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New insights into the activation, interaction partners and possible functions of MK5/PRAK

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1. ABSTRACT

MAP kinase-activated protein kinase 5 (MK5) was first described as a downstream target of the p38 MAP kinase pathway leading to its alternative acronym of p38-regulated/activated protein kinase (PRAK). However, since the discovery that MK5 is a *bona fide* interaction partner of the atypical MAP kinases ERK3 and ERK4 and that this interaction leads to both the activation and subcellular relocalisation of MK5, there has been considerable debate as to the relative roles of these MAPK pathways in mediating the activation and biological functions of MK5. Here we discuss recent progress in defining novel upstream components of the ERK3/ERK4 signalling pathway, our increased understanding of the mechanism by which MK5 interacts with and is activated by ERK3 and ERK4, and the discovery of novel interaction partners for MK5. Finally, we review recent literature that suggests novel biological functions for MK5 in a range of physiological and pathophysiological conditions including neuronal function and cancer.

2. INTRODUCTION

MAP kinase-activated protein kinase 5 (MK5) was first described as a serine/threonine protein kinase, which was closely related to MAP kinase-activated protein kinase 2 (MK2) and could be both phosphorylated and activated by p38 MAP kinase. The latter observation led to MK5 also being designated as p38-activated protein kinase or PRAK. Subsequent work demonstrated that MK5 interacted with the atypical MAP kinases ERK3 and ERK4 and that this interaction led to phosphorylation, activation and nuclear export of MK5. We previously reviewed the literature pertaining to the discovery, structure, expression and activation mechanism for MK5 (1). Here we provide a comprehensive update dealing with newly discovered aspects of MK5 regulation, the mechanism by which it can bind to regulatory kinases, novel substrates and possible biological functions for MK5.

3. PHOSPHORYLATION OF THE S-E-G MOTIF WITHIN THE ACTIVATION LOOP OF ERK3 AND ERK4 IS REQUIRED FOR BINDING, ACTIVATION AND CYTOPLASMIC RELOCALISATION OF MK5

While MK5 can be activated both by the classical MAP kinases p38 MAPKalpha/beta and the atypical MAP kinases ERK3 and ERK4, at least when these proteins are overexpressed in mammalian cells, only ERK3 and ERK4 have been found to form endogenous complexes with MK5 (2,3). Ectopically expressed MK5 is also readily activated by extracellular stimuli that activate the endogenous p38 MAPK signaling pathway, or by the overexpression of constitutively active mutants of either MKK3 or 6. However, as no extracellular stimuli have yet been linked to the activation of ERK3 and ERK4, the connection between extracellular stimuli and endogenous MK5 activation remains unresolved. Non-phosphorylated classical MAP kinases such as ERK1/2 are inactive mainly because interactions with potential substrates are hindered by the conformation of the activation

loop. The threonine and tyrosine residues within the activation loop of ERK1/2 are rapidly phosphorylated in response to extracellular stimuli and this induces a major conformational change, resulting in a 1000-fold increase in kinase activity (4). In contrast to ERK1/2, ERK3 and ERK4 contain the sequence S-E-G instead of T-X-Y in their activation loop. Furthermore, the single phospho-acceptor residues in ERK3 (S189) and ERK4 (S186) are constitutively phosphorylated in growing cells, indicating that these kinases are unresponsive to stimuli that would normally induce the phosphorylation and activation of conventional members of the MAP kinase family (5-7). The phosphorylation of the threonine and tyrosine in the activation loop of ERK2 has distinct functions during activation of the kinase. Phosphorylation of threonine 183 (corresponding to serine 189 in ERK3 and serine 186 in ERK4), has a central coordinating role in ERK2, promoting closure of the N and C lobes of the kinase, such that it becomes catalytically active. Phosphorylation of tyrosine 185 (substituted by a Glycine in ERK3 and ERK4) is thought to be important for substrate recognition (8). The phosphorylation of the single serine phospho-acceptor in the activation loop of both ERK3 and ERK4 is absolutely required for their ability to activate MK5 *in vitro* (6) and is also vitally important for the ability of ERK3 and ERK4 to form stable and active complexes with MK5 in mammalian cells (6,7). Thus, mutant forms of ERK3 and ERK4 in which the serine phospho-acceptor is substituted by alanine are unable to mediate the cytoplasmic relocalisation of ERK3/ERK4-MK5 complexes. This requirement for activation-loop phosphorylation for the interaction between the atypical map kinases and MK5 also differs from the interactions seen between the classical MAP kinases and MAPKAP kinases, such as MK2 and MK3, as these are mediated by the so called common docking domain and can occur independently of activation loop phosphorylation. Thus, the signaling pathway mediated through the atypical MAP kinases ERK3 and ERK4 operates with an extra level of control in order to be able to both activate and redistribute its downstream effector MK5.

4. PAK FAMILY KINASES ARE ACTIVATORS OF THE ERK3/ERK4-MK5 SIGNALLING AXIS

The threonine and the tyrosine residues within the activation-loop of the classical MAP kinases are phosphorylated by dual-specificity kinases of the MAP kinase kinase (MKK) family. The MKKs are highly specific and MAP kinases are their only known cellular substrates. The single phospho-acceptor site within the activation-loops of both ERK3 and ERK4 are crucial for both the activation and binding of MK5. However, these residues are not phosphorylated by any of the MKKs tested thus far (9). Several laboratories have shown that the serine phospho-acceptor within ERK3 and ERK4 is phosphorylated in trans in mammalian cells, but the identity of the kinase(s) responsible has been unclear. Recently, two groups using different experimental strategies, were able to identify the group 1 p21 activated kinases (PAKs) as *bona fide* ERK3 and ERK4 activation-loop kinases. Deleris and coworkers used a classical biochemical purification approach to identify the group 1 PAKs as activation-loop kinases for ERK3 and ERK4. PAK2 phosphorylated ERK3 on serine 189 and ERK4 on serine 186 *in vitro.* The ability of PAK2 to phosphorylate ERK3 at serine 189 *in vivo* was demonstrated by overexpression of ERK3 with PAK2 alone or together with activated mutants of Rac or Cdc42 (10,11). A role for PAKs in phosphorylation of the activation loop of ERK3 *in vivo* was further confirmed using siRNA knockdown of PAK1, 2 and 3 in cells overexpressing active RAC. Silencing of individual PAKs reduced Rac-induced ERK3 phosphorylation. However, all three PAKs had to be knocked down in order to see a complete inhibition of Rac-mediated ERK3 phosphorylation. De la Mota-Peynado *et al*., identified ERK3 as a substrate for PAK2 using a high-density protein microarray (10). They also demonstrated that PAK phosphorylated the activation loop of ERK3 and that PAK activity was important for ERK3 serine 189 phosphorylation *in vivo (10).* The p21 activated kinases are downstream effectors of the Rac and Rho family of small GTPases and activation of PAKs by overexpression of a constitutively active mutant of Rac1 resulted in both increased phosphorylation of ERK3 and ERK4 and the subsequent activation of MK5 (11). Conversely, inhibition of PAK kinase activity led to a reduction in ERK3 serine 189 phosphorylation and prevented the ERK3-mediated translocation of MK5 from the nucleus to the cytoplasm (10). Despite the clear identification of the PAKs as *bona fide* ERK3/ERK4 kinases, the physiological role of this emerging PAK1-3-ERK3/4-MK5 signalling axis lying downstream of Cdc42, Rac1 and Rho is as yet unclear. One prominent function of the PAKs, in addition to stimulation of cell proliferation and cell survival, is their role in cell motility. As MK5 has been reported to cause cytoskeletal changes it is tempting to speculate that ERK3/4-MK5 may be a downstream effector of the PAKs in regulation of the cytoskeleton and cell migration (12,13)

5. INTERACTIONS BETWEEN ERK3/ERK4 AND MK5 ARE MEDIATED BY A NOVEL SUBSTRATE-BINDING MOTIF

MK5 possesses a basic MAP kinase docking motif that overlaps its nuclear localization signal and mediates interactions with the acidic common docking (CD) motif of p38 alpha/beta MAPK (14,15). However, interactions between MK5 and either ERK3 or ERK4 occur completely independently of this MAP kinase docking motif. This is despite the fact that both ERK3 and ERK4 also contain a conserved common docking domain (3,16,17). Using a peptide array, Åberg and coworkers identified a novel motif in both ERK3 and ERK4 that is responsible for phosphorylation-dependent

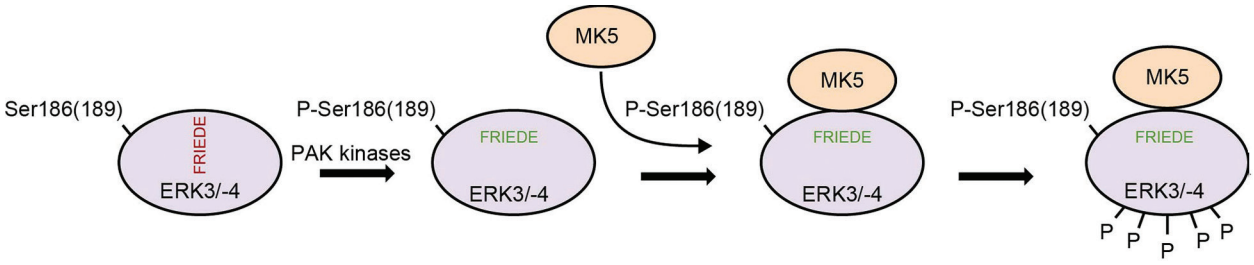

Figure 1. Phosphorylation of the S-E-G motif within ERK3 and ERK4 is required for binding to MK5. ERK3 and ERK4 are phosphorylated at their S-E-G motif (P-Ser189 and P-Ser 186 respectively) by activated PAK kinases. This phosphorylation leads to conformational changes in both kinases that expose the MK5-interacting FRIEDE motif. Complex formation between MK5 and either ERK3 or ERK4 also results in hyperphosphorylation of these atypical MAP kinases concomitant with both activation and relocalisation of MK5 from the nucleus to the cytoplasm.

interaction with MK5. This is termed the FRIEDE-motif and is located C-terminal to the common docking domain sequence in both ERK3 and ERK4 (18). A single mutation in either ERK3 or ERK4 in which the isoleucine within the FRIEDE motif is substituted by a lysine residue was sufficient to completely abrogate interaction with and activation of MK5. The region in MK5 responsible for interaction with the FRIEDE motif of ERK3 and ERK4 was mapped to the unique C-terminal extension within MK5, which is not conserved in the related kinases MK2 or MK3 and removal of the C-terminal 13 residues of MK5 abrogated the ability of MK5 to interact with both ERK3 and ERK4. However, this peptide alone was unable to bind to ERK3 or ERK4 with the whole C-terminal extension of MK5 being required for stable association with ERK3 and ERK4 (18). This contrasts with short peptides derived from the MAPK docking domains found in MK2 and MK3 as these are able to interact with their MAP kinase partners (19). The region where the FRIEDE motif within ERK3 and ERK4 is localized corresponds to the C-terminal extension in the classical MAP kinases. Structural data for ERK2 indicates that phosphorylation of threonine 183 within the activation loop induces conformational changes in this region that expose a hydrophobic surface proposed to facilitate the formation of ERK2 homodimers (8). Structural modeling of ERK4 using unphosphorylated and phosphorylated ERK2 as templates, indicates that the critical residues within the FRIEDE motif that are required for the interaction between ERK3/4 and MK5 may also become exposed at the surface of the protein when the serine phospho-acceptor within the S-E-G motif is modified (18). Thus the FRIEDE motif enables phosphorylation-dependent binding of MK5 to ERK3 and ERK4 (Figure 1). This mechanism is not shared with the related kinases MK2 and MK3 as these rely on the conventional common docking site to interact with the MAP kinases that activate them. Phosphorylation of the activation loop within both ERK3 and ERK4 is not only an important regulator of the catalytic activity of these kinases, but is also crucial for their role as scaffolds for MK5 and in determining the subcellular localization of both ERK3 and MK5 (18). Finally, the fact that both ERK3 and ERK4 also contain a conserved common docking domain indicates that these kinases have as yet undiscovered binding partners. Likely candidates include protein phosphatases that could act as negative regulators of activation loop phosphorylation.

6. UNCOVERING NEW SUBSTRATES AND FUNCTIONS FOR MK5

Since the discovery that MK5 is a physiological target and substrate for the atypical ERK3 and ERK4 MAP kinases a number of reports have linked MK5 with cellular substrates and functions. In certain cases there is an indication that these may involve the ERK3/ERK4-MK5 signalling axis.

Septins comprise a highly conserved family of GTP-binding proteins, which organize into higher order hetero-oligomeric filaments and can function as scaffolds for protein recruitment or provide barriers to diffusion in subcellular compartments. In humans, there are 13 septin proteins that are either expressed ubiquitously (Septins 2, 5-7,9-11) or in specific cells and tissues including lymphocytes, the CNS and testes (septins 1,3,4,8,12 and 14). They seem able to function in a wide range of cellular processes including the regulation of cell shape and actin dynamics, chromosome segregation, cytokinesis, DNA repair, membrane trafficking, autophagy, apoptosis and mitochondrial function (reviewed in (20). In a yeast two-hybrid screen looking for novel MK5 interaction partners, septin 8, a protein expressed in lymphocytes, CNS, eyes, the intestinal tract and placenta was identified as a binding partner for MK5 (21). While MK5 interacted with septin 8, it did not interact with septin 5 and a combination of GST-pulldown, co-immunoprecipitation, and FRET experiments indicated that the two proteins were *bona fide* interaction partners. Finally, MK5 was also able to phosphorylate septin 8, at least *in vitro,* on serine residues distinct from those previously identified in global phospho-proteomic analyses (21).

Shortly after publication of this report Mathias Gaestel's group, who had previously generated an MK5 knockout mouse, reported that these animals displayed an impairment of dendritic spine formation in

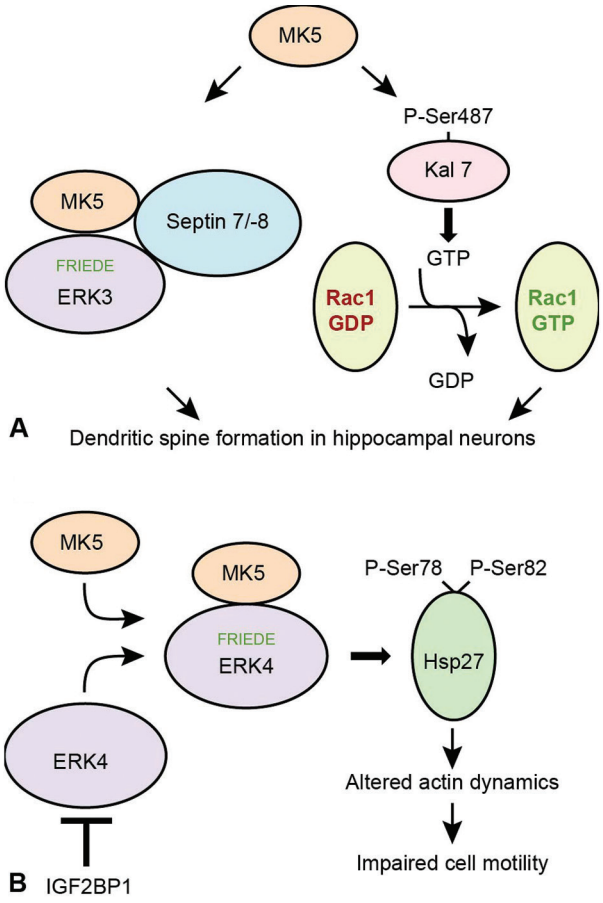

Figure 2. Novel functions and substrates for MK5. A, The ERK3-MK5 signaling axis regulates the formation of dentritic spines in hippocampal neurons. Both ERK3 and MK5 bind to distinct regions of Septin7 and -8. The resulting trimeric complex increases both dentritic branching and spine numbers when expressed in cultured primary murine hippocampal neurons. MK5 also regulates dendritic spine formation by binding to and phosphorylating the Rac1 GDP/GTP exchange factor Kal-7. This phosphorylation stimulates the exchange activity of Kal-7 leading to activation of Rac1. B, MK5 inhibits cell motility by phosphorylating Hsp27 at serine 78 and serine 82. Phosphorylated Hsp27 regulates the dynamics of the actin cytoskeleton by increasing the G-actin/F-actin ratio. Downregulation of ERK4 expression at the translational level by IGF2BP1 antagonizes the MK5-mediated inhibition of cell motility.

hippocampal neurons (22). This was intriguing as both ERK3 knockout mice and transgenic mice expressing a constitutively active form of MK5 display complex behavioural phenotypes, which might reflect changes in functional neuronal plasticity (23,24). As mice lacking MK5 also display reduced levels of ERK3, a Ras recruitment system (RRS)-based screen was performed using ERK3 as bait in yeast and septin 7 was identified as an ERK3 binding partner. Interestingly, septin 7 binds to a distinct region of the ERK3 C-terminus compared with MK5 and experiments in which septin 7 and MK5 were co-expressed with ERK3 revealed the association of all three proteins in cytoplasmic filamentous structures, indicating that ERK3 might bind to both MK5 and septin 7 simultaneously. Using double knockout ERK3/MK5 mouse embryonic fibroblasts (MEFs) it was shown that the distribution of endogenous septin 7 into filaments was weaker when compared with wild-type cells and that this defect could be rescued by co-expression of ERK3 and MK5. A conventional yeast 2 hybrid screen subsequently revealed that MK5 itself can bind to both septin 7 and septin 8, thus confirming the interaction previously observed by Shirayev *et al* (21). In addition, they identified an additional MK5 binding partner, the GDP exchange protein Kalirin-7 (Kal-7). Kal-7 is known to positively regulate dendritic spine formation via its activity as a GDP/GTP exchange factor for the Rac1 small GTPase (25). Furthermore, Kal-7 was phosphorylated *in vitro* by activated MK5 on serine 487, a modification, which had previously been implicated as a positive regulator of Kal-7 exchange activity (26). Finally, the co-expression of septin 7, ERK3 and MK5, but not septin 7 alone, in cultured primary murine hippocampal neurons increased both dendritic complexity (branching) and spine numbers. Taken overall, these data are a very strong indication that the ERK3-MK5 signalling axis plays a key role in septin-dependent regulation of dentritic spine formation and morphology (Figure 2A). The translocation of Kal-7 into dendritic spines leads to the Rac1-dependent activation of PAK. As mentioned previously, the PAK family of protein kinases were recently identified as activators of both ERK3 and ERK4 (10,11) raising the possibility of a positive feed-forward control of septin-mediated spine formation via the phosphorylation and activation of Kal-7 by MK5 (22). It will be very interesting to see if the role of the ERK3/ERK4-MK5 signalling axis extends to the regulation of any other septin-dependent functions, which have been identified in mammalian cells and tissues. One such septin-dependent function may be the regulation of immune cell biology. Both septins and ERK3 have recently been shown to be important for specific aspects of lymphocyte development and activation(2,27-29).

The oncofetal RNA–binding protein IGF2BP1 has been implicated in the formation of lamelipodia and invadopodia, both of which are implicated in mediating tumour cell motility. In an attempt to discover which mRNAs are targeted by IGF2BP1, both unbiased and candidate screening was carried out and the atypical MAP kinase ERK4 (MAPK4) was identified as a gene target, which is negatively regulated at the translational level by IGF2BP1 (12). In forging a link between ERK4 protein levels and the migration of cells it was hypothesised that down-regulation of ERK4 by IGF2BP1 might result in impaired activation of MK5 and a corresponding reduction in the levels of phosphorylated Hsp27. Because phosphorylation of Hsp27 is associated with modulation of the actin dynamics in cells the authors tested the effects of mutating the MK5 phospho-acceptor residues in Hsp27 to either mimic (S78/S82 to D78/D82) or abolish (S78/S82 to A78/A82) phosphorylation. They found that either knockdown of IGF2BP1, overexpression of ERK4/MK5 or

expression of Hsp27 D78/D82 caused redistribution of the actin cytoskeleton associated with up-regulated G-actin/F-actin ratios. In contrast, expression of Hsp27 A78/A82 did not. All of this implies that IGF2BP1 exerts its effects by antagonising the activation of MK5 and subsequent phosphorylation of Hsp27 (Figure 2B). In agreement with this both IGF2BP1 knockdown or expression of Hsp27 D78/D82 caused a reduction in cell motility as measured in wound healing assays and the migration defects caused by knockdown of IGF2BP1 could be reversed by concomitant siRNA-mediated knockdown of MK5. While the data are apparently consistent with the interpretation that MK5 mediated Hsp27 phosphorylation plays a key role in regulating actin dynamics and cell motility it is by no means clear from previous work that Hsp27 is a *bona fide in vivo* target for MK5. Hsp25, the murine homolog of human Hsp27, is phosphorylated *in vivo* by the p38-MK2 signalling axis and levels of Hsp25 phosphorylation and Hsp25 kinase activity are negligible in mice lacking MK2, indicating that MK5 is unable to substitute for this kinase or to phosphorylate Hsp25 *in vivo* (30). However, it should be noted that Serine 78 in human Hsp27 is not conserved in the murine protein, perhaps accounting for the discrepancies between results using genetically modified mice and cells derived from these animals and human tumour cell lines.

Recent work performed to study the role of the transcription factor FoxO1 in the regulation of recombination activating gene (Rag) expression during B cell development identified a novel phosphorylation site (Serine 215) within FoxO1, which appears to be essential for *Rag* gene transcription (31). This was detected by using FoxO1-deficient AMuLV-transformed B cell progenitors and screening a panel of FoxO1 mutants for the ability to rescue Rag gene transcription. Chromatin immunoprecipitation (ChIP) experiments revealed that while wild-type FoxO1 binds to several sites at the *Rag* locus a Ser215A mutant of FoxO1 failed to bind to DNA at these sites. Because MK5 had previously been demonstrated to phosphorylate Ser215 in the related transcription factor FoxO3a (32) overexpression or siRNA knockdown of MK5 was performed and found to down-regulate *Rag* mRNA expression as expected if MK5-dependent phosphorylation of FoxO1 were a requirement for Rag expression. Furthermore reciprocal expression and knockdown showed that MK5 required the presence of FoxO1 to mediate its effects. Finally, a number of *in vivo* assays were performed to ensure that these results could also be reproduced in primary (untransformed) cells with the result that in all cases MK5 was required for FoxO1-dependent Rag gene transcription (31). As yet it is unclear, which are the relevant upstream activators for MK5 in this FoxO1-dependent pathway. Rag expression is unaffected either by agonists (anisomycin) or specific chemical inhibitors (SB203580 or BIRB 796) of the p38 MAPK pathway, nor does siRNA-mediated knockdown of either ERK3 or the PAK kinases seem to affect Rag mRNA levels. Further work will be required to dissect out the relevant players in this pathway and it will be of great interest to determine if MK5 is also responsible for regulating FoxO1 activity in other cellular and physiological/pathophysiological contexts.

Recently, two new substrates of MK5 have been identified that strongly indicate that MK5 has a cytoprotective role under certain cellular conditions. In a two-hybrid screen Tang *et al* found DJ-1 to be an interaction partner of MK5 (33). DJ-1 is a protein with versatile functions that has been implicated in several processes including tumorigenesis, oxidative stress, and in the development of Parkinson's disease (reviewed in (34)). MK5 and DJ-1 colocalized in the nucleus upon oxidative stress in $MK5^{+/+}$ MEF cells. In contrast, barely any nuclear localization of DJ-1 was observed in $MK5^{-/-}$ cells upon H_2O_2 treatment and cell survival following this treatment was severly compromised is cells lacking MK5. Furthermore, it was demonstrated that MK5 facilitated DJ-1-mediated nuclear sequestering of the Daxx, which upon translocation to the cytoplasm leads to the induction of apoptosis by activating apoptosis signal-regulating kinase 1 (ASK1).

Motivated by findings that Hsp27 is a substrate of MK5, Kostenko *et al* recently conducted *in vitro* kinase assays using Hsp40, Hsp70, and Hsp90 as substrates (35). They found that MK5 phosphorylated Hsp40/DnaJB1 at several residues *in vitro*. This was confirmed in Hek293 cells overexpressing Hsp40/DnaJB1 and a GFP-fusion protein of a constitutively active mutant of MK5. Hsp40/DnaJB1 acts as a co-chaperone for Hsp70, a chaperone protein involved in protein folding that is upregulated during cellular stress (36). Hsp40/DnaJB1 recruits Hsp70 to misfolded proteins and stimulates its ATPase activity. Further experiments by Kostenko *et al* suggested that MK5 slightly increased the ATPase activity of the Hsp40:Hsp70 complex. MK5 also stimulated the ability of Hsp40/DnaJB1 to repress the transcriptional activity of heat shock factor 1 (HSF1). Further studies on endogenous proteins should be conducted to evaluate the role of MK5 in the regulation of Hsp70 and HSF1.

7. MK5 – FRIEND OR FOE IN CANCER?

In recent years, studies have been conducted to assess the role of MK5 in tumour initiation and development. Several lines of evidence from biochemical experiments, mouse cancer models as well as human tumour samples, suggest that MK5 can have either a tumour suppressor function or a tumour promoting activity, depending on the cell type or the stage of tumour development at which it is activated.

This was first demonstrated in a series of experiments performed by Peiqing Sun and coworkers

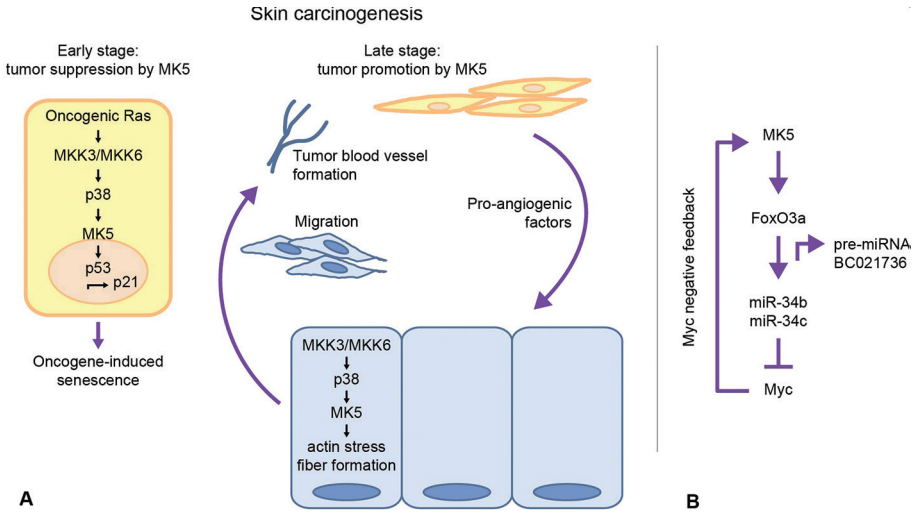

Figure 3. MK5 and tumourigenesis. A, MK5 has both an oncogenic and a tumour suppressive role in skin carcinogenesis depending on which stage of disease development it is expressed or activated. MK5 is required for oncogenic Ras-induced senescence in primary murine fibroblasts by a mechanism that requires p38 activity. MK5 stimulates the transcriptional activity of p53 leading to the increased expression of the cyclin-dependent kinase inhibitor p21. In contrast, at later stages of disease, MK5 expression in endothelial cells within the tumour microenvironment can promote cancer growth. MK5 is necessary for the migration of endothelial cells towards the tumour in response to tumour-secreted pro-angiogenic factors. At the site of the cancer, new blood vessels are created. B, Downregulation of Myc by MK5. MK5 phosphorylates the transcriptional activator FoxO3a at serine 215 leading to its nuclear accumulation. Here, FoxO3a stimulates the expression of the pre-miRNA BC021736 that is processed into miR-34b and miR-34c. miR-34b and miR-34c bind to the 3' UTR of the myc transcript and downregulate its expression at a post-transcriptional level. The MK5 promoter is itself a direct target of transcriptional activation by Myc. Therefore, by increasing the MK5 level in cells, Myc negatively regulates its own expression.

that employed a one-stage dimethylbenzanthracene (DMBA)-induced skin carcinogenesis model in mice. When combined with a phorbol ester (TPA) promoter, DMBA treatment is known to generate tumours in which more than 90% have a mutated and activated Ha-Ras oncogene (37). However, Sun *et al* found that knock-out mice lacking MK5 readily developed skin papillomas within one year after DMBA-treatment alone whereas more than 90% of treated wild-type mice remained healthy (37). Heterozygous $MK5^{+/-}$ mice were also found to be more prone to skin carcinogenesis in this model. They went on to show that MK5 is required for oncogenic Ras-induced senescence in primary murine fibroblasts by a mechanism that is dependent on p38 activity. In response to activated Ras, the p38-MK5 signaling axis stimulates the transcriptional activity of the key tumour suppressor protein p53 thereby increasing the level of the p21 protein in the cells. Interestingly, there is also strong evidence that Ser37 within the transcriptional activation domain of p53 is directly phosphorylated by MK5. Thus, Sun *et al* hypothesise that the tumour suppressor function of MK5 in this model is mediated by its ability to mediate oncogene-induced senescence by activating p53 (Figure 3A). Recently, it was reported that Tip60, a member of the MYST family of the histone acetyl transferases, is also activated by p38 upon oncogenic Ras-mediated senescence (38). Tip60 is a direct binding partner of MK5 and acetylates MK5 at Lys364. This modification seems to enhance the activity of MK5 towards Ser37 within p53.

Interestingly although MK5 acts as a tumour suppressor in the one-stage model of skin cancer described above, subsequent work showed that once a tumour had been formed it actually grew more slowly and was less likely to undergo oncogenic conversion to squamous cell carcinoma in mice lacking MK5 (39). When compared with tumours arising in wild-type animals, the skin papillomas in $MK5^{-/-}$ mice showed a complete lack of infiltrating blood vessels indicating that MK5 is required for tumour angiogenesis. This was further supported by the observation that skin papillomas from wild type mice efficiently formed tumours with a well-developed vasculature when transplanted into hosts expressing MK5. In contrast, the formation of blood vessels in tumours transplanted into MK5-deficient host animals was severely compromised and was accompanied by reduced tumour growth. This indicates that MK5 expression is needed in cells in the vicinity of the transplanted tumour in order to enhance angiogenesis and tumour growth. The formation of a tumour vasculature is dependent on the activation of endothelial cells in the tumour microenvironment by pro-angiogenic factors and their migration towards the tumour. Interestingly, primary vascular endothelial cells from MK5 null mice were unable to migrate towards pro-angiogenic factors, including VEGF, in supernatants harvested from skin papilloma cells. Upon activation of human umbilical vein endothelilal cells (HUVEC) with purified VEGF or supernatants harvested from skin papilloma cells, MK5 is indeed phosphorylated and activated in a p38-dependent fashion. This activation was

reported to be required for actin stress fiber formation, which is necessary for cell migration. A mechanism was proposed where MK5 is required for the activation of focal adhesion kinase (FAK) on focal contacts. Taken together, in skin tumour regions in mice, MK5 is activated in endothelial cells by tumour-secreted pro-angiogenic factors and this activation is necessary for their migration toward the tumour (Figure 3A).

A tumour suppressor role of MK5 has also been demonstrated in a mouse model of hematopoietic cancer overexpressing activated N-Ras (40). Deletion of MK5 in this model led to a decrease in the expression of senescence markers concomitant with hyperactivation of the JNK MAP kinase and increased cyclin D1 expression. In contrast to the murine skin model, MK5-mediated tumour suppression in this system does not seem to be dependent on p53 Ser37 phosphorylation.

Another key study indicating a link between MK5 and cancer was reported by Kress *et al*. (32) Here MK5 was identified in a human kinome siRNA screen in U2OS cells as a protein that can down-regulate the expression of the myc oncogene at the post-transcriptional level. MK5-mediated down-regulation of Myc was dependent on sequences in the 3'UTR of the myc transcript that contains binding sites for members of the miR-34 family of miRNAs. Activation of MK5 increased the expression of both miR-34b and miR-34c by stimulating the FoxO3a-dependent transcription of the pre-miRNA BC021736. It was subsequently shown that MK5 phosphorylated FoxO3a at Ser215 leading to its nuclear accumulation, increased DNA binding and thus transcriptional activity. Interestingly, the MK5 promoter itself was also shown to be a direct target of transcriptional activation by Myc. Therefore, a model of negative feedback regulation was proposed in which Myc modulates its own expression by increasing MK5 expression leading to FoxO3a-mediated transcription of the pre-miRNA for miR-34b and miR-34c (Figure 3B). Importantly, Myc and MK5 displayed reciprocal expression levels in colorectal tumours. MK5 was down-regulated in poorly differentiated adenocarcinomas (grade 3) relative to less aggressive, well and moderately differentiated samples (grade 1 and 2). In contrast, Myc was expressed at high levels in grade 3 tumours compared to normal colon epithelium.

The mTOR signaling pathway is frequently dysregulated in cancer (41-44). The mTOR kinase exerts its function in two distinct cellular complexes, mTORC1 and mTORC2. The mTORC1 complex, which in addition to mTOR consists of mLST8 and Raptor, is inhibited by the well-known immuno-suppressant and anti-cancer drug rapamycin, (43,45). The mTORC1 complex is regulated by a variety of physiological signals including nutritional and energy status, growth factors, and stresses and regulates cellular metabolism and growth (42-45). mTORC1 is activated by Rheb, a small GTPase of the Ras superfamily that binds to the complex in its GTP-bound state (46). Upon energy depletion, the mTORC1 complex is inhibited by a mechanism involving the tumour suppressing TSC1/TSC2/TBC1D7 complex (47,48). TSC2 is a GAP protein that stimulates the GTPase activity of Rheb, leaving it in an inactive GDP-bound state (49,50). Recently, it was demonstrated MK5 is necessary for the down-regulation of mTORC1 induced by certain forms of energy depletion (51). MK5-mediated down-regulation of mTORC1 was not dependent on TSC2 expression but rather involved a mechanism where MK5 phosphorylated Rheb directly at Serine 130 and reduced its ability to bind to GTP. Thus it could be envisaged that loss of MK5 or a reduced level of MK5 expression might also lead to increased mTORC1 activity and thus contribute to cancer development.

8. CONCLUSIONS

MK5 was originally discovered in 1998 in two separate screens aimed at identifying proteins sharing homology with MK2. For several years, its cellular function remained obscure as *in vitro* studies demonstrated that MK5 shared both upstream activators (p38 and ERK2) and downstream substrates (Hsp27) with its more widely studied relatives MK2 and MK3. However, the role of MK5 as a downstream target for p38 MAPK and its activity as an Hsp27 kinase is disputed. The subsequent identification of MK5 as a *bona fide* interaction partner and downstream target for the atypical MAP kinases ERK3 and ERK4 indicates that MK5 may not be regulated by the classical MAP kinase signaling pathway. However, the complete signaling pathway, which regulates the atypical MAP kinases ERK3 and ERK4, has still not been fully characterised. Some clues suggest that both MK5 and ERK3/4 may have a role in cell migration, but this has not yet been supported by any of the transgenic mouse models lacking ERK3, ERK4 or MK5. In fact, there is seemingly little phenotypic overlap between the mouse models lacking MK5, ERK3 or ERK4 generated so far. Unfortunately, mice lacking ERK3 are not viable, which limits our ability to compare mice lacking either ERK3 or MK5. Thus, conditional knock-out mouse models will be required in order to perform meaningful studies of the physiological links between ERK3 and MK5.

9. ACKNOWLEDGEMENTS

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