhibitors that inhibit MK2 activation or by analyzing efficacy of Chk1 or Wee1 inhibitors as these kinases have similar functions in cell cycle progression. Along these lines, Watterson et al. has recently demonstrated that anti-neuroinflammatory efficacy of BBB-permeable p38 MAPK inhibitors in the animal model of Alzheimer’s disease correlates with the inhibition of MK2 activity (Watterson et al., 2013).

**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 escape apoptosis. The implication that MK2 activity is necessary for G2/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 G2/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 outcome similar to inhibition of Chk1 or Wee1, both being targets of clinical investigation (Patil et al., 2013). Importantly, MK2-depleted mice are viable (Hegen et al., 2006; Kotlyarov et al., 2006).
1999; Ronkina 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, the knowledge whether p38 MAPK - MK2 inhibition could improve chemotherapy sensitivity is limited to in vitro data using only pharmacological inhibitors of p38 MAPK. Hirose et al. reported that U87 glioblastoma cells underwent a p38 MAPK-dependent G2/M arrest in response to the DNA methylating agent temozolomide (TMZ) (Hirose et al., 2003). Pharmacological blocking of p38 MAPK activity with SB203580 or siRNA knockdown bypassed the G2/M checkpoint resulting in increased sensitivity to cytotoxic action of TMZ. Interestingly, the p38 MAPK and Chk1 were found to work cooperatively to activate G2/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 G2/M arrest in U87 glioblastoma cells. Moreover, pharmacological inhibition of both pathways did not lead to greater bypass of TMZ induced G2/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 G2/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 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 above, 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 anti-cancer efficacy of Plk1 inhibition, without significant toxicity in non-transformed cells, has been demonstrated in oesophageal, prostate and brain cancer models (Bu et al., 2008; Guan et al., 2005; Liu and Erikson, 2003; Reagan-Shaw and Ahmad, 2005; Strebhardt, 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 S phase into an aberrant mitosis resulting in either failure of cytokinesis or cell death by an apoptotic mechanism. Likewise, Ferrao et al showed that inhibition of Chk1 effectively targeted lymphoma cells with MYC-driven intrinsic replicative stress but not normal B cells (Ferrao et al., 2012). Similarly, Wee1 inhibition by MK-1775 has been shown as a potent anticancer therapy approach independent of a genotoxic agent (Guertin et al., 2013).

Concluding remarks

Various functions of MK2 have been outlined herein that provide opportunities to intervene in the treatment of brain diseases (Figure 2). Current evidence suggests that MK2 contributes to neuroinflammation and plays a role in the pathophysiology of conditions such as Parkinson’s 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. While being 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 much similar to Chk1 could have positive implications in cancer therapy. Improving chemotherapy effectiveness by inhibiting MK2 has been already 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, nevertheless, needing to compete with high intracellular ATP, their BE is below the threshold required for therapeutic efficacy. Focusing on brain disorders, another challenge for MK2 inhibitors to overcome is the 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.

**Conflict of interest**

The authors declare no conflict of interest.
Acknowledgements

FMSG is supported by the University of Sydney Australian Postgraduate Award.

Authorship Contributions.

Wrote or contributed to the writing of the manuscript: Gurgis, Ziaziaris, Munoz.
References


Bachstetter A, Xing B, de Almeida L, Dimayuga E, Watterson DM and Van Eldik L (2011) Microglial p38alpha MAPK is a key regulator of proinflammatory cytokine up-
regulation induced by toll-like receptor (TLR) ligands or beta-amyloid (Abeta). *J Neuroinflammation* **8**: 79.


Guan R, Tapang P, Leuerson JD, Albert D, Giranda VL and Luo Y (2005) Small interfering RNA-mediated Polo-like kinase 1 depletion preferentially reduces the survival of p53-


chemical probes to address CNS protein kinase involvement in synaptic dysfunction.

*PLoS ONE* **8**: e66226.


MOL #90365

Footnotes

LM is supported by grant from Sydney Foundation for Medical Research and the University of Sydney Brown Fellowship.
Figure Legends

**Figure 1.** (A) Simplified structure of MK2 showing phosphorylation sites. Nuclear localisation sequence (NLS) and nuclear export sequence (NES) are amino-acid sequences that interact with importin-α or CRM/exportin-1, respectively, to facilitate shuttling of MK2 between nucleus and cytoplasm. (B) Proposed model for translocation of MK2. In resting cells, MK2 forms a tight complex with p38 MAPK that is predominantly located in the nucleus as a result of functional NLS in the inactivated MK2. After stimulation, MKK6 phosphorylates p38 MAPK which in turn activates MK2. Activation of MK2 results in NES being exposed and the complex of active p38 MAPK - MK2 is exported from the nucleus into the cytoplasm. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology (Gaestel, 2006), copyright 2006.

**Figure 2.** Signaling models of MK2-regulated neuroinflammation, actin remodeling and cell cycle control. (A) By activating AU-rich element (ARE) binding proteins, such as tristetraproline (TTP), heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0), polyA-binding protein-1 (PABP1) and human antigen-R (HuR), MK2 can regulate production of pro-inflammatory cytokines. This is achieved through stabilization of short-lived mRNAs containing AREs, that encode for cytokines such as IL-6, IL-8 and TNFα, leading to increased translation and secretion of these cytokines into the microenvironment. (B) Phosphorylation of Hsp27 by MK2 inhibits its chaperone activity and results in increased actin polymerisation and remodeling, leading to cell migration. In its unphosphorylated state, Hsp27 acts as an actin cap-binding protein inhibiting its polymerisation. (C) MK2 dependent phosphorylation of apoptosis-antagonising transcription factor (AATF) negatively regulates apoptosis. Phosphorylated AATF translocates from the cytoplasm into the nucleus where it binds to the PUMA, BAX, BAK promoter regions to repress p53-driven expression of these
pro-apoptotic proteins. (D) MK2 regulates cell cycle arrest and mitotic progression by modulating the activity of CDC25 phosphatases or Plk-1 kinase.

**Figure 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 activate 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 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 p38 MAPK - MK2 pathway is mediated by TAO kinases, which are activated by ATM and ATR through 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.
Figure 2

A  
**Inflammation**

- short-lived mRNA
  - ARE
  - stabilisation

  - TTP
  - P
  - PABP1
  - P
  - HuR
  - P
  - hnRNP A0

  → translation
  → IL-6, IL-8, TNFα

B  
**Actin remodeling / Migration**

- Hsp27

C  
**Apoptosis**

- cytoplasm
  - p-AATF
  - P

  → nucleus

- AATF
  - P

- p-AATF translocates to the nucleus

- PUMA
- BAX
- BAK

- pro-apoptotic
- p53-target genes

D  
**Cell cycle**

- Intra-S arrest
  - CDC 25A
  - P

- G2/M arrest
  - CDC 25B/C
  - P

- Mitotic progression
  - Plk1
  - P

- MK2
  - P
**double-strand DNA breaks (DSB)**

- ATM
- Chk2
- p53
- CDC 25A
- CDK4/6
- CDK2
- CDK1
- G1/S checkpoint

**single-strand DNA breaks (SSB)**

- ATR
- Chk1
- p38
- MK2
- CDC 25B/C
- 14-3-3
- CDC 25B/C
- Wee1
- G2/M checkpoint

**Cytoplasm**

- UV

**Cell Cycle Phases**

- G1
- S
- G2
- M

**Checkpoints**

- G1/S checkpoint
- Intra-S checkpoint
- G2/M checkpoint