MK2 and MK3 – a pair of isoenzymes?

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TABLE OF CONTENTS
1. Abstract
2. Introduction
3. Expression and Isoforms
4. Structure, activation and subcellular translocation
5. Substrates and functions
   5.1. Small mammalian heat shock protein
   5.2. ARE-mRNA binding proteins
   5.3. RSK
   5.4. TAB3
6. Use of MK2/3 knockout mice in animal models for human diseases
7. MK2/3 inhibitors for anti-inflammatory therapy
8. Perspective
9. Acknowledgements
10. References

1. ABSTRACT

The MAPK-activated protein kinases MK2 and MK3 form a pair of structurally and functionally closely related enzymes present in mammals and birds. Both protein kinases can bind to p38α MAPK and are activated by p38α via multiple proline-directed phosphorylations in a stress-dependent manner. Although the expression level and activity of MK2 is always significantly higher than that of MK3, the substrate spectrum of both enzymes is indistinguishable and covers proteins involved in cytokines production, endocytosis, reorganization of the cytoskeleton, cell migration, cell cycle control, chromatin remodeling and transcriptional regulation. Functional differences between MK2 and MK3 could result from the more prominent proline-rich SH3-targeting region in MK2, but are not reported so far. Since MK2 and MK3 are the main downstream targets of p38α responsible for posttranscriptional stimulation of cytokine biosynthesis, both enzymes are promising targets for the development of small molecule inhibitors which can be used in anti-inflammatory therapy. MK2-knockout mice show decreased LPS-induced cytokine biosynthesis and increased protection against collagen-induced arthritis. Recently generated MK2/3 double knockout mice show further reduction of LPS-induced cytokine production.

2. INTRODUCTION

Although stress-dependent phosphorylation of rat small heat shock protein (sHsp) has been originally described a quarter of a century ago (1, 2), the enzymes responsible for stress-dependent modification of mammalian sHsps in vivo have remained enigmatic for many years and a clear picture of in vivo sHsp-kinases has developed only slowly. First attempts to phosphorylate mouse Hsp25 in vitro demonstrated the ability of purified PKA and PKC to act as sHsp-kinases (3), but PKC and PKA inhibitors were not able to block sHsp phosphorylation by the endogenous kinase from Ehrlich ascites tumor cells (4). Partial purification of the stress-induced sHsp-kinase (s) revealed a novel kinase distinct from PKA, PKC and RSK that is regulated by phosphorylation (5).

By chance, some left over recombinant Hsp25 (mouse sHsp) (6), which was originally sent to Graham Hardie for testing it as a substrate for AMPK, was also tested as a substrate for a newly purified kinase from Philip Cohen’s lab designated MAPK-activated protein kinase 2 (MAPKAPK2, MK2) (7). Surprisingly, Hsp25 and Hsp27 (human sHsp) were proven to be ideal substrates for MK2 and, furthermore, purification of MK2 from rabbit muscle
MK2 and MK3 – a pair of isoenzymes?

directly paralleled the purification of sHsp kinase activity, strongly suggesting that MK2 is the sHsp kinase of mammalian cells (8). The primary structure of MK2 was determined by cloning its cDNA revealing a proline-rich N-terminal domain and a C-terminus with a putative nuclear localization signal (NLS) (9, 10). MK2’s catalytic domain was found to be most similar to calcium/calmodulin-dependent protein kinases although it was clear that this enzyme is not activated by calcium, but by phosphorylation. Since MK2 was described to be activated by classical MAPK similar to RSK2 (designated MAPKAPK1 at this time) (7), it was still puzzling how stress-dependence of sHsp phosphorylation was archived. This issue was solved when two groups in parallel demonstrated that MAPKAPK2 is activated by a new stress-activated MAPK, designated p40 MAPK (11) or MAPKAPK2 reactivating kinase (RK) which was similar to the stress-activated kinase HOG1 of *Saccharomyces cerevisiae* (12) and which is now known as p38 MAPK (13).

MK3 was also discovered in parallel by two independent groups and by two different ways. In one approach, the search for binding partners of a catalytically inactive p38 MAPK in a Y2H screen lead to the identification of a cDNA which coded for a protein kinase with about 70% identity to MK2 at the amino acid level (14). In the second approach, putative tumor suppressor genes were searched by analyzing genes in a specific region of human chromosome 3p which is deleted in small cell lung cancer. One of the genes identified in this region displayed high homology to MK2, was originally designated 3pK (chromosome 3p kinase) and turned out to be identical to MK3 (15).

A decade ago and, again, by two groups in parallel, another protein kinase with partial similarity to MK2 and MK3 was identified and termed PRAK or MAPKAPK5 (16, 17). Since this enzyme does not act as a stress-activated sHsp-kinase in *in vivo* (18) and since its activator and activation mechanism are different from MK2 and MK3 (19, 20), this protein kinase is discussed in a separate review of this special issue (Perander et al., 2008).

Homologue kinases of MK2 and MK3 lacking the proline-rich N-terminal domain of MK2 were identified in insects, nematodes and sea urchin (designated MK4 in this organism (21)), while in vertebrates full length homologues of MK2 are present. In worms, insects and lower vertebrates there is only one homologous kinase, while MK2 and MK3 co-exist in birds and mammals indicating a relatively late gene duplication event (22).

### 3. EXPRESSION AND ISOFORMS

MK2 and MK3 mRNAs are present in most human and mouse tissues analyzed (9, 10, 15). MK2 and MK3 proteins are ubiquitously expressed, but the levels of MK2 expression is significantly higher than MK3 levels (23). Furthermore, two protein isoforms have been described for MK2, which migrate as two distinct bands in SDS–PAGE (mouse: 45 and 55 kDa (24); rabbit: 53 and 60 kDa (7)). Correspondingly, two biochemically distinct forms (p43 and p49) of MK2 have also been detected in rat cardiac myocytes (25). Both isoforms of MK2 show bands of comparable intensity in SDS-PAGE, bind to p38α and show p38α-dependent activation (23) and are absent in MK2 knockout cells (26). So far, it is not clear whether these MK2 isoforms reflect a differential splicing event, post-translational modifications or proteolysis. For human MK2, an alternative cDNA coding for a truncated and modified C-terminus (MK2short, MK2S in Figure 1) has been identified (27). However, since this cDNA is very rare in EST-libraries, it is unlikely to account for the two comparable protein bands in SDS-PAGE.

### 4. STRUCTURE, ACTIVATION AND SUBCELLULAR TRANSLOCATION

MK2 is highly homologous to MK3 (75% amino acid identity for the human enzymes) suggesting that MK2 and MK3 form a pair of related kinases. Human MK2 features 400 residues with an N-terminal proline-rich region, that is able to bind SH3 domains (28) (residues 10-44), a catalytic kinase domain (residues 51-325), and a C-terminal autoinhibitory region (residues 328-364) (cf. Figure 1) (29). The kinase domains of MK2 and MK3 are most similar (35 to 40% identity) to CaMK and the CTKD (29). The kinase domains of MK2 and MK3 form a pair of related kinases. Human MK2 and MK3 – a pair of isoenzymes?
MK2 and MK3 – a pair of isoenzymes?

| Figure 1 | Amino acid sequence alignment of human MK2 (1-400), MK3 (1-382) and MK2S (1-370), an amino acid sequenced with a different C-terminus derived from a 3' altered cDNA. The proline-rich motif of MK2, the proline-directed phosphorylation sites of MK2 (T222, S272, T334) and MK3 (T201, S251, T313), the central tryptophan residue of the autoinhibitory helix (Al), the nuclear export (NES) and import signal (NLS) are indicated. The NLS overlaps with the p38-binding site. Subdomains of the kinase catalytic domain (gray) are indicated by Roman numbers. |
| | Accessible the substrate peptide binding site of MK2, therefore representing a so-called bifunctional switch (22, 32). In contrast to regulated NES function, the C-terminal bipartite NLS is active independently of the phosphorylation state, enabling this kinase for active shuttling between nucleus and cytoplasm. However, because of partial overlapping of the NLS with the p38 docking site it remains unclear how the p38 binding interferes with re-localisation of endogenous MK2. |
| It was shown that MK2 and p38α form a stable complex (31, 39). The knockout studies demonstrated decreased levels of p38α in MK2-deficient cells (40), as well as reduction of MK2 expression in p38α knockout cells (41). Since the catalytic activity of MK2 is not required for p38 stabilization, mutual stabilization by complex formation and/or intracellular relocalisation was proposed (22). Characterisation of the p38α-MK2 interaction using different methods show, that the heterodimer is a stable, high affinity complex with a Kd of 2.5 nM (39) or even 0.5 nM (42) and phosphorylation of MK2 attenuates the affinity of the binary MK2:p38α complex by an order of magnitude. Importantly, the 3D structure of the p38:MK2 complex was recently resolved with 4.0Å (PDB: 2ONL) (43) and 2.7Å (PDB: 2OZA) resolution (44), respectively. Interacting p38α and MK2α |

**Proline-rich**

| MK2 | 1  MLSNSQGSPPPVPP-APPPQOPTALPHPAOQPQPPFQPHVKSGLQI 54 |
| MK2S | 1  MDGTAEEQGPVPPPVAPGPGGLGAPGRRP 34 |
| MK3 | 55  KNAIIDDYKVSTGVVLGLNGKVLQIFNKRTQFALKMLQCPKARRVELHWWASQC 114 |
| MK3S | 35  KKYAVTDDYQLSCKVQLGNGKVLQCHKRRTQGKCALKLYDSPKARQVFDHWHQASG 94 |

**S272**

| MK2 | 175  QYLSINTAHRLVKPENLYTSKRPNAILTLDVFAKETTSHNMRCTCYTPYVAPEV 234 |
| MK3 | 155  QFHLISHNIAHRDVKPLENYTSKEKDAVLTAELDVFKAETTQ-NALDCYTTPYVAPEV 213 |

**S251**

| MK2 | 235  LGPEKYDKSCDMWLGLVINYILLGCYPPFYSNHGLIAEGMKTIRKMGQYEFNPEWSEV 294 |
| MK3 | 214  LGPEKYDKSCDMWLGLVINYLLLCCYPPFYSNHGLIAEGMKTIRKMGQYEFNPEWSEV 273 |

**T201**

| MK2 | 295  SEEVKLIRHNLKLTPTQRMITEPFMNHEFWIQSTVPTLLTSVEKDETREDVKVE 354 |
| MK2S | 295  SEEVKLIRHNLKLTPTQRMITEPFMNHEFWIQSTVPTLLTSVEKDETREDVKGE 354 |
| MK3 | 274  SEADAQLKLLKTDPTELTRITQFMNHEFWIQSMVPTCTHLTARVLQEDKDKDDEVKE 333 |

**S334**

| MK2 | 355  BMTSAPAMPEVDYEQIRIKSTEDASNPGLILKIFARKAREEAAAH 400 |
| MK3 | 334  BMTSAPAMPEVDYQRIKSTEDASNPGLILKIFARKAREEAAAH 382 |
| MK2S | 355  CLHDKNSQATWTLRL 370 |
Figure 2. The MK2 activation and nuclear-cytoplasmic translocation cycle based on the recent p38/MK2 heterodimer structure where p38 masks the NES (17). Inactive MK2 forms a stable complex with p38 in the nucleus. Activated MKK3/6 displaces MK2 from p38 and phosphorylates the latter on the TGY motif. Activated p38 transiently interacts with MK2, leading to the phosphorylation of MK2 on T222 and T334 (and, may be, also at S272). Phosphorylation of T334 induces conformational changes in hinge region, resulting in the exposure/activation of previously masked NES. Strong NES defies constitutively active NLS causing MK2 translocation to the cytoplasm, which is reversible as soon as MK2 becomes dephosphorylated. In the nucleus, inactive MK2 can again undergo association with dephosphorylated p38. Structure of MK2:p38 complex is based on PDB: 2OZA (17).
5. SUBSTRATES AND FUNCTIONS

MK2/3 are activated in vivo by stimulation of the p38 MAPK cascade and participate in diverse cellular processes such as cytokines production (23, 26), endocytosis (47), reorganization of the cytoskeleton (48), cell migration (49-51), cell cycle control (52), chromatin remodeling (45, 46) and gene expression (Ronkina and Gaestel, unpublished data). The optimal phosphorylation site motif for MK2/3 has been defined as ϕ-X-R-X- (L,N)-(pS/pT)- (I,V,F,L)-X, where ϕ represents a hydrophobic amino acid (15, 52, 53). MK2 and MK3 have similar kinetic constants for the phosphorylation of peptide substrates and phosphorylate the same serine residues in Hsp27 and Hsp25 (also known as Hspb1) with the same relative rates (53). However, the level of in vivo activity of MK2 is always markedly higher than that of MK3. Accordingly, it was shown that MK3 could have significant effects only in an MK2-free background. Consistently, MK2/MK3 double-knockout mice showed further reduction of TNF production and expression of p38 and TTP compared to MK2 single knockout mice. This finding, together with the observation that ectopically expressed MK3 can rescue MK2 deficiency similarly to ectopic MK2, indicates that both kinases share physiological functions in vivo but are expressed to different levels (23).

Interestingly, the dramatic reduction of p38 protein levels in MK2/3 double knockout cells resulted also in attenuation of p38 functions besides the MK2/3 signaling pathway. For example, stress-induced, but not mitogen-induced, phosphorylation and activation of another direct p38 downstream substrate, MSK (Mitogen- and Stress-activated protein Kinase, see review of J.S.C. Arthur within this special issue), is significantly reduced in MK2/3 double knockout macrophages and MEF cells, indicating that residual p38 is not sufficient to fulfill its function completely (Ronkina, Arthur and Gaestel, unpublished data). p38 protein levels and catalytic function could be rescued by expression of exogenous MK2, MK3 or by catalytic dead mutant of MK2 (23, 54). Restoration of p38 protein levels by ectopically expressed MKs or by catalytic pathway. For example, stress-induced, but not mitogen-

MK2 and MK3 – a pair of isoenzymes?

specific subcellular staining of endogenous MK2 and MK3 was not reported in the literature until now.

5.1 Small mammalian heat shock protein

One of the major in vivo substrates of MK2/3 is the small heat shock protein Hsp25/27. Mammalian Hsp25/27 are rapidly phosphorylated by MAPKAP kinase 2/3 at two or three serine residues in response to various extracellular stresses (7, 53). Upon phosphorylation large shSp oligomers dissociate to tetramers and lose their chaperone activities and ability to protect against oxidative stress effectively (55, 56). On the other hand, it was shown that MK2/3 dependent Hsp25/27 phosphorylation increases the stability of the actin microfilaments providing homeostatic functions at the actin cytoskeleton level during stress (48). With regard to the functional versatility ascribed to Hsp25, it is rather surprising that studies with Hsp25 knockout mice do not show any notable effects of Hsp25 deficiency on ontogenesis and tissue physiology under normal conditions. Nevertheless the same studies demonstrate a critical role of Hsp25 in thermotolerance development and protection of cells from apoptosis under thermal stress (57).

5.2 ARE-mRNA binding proteins

MK2 knockout mice show decreased production of inflammatory cytokines such as TNF and IL-6 upon LPS-stimulation (26). Impaired cytokine production make MK2 deficient mice less sensitive to LPS-induced endotoxic shock, but more susceptible to bacterial infection (58). Lack of MK3 alone does not lead to any notable changes in inflammatory response, but absence of both, MK2 and MK3, results in further reduction of TNF production compared to MK2 single knockout. This finding suggests that MK2 and MK3 function additively in stress-induced cytokine production (23). MK2 was shown to regulate TNF and IL-6 production posttranslationally by increasing the stability and translation of their mRNAs. Such regulation depends on AU-rich elements (AREs) within the 3'-untranslated regions of these mRNAs (59, 60). A similar mechanism of MK2/3-dependent regulation was shown also for other inflammatory mediators such as COX-2 (cyclooxygenase 2), MIP-2 (macrophage inflammatory protein-2), GM-CSF (granulocyte-macrophage colony-stimulating factor), CXCL-1 (C-X-C motif ligand 1) (23, 61, 62).

There are some ARE-binding proteins involved in posttranscriptional regulation of ARE-containing mRNAs which were described to be MK2/3 substrates. Among them: tristetraprolin (TTP) (63, 64), heterogeneous nuclear ribonucleoprotein (hnRNP) A0 (62), polyA-binding protein 1 (PABP1) (61) and human R-antigen (HuR) (65). Phosphorylation of ARE-binding proteins by MK2/3 could influence their stability, and/or ARE-binding affinities, and/or their interaction with other proteins. TTP, a zinc-finger protein that binds to ARE containing mRNAs and causes their destabilization by targeting them to exosomes (66) or proteasomes (67), was shown to be major target of MK2 involved in the posttranscriptional regulation of TNF. Phosphorylation of TTP by MK2 increases its stability and binding to 14-3-3 proteins and reduces its ARE affinity leading to inhibition of TTP-dependent degradation of ARE-containing transcripts (64, 68, 69).

5.3 RSK

MK2 and MK3 additively contribute to Toll-like receptor (TLR) signalling response in dendritic cells (DC) trough phosphorylation of RSK (p90 ribosomal S6 kinase, see review of Carrière et al., this special issue) at S386 which is pivotal for RSK activation (47). Whereas lack of only MK2 or MK3 alone does not lead to remarkable
MK2 and MK3 – a pair of isoenzymes?

endocytic defects, dual deficiency of both MK2 and MK3 significantly attenuates TLR4-induced edocytosis in DC. The involvement of MK2/3 in TLR-driven endocytosis is cell type specific and was observed only in DC. In other cell types, RSK autophosphorylation at S386 is catalysed by Erk1/2-activated C-terminal kinase domain (CTKD) of RSK. Thus, direct phosphorylation of S386 by MK2/3 in DC bypasses the necessity of the CTKD phosphorylation by Erk1/2. Taking into account the critical importance of DC for effective triggering of innate immune response, it could be supposed that even light stimuli which weakly activate Erk1/2 or p38 should still be able to induce endocytosis in DC. This could be a possible explanation for unordinary signalling mechanism evolved by DC and not by other cell types.

5.4 TAB3

IL-1 induced phosphorylation of TAB3 (TAK1-binding subunit 3) at S506 is mediated by MK2/3 and does not occur in MK2/3 double knockout MEFs (70) TAB3 is structurally related to TAB2 and demonstrates functional redundancy to TAB2 in mediation of IL-1 induced activation of TAK1 (71). IL-1 stimulates binding of TAB2/TAB3 to K63-linked polyubiquitinated TRAF6 and facilitates the formation of TRAF6-TAB2/TAB3-TAK1 complex, leading to the activation of TAK1 by auto-phosphorylation (72). How MK2/3-dependent phosphorylation of TAB3 influences its function is not clear so far.

6. USE OF MK2/3 KNOCKOUT MICE IN ANIMAL MODELS FOR HUMAN DISEASES

Stress-induced p38-MK2/3 activation has been implicated in various pathological conditions (73). The usage of specific p38 inhibitors helped to determine whether this signalling pathway contributes to designated pathologies, but side effects of p38 MAPK inhibitors, such as for example hepatotoxicity (74), limit their application in experimental disease models. Therefore, MK2/3 knockout mice, which are viable in contrast to the embryonic lethal p38 alpha knockout, become a very useful, if not indispensable, tool for the analysis of the functional role of MK2/3 in different mouse models for human diseases. The reaction to bacterial LPS/galactosamine is a well characterized stress response in mice and results in endotoxic shock mediated by inflammatory cytokines, especially the toxic TNF response in liver (75). In contrast to WT littersmates, MK2/-/- mice were partially protected and more than 50% survived endotoxic shock (26). Production of cytokines such as TNFα and interleukin (IL)-6 upon LPS-stimulation is severely affected. It is now clear that MK2 is the main target of p38α in the signalling pathway which regulates the stability and translation of TNFα and IL-6 mRNAs through a process that involves the AU-rich elements in the 3’ non-coding region of these mRNAs (59).

Interestingly, MK2 knockout mice demonstrate increased resistance to collagen-induced arthritis which is the murine model of rheumatoid arthritis (76). MK2 mediates joint inflammation by upregulation of inflammatory cytokines such as TNF-α and IL-6. Significantly reduced incidence and severity of collagen-induced arthritis in MK2 deficient mice disclose MK2 as a key player in the pathogenesis of this systemic autoimmune inflammatory disease. Atherosclerosis is another chronic inflammatory disease, where MK2 was shown to play a functional role (77). MK2 promote recruitment of monocytes into the vessel wall, by increasing VCAM-1 (vascular cell adhesion molecule) and MCP-1 (monocyte chemoattractant protein) expression in endothelial cells upon feeding an atherogenic diet of hypercholesterolemic mice (ldlr/-). Consequently, MK2-deficiency of hypercholesterolemic mice (ldlr/-/mk2/-) decreases the diet-induced accumulation of lipids, macrophages and foam cells in the aorta and thus ameliorates atherosclerosis.

In the model of experimental asthma, the endothelial permeability as well as expression of particular chemokines (IL-4, IL-5, IL-10, IL-13, MCP-1) and adhesion molecules (VCAM-1) were reduced in the lung of allergen treated MK2 knockout mice indicating that MK2 is essential for the development of the local lung inflammation (78). In the model of pulmonary fibrosis, lack of MK2 prevents myofibroblast formation and in this way exacerbates fibrosis, suggesting that MK2 mediates myofibroblast differentiation in fibrotic injury (79, 80). The increased Hsp25 phosphorylation in early diabetic nephropathy was almost completely inhibited in MK2 knockout mice (81). The suppression of MK2-induced Hsp25 phosphorylation, however, did not prevent MK2-deficient mice from renal hypertrophy, glomerular hyperfiltration, renal fibrosis and albuminuria in the early diabetic state. This observation challenges the finding that phosphorylation of Hsp25 is responsible for abnormalities in glomerular hemodynamics in the early stages of diabetes mellitus (82). The increased Hsp25 phosphorylation in the diabetic state was markedly reduced, but not completely abolished in the MK2 knockout mice suggesting that other kinases, most probably MK3, might compensate for the loss of MK2.

MK2 was shown to be responsible for generating ischemia-induced cardiomyocyte apoptosis and in accordance with this observation, the hearts of the MK2 knockout mice were resistant to myocardial ischemic reperfusion injury (83). While the hearts of the MK2 knockout mice were protected form myocardial ischemic mycardial injury, the cardioprotective effects of ischemic preconditioning was markedly reduced in the absence of MK2 (84). Lack of MK2 in mice subjected to focal cerebral ischemia protects brain from neurological deficits and results in prominent reduction of infarct expanses (85). Furthermore, the crucial role of MK2 in microglial neuroinflammation and neurodegeneration was demonstrated in microglia – neuron coculture with possible relevance to Alzheimer disease (AD) (86). Upon LPS + IFN-γ or β-stimulation, MK2 deficient microglia produces decreased amount of neurotoxic pro-inflammatory mediators such as TNF, CXCL-1 and MIP-1. Consistently, MK2 deficient microglia is not able to induce neuronal cell injury. In agreement with that, lack of MK2 in microglia protects neurons from inflammation-induced neurotoxicity. In the
MK2 and MK3 – a pair of isoenzymes?

cortical tissue of TASTPM mice, a transgenic model of Alzheimer's disease (AD), enhanced production of the same cytokine set as well as up-regulation of MK2 expression and activity has also been demonstrated. However, direct toxicity of one or combined chemokine/cytokine on cortical neurons was not demonstrated in this study. Crossing TASTPM mice on MK2-deficient background could be useful to validate MK2 role in this AD model.

Thus MK2 and MK3, as key drivers of inflammatory response, represent an attractive therapeutic target for the inhibitors that could ameliorate numerous inflammatory diseases, including rheumatoid arthritis, atherosclerosis and asthma. However, intact cytokine response is also essential for efficient host defence against invading pathogens. For example, global suppression of cytokine production via anti-inflammatory glucocorticoid therapy strongly increases susceptibility to microbial infection. Consistent with the affected cytokine synthesis, MK2 ablation may result in increased susceptibility to infections as well. Indeed, MK2−/− mice displayed significantly increased mortality when infected with a facultative intracellular bacterium Listeria monocytogenes reflected by significantly higher bacterial loading in spleen and lung (58). Additionally to impaired TNFα and IFNγ production, MK2-deficient macrophages displayed affected phagocytosis.

Apart from inflammation and infection, a strong activation of p38/MK2/3 axis accompanies genotoxic stress and treatment with cytostatica, such as cisplatin or doxorubicin, raising the question whether this activation modulates apoptosis or cell cycle checkpoint control. MK2-deficient mouse cells show increased apoptosis after UV irradiation and elevated levels of p53 protein (87). In an in vitro kinase assay, MK2 is able to phosphorylate HDM2 on S157 and S166. The consequence of these modifications is enhanced ability of MDM2 (HDM2) to degrade p53 and, hence, lack of this modification in MK2-deficient cells causes increased stability of p53. However, stress-dependent reduction of p53 level is not completely abrogated in MK2-deficient cells, indicating existence of additional mechanism responsible for the downregulation of p53 at the end of the stress response (87). Since several years, it was known that inhibition of p38 pathway in UV treated cells resulted in loss of G2/M checkpoint control (88). Interestingly, MK2 is able to phosphorylate CDC25B, creating 14-3-3 binding sites and inhibiting G2/M progression similar to CHK1 and CHK2, which are activated by ATM/ATR in γ-irradiated cells (52). Moreover, in the absence of p53, MK2 activation was reported to induce Cdc25A-mediated S phase checkpoint in cisplatin treated cells and Cdc25B-mediated G2/M checkpoint after doxorubicin treatment (89). Consequently, depletion of MK2 in p53−/− cells using RNAi resulted in an increase in the sensitivity to both chemotherapeutic agents in cell culture as well as in a tumor model in vivo, probably due to increased mitotic catastrophe (89). The two latter evidences are only indicative and the role of MK2 as a tumor promoter or checkpoint kinase should be validated using MK2-deficient animals and established disease models.

Summing up, MK2 knockout mice - and in future also MK2/3 double knockout mice - represent a profitable tool for evaluation of the impact of MK2/3 on the development of different pathologies. However, for the interpretation of the results obtained in these models, attenuated p38 activity due to ablation of MK2/3 should also be considered.

7. MK2/3 INHIBITORS FOR ANTI-INFLAMMATORY THERAPY

In the Christmas issue of Nature in 1994, a bicyclic imidazole, which could act as an efficient cytokine-suppressive anti-inflammatory drug in monocyte culture, was identified as ATP-competitive inhibitor of p38 MAPK (90). This finding initiates the development of different generations of p38 MAPK inhibitors starting with the well known compound SB203580 and leading to more potent and specific inhibitors such as VX-745 and BIRB-796 (91). However, due to liver toxicity and other unexpected side effects, until now none of these p38 MAPK-inhibitors had made it to the clinics (92). Meanwhile, the central role of MK2/3 in posttranscriptional control of inflammatory cytokine production and the viability of the MK2/3 double knockout mice (23), compared to the embryonic lethality of the p38α knockout mice (93), qualifies MK2/3 as a promising target for the development of small molecule inhibitors which can be used as anti-inflammatory therapeutics. Accordingly, screens for small molecule inhibitors for MK2 and MK3 were carried out by various pharmaceutical companies, but the first outcome was rather scanty. Obviously, the ATP-binding pocket of MK2 is shallow and the structural change of the catalytic domain itself from its inactive to its active conformation is small and not easy to be locked by an inhibitor. MK2 activation is probably more regulated by the C-terminal domain and its interaction with the catalytic domain (29, 94) and by protein-protein-interactions within the stable MK2/3/p38 MAPK complex (44). However, some effective small molecule inhibitors for MK2/3 were identified (reviewed in (91)). Recently, pyrrolopyridins and carboline analogs were described as potent and rather selective inhibitors with an IC50 in the low nanomolar range and the 3D structures of the inhibitors bound to MK2 fragments were determined (34, 35). Both classes of inhibitors bind near the P-loop and within the hinge region of MK2 which is a larger pocket than in most other kinases (34, 35). The inhibitors are active in blocking LPS-stimulated TNF production in U937 (35, 95) and THP-1 cells (34) at least in the low micromolar range. Moreover, the pyrrolopyridin “compound 23” when given orally to rats 2 h prior to LPS challenge can suppress more than 80% of TNF production in vivo (35).

In the present time the declared targets for developing of small molecule inhibitors for MK2 and MK3 are inflammatory diseases. However, since the disruption of the MK2 signalling pathway enhances sensitivity to chemotherapeutic agents (89), MK2 inhibitors could also be of use in combination with conventional cancer chemotherapy.
8. PERSPECTIVE

So far, MK2 and MK3 appear as structurally and functionally closely related isoenzymes with a seemingly identical manner of p38-dependent activation and an indistinguishable substrate spectrum. In addition, a common physiological function in posttranscriptional regulation of cytokine biosynthesis via TTP and stabilization of p38 has been demonstrated (23) making both kinases promising targets for anti-inflammatory therapy. However, the more prominent proline-rich region in MK2 compared to MK3, which could be of importance for subcellular targeting (28) and seems to be necessary for regulation of cell migration (40), might be the basis for some functional differences between both protein kinases. For analysis of these differences in targeting and for a further understanding of activation-dependent subcellular translocations of MK2/3 in concert with p38, antibodies which allow the subcellular detection of the endogenous kinases will be essential. Finally, the role of MK2 and MK3 in tumorigenesis and cell cycle control will also require further analysis.

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10. REFERENCES


MK2 and MK3 – a pair of isoenzymes?


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