Regulatory Roles of MAPK Phosphatases in Cancer

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The mitogen-activated protein kinases (MAPKs) are key regulators of cell growth and survival in physiological and pathological processes. Aberrant MAPK signaling plays a critical role in the development and progression of human cancer, as well as in determining responses to cancer treatment. The MAPK phosphatases (MKPs), also known as dual-specificity phosphatases (DUSPs), are a family of proteins that function as major negative regulators of MAPK activities in mammalian cells. Studies using mice deficient in specific MKPs including MKP1/DUSP1, PAC-1/DUSP2, MKP2/DUSP4, MKP5/DUSP10 and MKP7/DUSP16 demonstrated that these molecules are important not only for both innate and adaptive immune responses, but also for metabolic homeostasis. In addition, the consequences of the gain or loss of function of the MKPs in normal and malignant tissues have highlighted the importance of these phosphatases in the pathogenesis of cancers. The involvement of the MKPs in resistance to cancer therapy has also gained prominence, making the MKPs a potential target for anti-cancer therapy. This review will summarize the current knowledge of the MKPs in cancer development, progression and treatment outcomes.

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OVERVIEW OF THE MAPKs

The evolutionarily conserved mitogen-activated protein kinase (MAPK) cascade coordinately regulate a wide array of cellular processes such as cell proliferation, differentiation, metabolism, motility, survival, apoptosis, and are therefore important for numerous physiological processes including innate and adaptive immune responses (1-3).

There are 3 main groups of MAPKs in mammalian species; the extracellular signal-regulated protein kinases (ERK1/2), the p38 MAP kinases (isoforms α, β, γ, δ) and the c-Jun NH2-terminal kinases (JNK1/2/3) (Fig. 1) (4). These MAPK pathways can be activated by various cell stimuli, including growth factors, cytokines and cellular stress, which leads to a cascade of sequential phosphorylation events, beginning with the phosphorylation of MAPK kinases (MAPKKs) on their Ser/Thr residues by MAPK kinase kinases (MAPKKKs) (5). Activated MAPKKs in turn phosphorylate MAPKs at the adjacent threonine and tyrosine residues in the conserved Thr-X-Tyr (TXY) motif within their activation loop. The amino acid denoted X corresponds to glutamic acid in ERK, proline in JNK or glycine in p38 (6-8).

MAPKs respond to a wide range of extracellular and intracellular changes. Activated MAPKs can phosphorylate/activate a wide array of downstream targets, including protein kinases and transcription factors that facilitate the
transcription of MAPK-regulated genes (3,4,9,10). The cellular response of the MAPK pathways depends on the nature of the stimulus and the duration of the signal (11). In general, the ERK pathway is mainly involved in growth, differentiation, and development, while JNK and p38 MAPK pathways are preferentially involved in inflammation, apoptosis, growth, and differentiation (10). In immune cells, the MAPK pathways are downstream targets of cell receptor signaling, working cooperatively to regulate various cellular responses such as cell proliferation, differentiation, effector function, and cell death (Fig. 1) (3,12).

MAPK SIGNALING PATHWAYS IN CANCER

Perturbations in MAPK signalling are a feature in cancer development and progression. Although the ERK1/2 pathway is commonly linked with cell growth, recent studies have implicated this pathway in other aspects of the phenotype of tumors. As such, aberrant ERK1/2 activation has been implicated in one-third of all human cancers (13). Most oncogenic activities that lead to constitutive activation of ERK1/2 signalling occur upstream of ERK1/2 activation, including overexpression of receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), activating mutations in receptor tyrosine kinases, sustained autocrine or paracrine production of activating ligands, as well as Ras mutations and B-Raf mutations, resulting in enhanced or constitutive downstream activation of the Raf-MEK-ERK pathway (9,13-15). Phosphorylation of ERK1/2 via the Ras/Mek/ERK pathway cascade induces the activation of transcription factors NF-κB, AP-1, and ETS, resulting in the induction of downstream components, c-Fos, cyclin D1, and c-Myc, which are important cell proliferation and growth regulation factors (16,17). Dysregulation of this pathway has been demonstrated in a variety of malignancies, including hepatocellular carcinoma, gastric adenocarcinoma, and renal cell carcinoma. Additionally, ERK1/2 promotes cell survival by inhibiting apoptosis in response to a wide range of stimuli, such as TNFα, Fas ligand, TRAIL, radiation, osmotic stress, hypoxia, growth factor withdrawal, nitric oxide, hydrogen peroxide, matrix detachment and chemotherapeutic agents (18).

The role of the stress activated MAPKs, including JNK and p38, in cancer is complex and in some cases controversial. Increased levels of phosphorylated p38 have been linked to various malignancies, including follicular lymphoma, lung, thyroid and breast carcinomas, as well as glioma and head and neck squamous cell carcinomas (2). In contrast, studies using mice with disrupted genes of the p38 kinases, MKK3 and MKK6, or the p38α gene demonstrated enhanced transforming potential of fibroblasts, indicating a role of the p38 MAPK pathway in tumor suppression (19-21). The p38 pathway has also been im-
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Figure 2. Inactivation of MAPKs by MKPs (adapted from [42]).

MAPK PHOSPHATASES (MKPs)

The magnitude and duration of MAPK activation determine the signaling output and are thus crucial in numerous biological processes (39). Abnormal MAPK signaling has been implicated in inappropriate cellular immune response and human malignancies. The MAPK phosphatase (MKP) or dual specificity phosphatase (DUSP) protein family has been identified as major negative regulators of MAPKs (40,41). MAPK inactivation occurs through dephosphorylation of threonine and/or tyrosine residues within the Thr-X-Tyr motif located in the MAPK activation loop (Fig. 2).

The MKPs share a common structure that comprises a C-terminal catalytic domain, which shares sequence similarity to the prototypic VH-1 DUSP encoded by vaccinia virus, and an N-terminal non-catalytic domain containing both a conserved cluster of basic amino acid residues involved in MAPK recognition (40,42,43). MKPs are highly specific for MAPKs over other kinase substrates, but vary in their substrate specificities among the members of the MAPK family and subcellular localizations.

The biological outcome of MAPK activation is dependent on the stimuli, the strength/duration of the signal and cell type/tissue specificity. For instance, transient JNK activation promotes cell survival, while prolonged JNK activation induces cellular apoptosis (38). This reflects a balance between the various upstream activators and negative regulatory mechanisms, which oppose pathway activation. The ability of the MAPK pathways to be activated by a variety of stimuli and to activate various downstream targets shows that they are important components in cell signalling and must be tightly regulated.
MKPs IN CANCER

The role of MKPs in cancer has been less well-defined than their associated MAPKs despite increasing evidence that disturbances in MKP function may play a role in cancer development. Studies have correlated loss of MKPs with cancer development or progression as a result of loss of negative regulation of MAPKs. However, gain of MKP expression has also been associated with cancer progression, drug resistance and poor patient prognosis (44).

Due to the emerging role of the MKPs in regulating cancer cell growth and death, there has been increasing interest in studies regarding the role of MKPs in cancer. This often begins with the establishment of a clinical correlation between tissue expression of a particular MKP, the activity of the corresponding MAPK substrate and effect on the disease. Following this, studies in cancer cells seek to correlate MKP expression and function to a tumor phenotype in vitro. Lastly, in vivo studies utilize mouse xenograft models to assess tumor formation and growth, or specific mouse models carrying one or more mutations or deletions of MKPs to examine specific types of cancers. However, despite this increase in MKP studies, the role of the MKPs in human cancers remains poorly defined.

ROLE OF MKP-1/DUSP1 IN CANCER

MKP-1 was the first MKP to be discovered and has been shown to be involved in various functions such as metabolic signaling (79), skeletal muscle function (80), inflammatory response (81,82) and cancer (83). The study of MKP-1 has been the most advanced in the context of cancers compared to the other MKPs. Studies have revealed that MKP-1 was over-expressed in many cancer types such as breast (48), lung (45), prostate (84), ovarian (49), pancreatic (47), and gastric adenocarcinoma (Table 1) (46). Clinical studies have also shown that MKP-1 expression is correlated with cancer progression and may be a useful prognostic marker. For instance, MKP-1 expression was found to be highly expressed in the malignant tissues of human breast cancer patients compared to non-malignant samples (85,86). The increased expression of MKP-1 correlated with reduced JNK activity, suggesting that therapies that reduce the expression or activity of MKP-1 might enable the expression of the pro-apoptotic signaling by JNK in malignant cells. In pancreatic cancer, MKP-1 mRNA and protein levels are elevated, and down-regulation of MKP-1 expression in pancreatic cancer cells resulted in reduced anchorage-dependent and independent tumor growth (47). The increased levels of MKP-1 expression were found to have an inverse correlation with both JNK activity and apoptosis markers, indicating that MKP-1 might mediate anti-apoptotic effects in pancreatic cancer via its regulation of JNK activity. In HCC, decreased MKP-1 expression in tumor cells was found to be extensively correlated with improved disease-free survival rates, and reduced serum alpha-fetoprotein levels and tumor size (50). In primary ovarian tumors, moderate to strong expression of MKP-1 was detected in 57.6% of invasive ovarian carcinomas and MKP-1 expression was a prognostic marker for shorter progression-free survival (49).

MKP-1 has also been assessed in relation to cancer therapy and resistance. In breast cancer, several other studies have indicated that MKP-1 may be an adverse prognostic factor in breast cancer patients and contributes to chemoresistance (85,87,88). Studies have shown that MKP-1 overexpression lead to increased resistance to cisplatin in human lung cancer cell lines while knockdown of MKP-1 enhanced cisplatin-mediated apoptosis in the lung (89). Additionally, a recent study reported that MKP-1 translocates to the mitochondria after cell irradiation and inhibits the pro-apoptotic activities of JNK in breast cancer cell lines (86). In colorectal cancer (CRC), it has been shown that MKP-1 overexpression was associated with resistance to cetuximab-based chemotherapy in KRAS wild-type patients with metastatic CRC, indicating a role for MKP-1 as a negative predictive biomarker of response to cetuximab (90). In HCC cell lines, chemotherapy in combination with knockdown of MKP-1 resulted in significantly greater efficacy than chemotherapy alone (91). In lung cancer cell lines, silencing (siRNA) of MKP-1 expression resulted in enhanced activation of JNK and p38 by cisplatin compared to the parental cells, and this correlated with an increase in sensitivity to cisplatin (45). Cisplatin has been shown to induce MKP-1 in lung and ovarian cancer cell lines and this occurs via an ERK-dependent pathway (92,93). The studies demonstrated that ERK is able to phosphorylate MKP-1, resulting in stabilization of the protein and resistance to ubiquitin-mediated degradation. MKP-1 overexpression also protected breast cancer cells from apoptosis triggered by doxorubicin, mechlorethamine and paclitaxel.
Table I. Summary of effects of MKPs in human cancer

| Gene/MKP | Alterations/clinical outcome |
|----------|-----------------------------|
| DUSP1/MKP1 | - Up-regulated in NSCL (non-small cell lung) (45), gastric- (46), pancreatic- (47), and breast-carcinomas (48)  
- Expression is inversely proportional to tumor grade in colon-, prostate-, bladder-, breast- and hepatocellular-carcinoma (44)  
- Up-regulated in ovarian-carcinomas. Expression correlated with shorter progression free survival (49)  
- High expression correlated with poor prognosis in breast carcinomas (48)  
- In hepatocellular carcinomas, expression correlated with poorer disease-free survival and survival rates (50) |
| DUSP2/PAC1 | - High expression in serous ovarian carcinoma; down-regulated after chemotherapy. High expression correlated with poorer overall survival (51).  
- Down-regulated in acute leukemia (52) |
| DUSP4/MKP2 | - Up-regulated in liver- (53), breast- (48), and in pancreatic cancer cell lines (54)  
- In lung adenocarcinoma, gene deletion correlated with better overall survival (56) |
| DUSP5 | - Over-expressed in dysplastic tissue and pancreatic carcinoma in situ (60)  
- Down-regulated in invasive pancreatic carcinoma (61) and in high-grade lung carcinomas (62)  
- Down-regulated in keratinocytes and breast cancer cells by the tumor promoter palytoxin (63) and in ovarian cancer cells by oxidative stress (64)  
- Up-regulated in HER2 + breast cancers (65) and in breast tumors resistant to tamoxifen therapy (66)  
- Down-regulated in oesophageal and nasopharyngeal carcinomas (NPC) (67) |
| DUSP6/MKP3 | - Elevated levels in acute myeloid leukaemia and acute lymphoblastic leukemia (68) |
| DUSP7/MKP-X | - Up-regulated by calcitriol (70), anti-inflammatory phytochemicals (71) and Vitamin D (72) in prostate cancer cells  
- In colorectal carcinoma (73) |
| DUSP9/MKP4 | - Down-regulated in skin squamous cell carcinoma (SCC) (69) |
| DUSP10/MKP5 | - Frequent deletions in leukemia (74), NSCL cancer (75) and prostate carcinoma (76) |
| DUSP16/MKP7 | - Down-regulated by miR-24 in AML (77)  
- Down-regulated in Burkitt’s lymphoma through hypermethylation (78) |

(94). Importantly, these studies show that MKP-1 conferred resistance to these chemotherapeutic agents by inhibiting JNK pathway (79,89,94). Furthermore, knock-down of MKP-1 in breast cancer cell lines was able to promote tamoxifen-induced cell death via JNK (95). Interestingly, another DNA damaging agent, camptothecin, was found to induce apoptosis in the HCT116 CRC cell line by down-regulating MKP-1 expression and a reciprocal prolonged induction of ERK activation in the nucleus (96). This suggests that camptothecin treatment promotes antitumor effects by inhibition of MKP-1 activity on the ERK pathway. Together, these studies provide evidence of MKP-1 in mediating resistance to chemotherapy by regulating the MAPK pathways. However, the specific MAPK pathway regulated by MKP-1 in conferring chemo-resistance seems to be dependent on cell/tissue type and chemotherapeutic agent used. Overall, these findings suggest that MKP-1 may play a critical role in cancer development, may be a useful marker for predicting the survival of cancer patients and may be significant in determining treatment outcomes.

ROLE OF MKP-2/DUSP4 IN CANCER

Like MKP-1/DUSP1, MKP-2/DUSP4 is also predominantly localized in the nucleus (97). However, its involvement in cancer is less well-known compared to MKP-1. Genetic studies have provided evidence of increased mRNA levels of MKP-2 in hepatomas (53), pancreatic cancers (54), familial modulatory thyroid carcinoma (FMTC), multiple endocrine neoplasia (MEN), and papillary thyroid carcinoma (98). Moreover, MKP-2 expression was increased 3-fold in human breast cancer samples as compared to non-malign-
nant samples where it appears to be co-expressed with MKP-1 (48). In pancreatic cancer cell lines harboring activating mutations in K-Ras, MEK-dependent over-expression of MKP-2 has been reported (54). There has also been evidence of MKP-2 over-expression in benign serous borderline tumors (SBT) in the ovary (44). The expression levels of MKP-2 were found to be higher in SBT compared with serous carcinomas (SCAs) and it has been implied that MKP-2-mediated suppression of ERK-dependent events associated with degradation of the extracellular matrix may be responsible for the more benign behavior of SBT (55). A recent study by Gröschl et al., discovered that MKP-2 was highly expressed in CRC with high frequent microsatellite instability (MSI-H) compared to microsatellite stable (MSS) CRC (99). Additionally, overexpression of MKP-2 in CRC cell lines led to increased proliferation, possibly by stimulating growth promoting genes such as MYC and cyclin D1 (99). MKP-2 was also found to be highly expressed in intestinal epithelial cells expressing oncogenic forms of KRAS and BRAF, and in KRAS and BRAF mutant CRC cells, with corresponding nuclear ERK inhibition (100). It is possible that sustained nuclear ERK activation is unfavourable for the cancer cells and thus the nuclear ERK phosphatases may be an alternative target in treating CRC displaying aberrant KRAS/BRAF/ERK signalling. In line with this, inhibition of MKP-2 attenuated the in vitro and in vivo proliferation of mammary tumor cells derived from a RET-MEN2A transgenic mouse by suppressing cyclin B1 expression, and leading to dysregulation of the cell cycle (98).

In contrast, several other studies have implicated MKP-2 as a negative regulator of cancer. Screening studies have suggested that MKP-2 may be a tumor suppressor/cancer susceptibility gene linked to chromosome 8p, which is frequently mutated in breast and prostate cancers (57). Low levels of MKP-2 expression also correlated with higher post neo-adjuvant chemotherapy cancer cell proliferation and a reduction in recurrence-free survival in breast cancer patients (101). Overexpression of MKP-2 in breast cancer cell lines reduced ERK activation and increased cell killing by docetaxel (101). In addition, EGFR-mutations have associated with loss of MKP-2 within the 8p locus in lung cancer (56). This is supported by another study that observed high rates of loss of heterozygosity (LOH) at markers adjacent to or within MKP-2 in breast cancer cell lines, supporting their status as candidate tumor suppressor genes (102). Furthermore, MKP-2 was found to be a common epigenetically silenced gene in glioma and overexpression of MKP-2 inhibited glioblastoma cell growth (58). More recently, MKP-2 was found to be induced by tamoxifen in breast cancer (MCF-7) cells and MKP-2 overexpressing MCF-7 cells have increased sensitivity to tamoxifen treatment by inhibiting ERK-mediated proliferation and tamoxifen resistance (103). Interestingly, expression of MKP-2 was reported to rescue 293T cells from apoptosis upon UV or cisplatin exposure by negative regulation of JNK activation but not through regulation of ERK (104). These findings indicate that MKP-2 may have both positive and negative regulatory roles in cancer development although the mechanisms are still poorly understood.

ROLE OF MKP-3/DUSP6 IN CANCER

MKP-3/DUSP6 is the best characterized of the cytoplasmic phosphatases and specifically targets ERK1/2 over the stress-related MAPKs (105). A tumor suppressive function of MKP-3 which is mainly through negative regulation of ERK has been supported by studies on various cancers including pancreatic cancer, lung cancer, nasopharyngeal carcinoma (NPC), breast cancer as well as head and neck cancer. For instance, Mkp-3 is localized on chromosome 12q21, one of the regions of frequent allelic loss in pancreatic cancer cell lines, suggesting that this molecule may play a role in cancer pathogenesis (61). In a majority of these cell lines, reduced levels of MKP-3 was accompanied by activating mutations in KRAS (61). MKP-3 expression was slightly upregulated in primary pancreatic cancer tissues and dysplastic in situ carcinoma cells, but down-regulated in invasive carcinoma, particularly in the poorly differentiated type (60). The loss of MKP-3 and gain-of-function mutations of KRAS may synergistically lead to activation of the ERK1/2 pathway, thus contributing to the development of the invasive phenotype (60). MKP-3 was also observed to be induced in myeloma cells harbouring a constitutively active mutant NRAS gene, and in human melanoma cell lines with activating mutations in BRAF (106-108). These studies suggest that the expression of MKP-3 in response to activated RAS or RAF might serve as a negative feedback to the RAS/RAF/MEK/ERK1/2 pathway.

In primary lung cancers, decreased MKP-3 expression was associated with increase in growth activity and histo-
logical grade of tumors. MKP-3 expression levels were also found to be weaker in most lung cancer cell lines compared to immortalized airway epithelial cells, and forced expression of MKP-3 was able to suppress the growth of these cancer cell lines (62). In support of this, siRNA knockdown in high-MKP-3-expressing lung cancer cells showed enhanced ERK signaling and cellular proliferation, whereas MKP-3 overexpression in low MKP-3-expressing lung cancer cells resulted in attenuated ERK activation, growth arrest and apoptosis (109).

In esophageal squamous cell carcinoma (ESCC) and NPC, MKP-3 was found to be down-regulated in 40% of ESCC biopsies and in 75% of NPC biopsies compared to their normal counterparts (67). The study also showed increased ERK activity in ESCC and NPC cell lines expressing low levels of MKP-3 compared to immortalized esophageal and nasopharyngeal epithelial cell lines, indicating negative regulation of ERK by MKP-3. On the other hand, MKP-3 overexpression in ESCC and NPC cell lines resulted in reduced cancer cell proliferation and migration/invasion in vitro, as well as reduced tumor growth in vivo (67). The tumor suppressive function of MKP-3 in ESCC was further supported by a recent study showing that MKP-3 expression was negatively correlated to pathological grade and that exogenous MKP-3 expression in ESCC cell lines markedly increased apoptosis in vitro (110).

Interestingly, MKP-3 has been shown to be a ΔNp63 α-targeted gene and important for ΔNp63 α-mediated inhibition of cancer invasiveness and metastasis via inhibition of ERK (111). Knockdown of ΔNp63 α resulted in reduced MKP-3 expression, increased ERK1/2 activation and increased cell migration and invasion which can be rescued by forced expression of MKP-3. In addition, increased expression of Rab2A, a small GTPase critical for vesicle trafficking, has been shown to be correlated with poor clinical outcomes in human breast cancer patients (112). It was found that Rab2A binds to ERK to protect active ERK from dephosphorylation by MKP-3. This in turn promotes the nuclear translocation of β-catenin and tumorigenesis of BCSCs in human primary breast cancer (112).

Furthermore, studies have suggested that MKP-3/DUSP6 is involved in chemoresistance via regulation of ERK activation. In ovarian cancer, forced expression of MKP-3 or treatment with an ERK inhibitor enhanced cisplatin sensitivity, whereas MKP-3 knockdown resulted in reduced cisplatin-induced cell death (64). In melanoma cells, MKP-3 overexpression prevented ERK-mediated induction of nucleotide excision repair proteins, ERCC1 and XPF, by cisplatin, resulting in increased sensitivity to cisplatin (113). In contrast, MKP-3 overexpression promoted resistance to cisplatin-mediated cell death in in vitro and in vivo models of glioblastoma, indicating that MKP-3 may function as a tumor promoter in these cancers (114). Since ERK activation has been shown to be responsible for mediating apoptosis in glioblastomas (115), this correlates with the chemo-protective function of MKP-3 in this cancer. This provides evidence that the role of MKP-3 is dependent on the function of ERK in triggering apoptosis or promoting resistance in response to DNA damaging agents.

**ROLE OF MKP5/DUSP10 IN CANCER**

MKP5 was originally identified as a phosphatase capable of regulating JNK and p38 MAPks, but not ERK1/2, when overexpressed in cell lines (116,117). However, studies using MKP5 deficient mice from our group demonstrated that this protein is able to dephosphorylate all the three groups of MAPks as well as IRF3 in a cell/tissue and context-dependent manner (118). By regulating JNK in immune cells such as T cells, MKP5 plays a significant role in both innate and adaptive immune responses (119). In neutrophils, MKP5 selectively inhibits p38 activation and suppresses the expression of inflammatory mediators such as superoxide (120). In adipose tissue, increased p38 activation was detected in aged MKP5 deficient mice, which is correlated with the development of visceral obesity and adipose tissue inflammation in the mice (121). In intestinal epithelial cells, MKP5 was found to suppress ERK activation and cell proliferation (122). Moreover, this protein was found to directly interact and dephosphorylate IRF3 in macrophages in response to RNA virus infection, thereby inhibiting type I interferon responses (118). These studies demonstrate the important role of this molecule in various physiological and pathophysiological conditions.

MKP5 is emerging as an important player in cancer as well. In the HCC cell line HepG2, environmental carcinogens upregulate microRNA(miR)-181 to enhance p38 activation by targeting MKP5 (123). Inhibition of miRNA-181 led to enhanced expression of MKP5 and a corresponding suppression of p38 activation, which in turn
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reduced cancer cell migration. In pancreatic cancer cell lines, miRNA-92a targets MKP5 to promote JNK activation, thereby enhancing cell proliferation (124). These studies suggest that MKP5 is possibly a tumor suppressor in HCC and pancreatic cancer by inhibiting p38 or JNK.

Nomura and colleagues reported that MKP5 was frequently upregulated in CRC but not in lung carcinoma, breast cancer or glioblastomas (73). Intriguingly, they found that MKP5 functions as a scaffold protein and cytoplasmic anchor for ERK, preventing nuclear translocation of phospho-ERK, thereby inhibiting ERK-dependent transcription and cancer progression. Our study on MKP5 in intestinal epithelial cells and tumorigenesis demonstrated that this protein suppresses intestinal epithelial cell (IEC) wound healing and inhibits tumorigenesis by suppressing ERK-mediated signaling (122). We showed that loss of MKP5 in mice resulted in increased cell proliferation and wound healing due to upregulation of ERK activation and KLF5 expression. While these factors are important in intestinal healing and barrier function maintenance after injury, unregulated cell proliferation may result in cancer development. Indeed, MKP5 knockout mice developed more colon tumors with increased severity associated with increased intestinal epithelial cell proliferation and migration in response to dextran sodium sulphate (DSS)/azoxymethane (AOM) treatment compared to WT mice. In addition, overexpression of MKP5 in human CRC cells resulted in reduced tumor formation in immune deficient mice. Furthermore, high MKP5 expression is associated with better survival in CRC patients (122). These findings established MKP5 as a tumor suppressor in CRC, and a potential target for the development of therapeutic methods to improve CRC patient outcomes.

ROLE OF MKP7/DUSP16 IN CANCER

MKP7 (DUSP16) was identified as a phosphatase specific for p38 and JNK in vitro (125) although some groups have reported MKP7 to be specific only for JNK (126). However, using MKP7 deficient cells, we demonstrated that this protein inactivates ERK and JNK in T cells in response to T cell receptor stimulation to specifically regulate Th17 response (127). In addition to its function in immunity, a regulatory role of MKP7 in cancer has been implicated. For instance, studies have suggested that MKP7 may be downregulated in cancers by genetic or other mechanisms. In this respect, it is worth noting that the MKP7 gene is located on chromosome 12p, an area of frequent chromosomal loss in human tumors such as leukemia (74), NSCL cancer (75) and prostate carcinoma (76). Furthermore, MKP7 overexpression, through inactivation of the JNK pathway, has been shown to reduce Rat-1 cell growth and suppress transformation capabilities of BCR-ABL oncogene transformed cells in vitro and in vivo (74). Downregulation of MKP7 by miR-24, whose expression is induced by compromised RUNX function, has also been linked to development of acute myeloid leukemia (77). The reduction of MKP7 expression led to increased JNK and p38 phosphorylation, which in turn stimulated myeloid cell growth and inhibited differentiation. There was also evidence showing that MKP7 may regulate sensitivity of cancer cells to anti-cancer therapies. For instance, methylation-dependent transcriptional silencing of MKP7 in Burkitt’s lymphoma cells, but not other B cell lymphomas, abrogated the negative regulation of JNK activity to enhance the sensitivity of cells to chemotherapeutic agents that activate JNK, such as doxorubicin, sorbitol and cisplatin (78). Moreover, a study demonstrated that a novel anticancer agent, NSC-741909, induced ROS-mediated inactivation of MKP7 in NSCL cancer cell lines to activate the JNK signaling pathway and apoptosis (128).

OTHER MKPs IN CANCER

Several other MKPs, including PAC-1/DUSP2, MKP4/DUSP9, and MKP-X/DUSP7, have also been implicated in the development of cancers. For example, in ovarian carcinoma, high levels of PAC1 mRNA predicted significantly worse overall survival as compared to low expression (51). Interestingly, in acute leukaemia, loss of DUSP2/PAC-1 expression has also been associated with constitutive ERK activation (52), suggesting that PAC-1 may function as a tumor suppressor. For MKP4/DUSP9, a tumor suppressive function has been implicated. It was reported that loss of MKP4 was associated with squamous cell carcinoma (SCC) but not in benign papillomagenic lineages (69). Overexpression of MKP4 resulted in tumor cells microtubule disruption and cell death. Finally, MKP-X/DUSP7 has been found to be constitutively highly expressed in bone marrow and peripheral leukocytes from acute myeloid leukaemia (AML) and acute lymphoblastic leukemia (ALL) patients, whereas very low levels of
MKP-X was detected in the bone marrow of healthy patients (68), suggesting the possible involvement of this molecule in the pathogenesis of leukaemia.

TARGETING MKPs FOR CANCER TREATMENT

Aberrant expression of various MKPs/DUSPs has been observed in human cancers. However, whether overexpression or loss of expression of the MKPs contributes to malignant phenotype depends on the specific MKP and cancer/tissue type. The use of genetic knockout mouse models for individual MKPs allows for the study of whether gain/loss of expression is associated with the development and progression of cancers. These studies also help in investigating the roles of MKPs in mediating the sensitivity of tumors to chemical and physical agents used in both conventional and novel cancer therapies. For example, MKP-1, MKP-3 and MKP7 have been linked to resistance to chemotherapeutic treatment thus providing possible targets for the design of new therapies.

Therefore studies have been done to identify phosphatase inhibitors specific for individual MKPs which may have anti-tumor properties. Sanguinarine was identified as a potent and selective inhibitor of MKP-1 in HeLa and PANC-1 cancer cell lines (129). Along with its analogue, chelerythrine, sanguinarine was able to inhibit MKP-1 \textit{in vitro} and enhanced phosphorylation of ERK and JNK (129). Thus sanguinarine and its analogs may serve as novel MKP-1 inhibitors in the treatment of human cancers. Another MKP-1 inhibitor, triptolide, has been shown to enhance cisplatin-mediated apoptosis in osteosarcoma (130) and ovarian cancer cells (93). A group of uracil quinolones have also been identified by Arnold et al., from a chemical library of MKP-1 inhibitors which may be useful in studying the role of MKP-1 in cancers (131). Two of the pyrrole carboxamides, PSI2106 and MDF2085 have also been identified as selective inhibitors of MKP-1 but their role in cancer therapy has not been examined (132). In addition, using a high-content, fluorescence-based chemical complementation assay for MKP activity, Vogt and Lazo identified NSC95397, a compound which inhibited both MKP-1 and MKP-3 \textit{in vitro}, and restored paclitaxel sensitivity in breast cancer cells exposed to dexamethasone (133). Thus the use of specific inhibitors targeting the MKPs may provide novel and specific therapies against cancers.

CONCLUSION

The MAPK protein family plays a diverse and at times contradictory role in the development of human cancers. As negative regulators of the MAPKs, the MKPs/DUSPs thus play important roles in cellular responses that are integral to cancer development and progression. Studies have suggested that the MKPs are potential biomarkers in cancers and MKP expression is often correlated with patient prognosis and chemotherapeutic sensitivity. However, the correlation between an MKP and a tumor phenotype may vary depending on the type of cancer. Further studies have to be conducted to discern the expression levels of the MKPs and how they affect the downstream signaling pathways and responses in human cancers. The understanding of how abnormalities in MKP expression and the regulation of the MAPK pathways by these phosphatases may thus be critical to developing more effective therapies against cancer.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

REFERENCES

1. Dickinson, R. J., and S. M. Keyse. 2006. Diverse physiological functions for dual-specificity MAP kinase phosphatases. \textit{J. Cell Sci.} 119: 4607-4615.
2. Wagner, E. F., and A. R. Nebreda. 2009. Signal integration by JNK and p38 MAPK pathways in cancer development. \textit{Nat. Rev. Cancer} 9: 537-549.
3. Zhang, Y. L., and C. Dong. 2005. MAP kinases in immune responses. \textit{Cell. Mol. Immunol.} 2: 20-27.
4. Chen, Z., T. B. Gibson, F. Robinson, L. Silvestro, G. Pearson, B. Xu, A. Wright, C. Vanderbilt, and M. H. Cobb. 2001. MAP kinases. \textit{Chem. Rev.} 101: 2449-2476.
5. Cargnello, M., and P. P. Roux. 2011. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. \textit{Microbiol. Mol. Biol. Rev.} 75: 50-83.
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and M. R. Rosner. 2004. ERK7 expression and kinase activity is regulated by the ubiquitin-proteasome pathway. J. Biol. Chem. 279: 23073-23081.

7. Kyriakis, J. M., P. Banerjee, E. Nikolakaki, T. Dai, E. A. Rubie, M. F. Ahmad, J. Avruch, and J. R. Woodgett. 1994. The stress-activated protein kinase subfamily of c-Jun kinases. Nature 369: 156-160.

8. Lechnor, C., M. A. Zahalka, J. F. Giot, N. P. Moller, and A. Ulrich. 1996. ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation. Proc. Natl. Acad. Sci. U. S. A. 93: 4355-4359.

9. Dhillon, A. S., S. Hagan, O. Rath, and W. Kolch. 2007. MAP kinase signalling pathways in cancer. Oncogene 26: 3279-3290.

10. Wada, T., and J. M. Penninger. 2004. Mitogen-activated protein kinases in apoptosis regulation. Oncogene 23: 2838-2849.

11. Engelberg, D. 2004. Stress-activated protein kinases - tumor suppressors or tumor initiators? Semin. Cancer Biol. 14: 271-282.

12. Liu, Y., E. G. Shepherd, and L. D. Nelin. 2007. MAPK phosphorylation-regulating the immune response. Nat. Rev. Immunol. 7: 202-212.

13. Schubbert, S., K. Shannon, and G. Bollag. 2007. Hyperactive Ras in developmental disorders and cancer. Nat. Rev. Cancer 7: 295-308.

14. Goffin, J. R., and K. Zhuk. 2013. Epidermal growth factor receptor: pathway, therapies, and pipeline. Clin. Ther. 35: 1282-1303.

15. Normanno, N., L. A. De, C. Bianco, L. Strizzi, M. Mancino, M. R. Maiello, A. Carotenuto, F. G. De, F. Caponigro, and D. S. Salomon. 2006. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene 366: 2-16.

16. Bancroft, C. C., Z. Chen, G. Dong, J. B. Sunwoo, N. Yeh, C. Park, and W. C. Van. 2001. Coexpression of proangiogenic factors IL-8 and VEGF by human head and neck squamous cell carcinoma involves coactivation by MEK-MAPK, and IKK-NF-κB signal pathways. Clin Cancer Res. 7: 435-442.

17. Treinies, I., H. F. Paterson, S. Hooper, R. Wilson, and C. J. Marshall. 1999. Activated MEK stimulates expression of AP-1 components independently of phosphatidylinositol 3-kinase (PI3-kinase) but requires a PI3-kinase signal to stimulate DNA synthesis. Mol. Cell. Biol. 19: 321-329.

18. Lu, Z., and S. Xu. 2006. ERK1/2 MAP kinases in cell survival and apoptosis. IUBMB Life 58: 621-631.

19. Branco, D., N. Tanaka, A. Jaeschke, J. J. Ventura, N. Kelkar, Y. Tanaka, M. Kyuuma, T. Takeshita, R. A. Flavell, and R. J. Davis. 2003. Mechanism of p38 MAP kinase activation in vivo. Genes Dev. 17: 1969-1978.

20. Bulavin, D. V., C. Phillips, B. Nannenga, O. Timofeev, L. A. Donehower, C. W. Anderson, E. Appella, and A. J. Fornace, Jr. 2004. Inactivation of the Wip1 phosphatase inhibits mammalian tumorigenesis through p38 MAPK-mediated activation of the p16(Ink4a)-p19(Arf) pathway. Nat. Genet. 36: 343-350.

21. Timofeev, O., T. Y. Lee, and D. V. Bulavin. 2005. A subtle change in p38 MAP kinase activity is sufficient to suppress in vivo tumorigenesis. Cell Cycle 4: 118-120.

22. Han, J., and P. Sun. 2007. The pathways to tumor suppression via route p38. Trends Biochem. Sci. 32: 364-371.

23. Ventura, J. J., S. Tenbaum, E. Perdiguerio, M. Huth, C. Guerra, M. Barbacid, M. Pasparakis, and A. R. Nebreda. 2007. p38α MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. Nat. Genet. 39: 750-758.

24. Cellurale, C., G. Sabio, N. J. Kennedy, M. Das, M. Barlow, P. Sandy, T. Jacks, and R. J. Davis. 2011. Requirement of c-Jun NH(2)-terminal kinase for Ras-initiated tumor formation. Mol. Cell. Biol. 31: 1565-1576.

25. Chang, Q., Y. Zhang, K. J. Beczhold, D. Bhatia, H. Zhao, J. Chen, V. Castranova, X. Shi, and F. Chen. 2009. Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. J. Hepatol. 50: 323-333.

26. Hui, L., K. Zatloukal, H. Scheuch, E. Stepien, and E. F. Wagner. 2008. Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 downregulation. J. Clin. Invest. 118: 3943-3953.

27. Kennedy, N. J., and R. J. Davis. 2003. Role of JNK in tumor development. Cell Cycle 2: 199-201.

28. Derijard, B., M. Hibi, I. H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R. J. Davis. 1994. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell 76: 1025-1037.

29. Johnson, R., B. Spiegelman, D. Hanahan, and R. Wisdom. 1996. Cellular transformation and malignancy induced by ras require c-jun. Mol. Cell. Biol. 16: 4504-4511.

30. Sakurai, T., S. Maeda, L. Chang, and M. Karin. 2006. Loss of hepatic NF-kappa B activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. Proc. Natl. Acad. Sci. U. S. A. 103: 10544-10551.

31. Chen, N., M. Nomura, Q. B. She, W. Y. Ma, A. M. Bode, L. Wang, R. A. Flavell, and Z. Dong. 2001. Suppression of skin tumorigenesis in c-Jun NH(2)-terminal kinase-2-deficient mice. Cancer Res. 61: 3908-3912.

32. She, Q. B., N. Chen, A. M. Bode, R. A. Flavell, and Z. Dong. 2002. Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res. 62: 1343-1348.

33. Tournier, C., P. Hess, D. D. Yang, J. Xu, T. K. Turner, A. Nimnuan, D. Bar-Sagi, S. N. Jones, R. A. Flavell, and R. J. Davis. 2000. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Science 288: 870-874.

34. Wang, Y., Q. Y. He, S. W. Tsao, Y. H. Cheung, A. Wong, and J. F. Chu. 2008. Cytokertin 8 silencing in human nasopharyngeal carcinoma cells leads to cisplatin sensitization. Cancer Lett. 265: 188-196.

35. Picco, V., and G. Pages. 2013. Linking JNK Activity to the DNA Damage Response. Genes Cancer 4: 360-368.

36. Sun, T., D. Li, J. Wang, L. Xia, J. Ma, Z. Guan, G. Feng, and X. Zhu. 2011. c-Jun NH2-terminal kinase activation is essential for up-regulation of LC3 during ceramide-induced autophagy in human nasopharyngeal carcinoma cells. J. Transl. Med. 9: 161.

37. Xu, P., M. Das, J. Reilly, and R. J. Davis. 2011. JNK regulates FoxO-dependent autophagy in neurons. Genes Dev. 25: 310-322.

38. Ventura, J. J., A. Hubner, C. Zhang, R. A. Flavell, K. M. Shokat, and R. J. Davis. 2006. Chemical genetic analysis of the time course of signal transduction by JNK. Mol. Cell 21: 701-710.

39. Ebisu, M., K. Kondoh, and E. Nishida. 2005. The duration, magnitude and compartmentalization of ERK MAP kinase activ...
Keyse, S. M. 2008. Dual-specificity MAP kinase phosphatases.

Owens, D. M., and S. M. Keyse. 2007. Differential regulation of MAP kinase phosphatases. Cell. Signal. 16: 769-779.

Kondoh, K., and E. Nishida. 2007. Regulation of MAP kinases by MAP kinase phosphatases. Biochem. Biophys. Acta 1773: 1227-1237.

Owens, D. M., and S. M. Keyse. 2007. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene 26: 3203-3213.

Keyse, S. M. 2008. Dual-specificity MAP kinase phosphatases (MKPs) and cancer. Cancer Metastasis Rev. 27: 253-261.

Vicent, S., M. Garayoa, J. M. Lopez-Picazo, M. D. Lozano, G. Toledo, F. B. Thunnissen, R. G. Manzano, and L. M. Montuenga. 2004. Mitogen-activated protein kinase phosphatase-1 is overexpressed in non-small cell lung cancer and is an independent predictor of outcome in patients. Clin. Cancer Res. 10: 3639-3649.

Bang, Y. J., J. H. Kwon, S. H. Kang, J. W. Kim, and Y. C. Yang. 1998. Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. Biochem. Biophys. Res. Commun. 250: 43-47.

Liao, Q., J. Guo, J. Kleeff, A. Zimmermann, M. W. Buchler, M. Korc, and H. Fries. 2003. Down-regulation of the dual-specificity phosphatase MKP-1 suppresses tumorigenicity of pancreatic cancer cells. Gastroenterology 124: 1830-1845.

Wang, H. Y., Z. Cheng, and C. C. Malbon. 2003. Overexpression of mitogen-activated protein kinase phosphatases MKP1, MKP2 in human breast cancer. Cancer Lett. 191: 229-237.

Denkert, C., W. D. Schmitt, S. Berger, A. Reles, S. Pest, A. Siegert, W. Lichtenegger, M. Dietel, and S. Hauptmann. 2002. Expression of mitogen-activated protein kinase phosphatase-1 (MKP-1) in primary human ovarian carcinoma. Int. J. Cancer 102: 507-513.

Tsujita, E., A. Taketomi, T. Gion, Y. Kuroda, K. Endo, A. Watanabe, H. Nakashima, S. Aishima, S. Kohnoe, and Y. Maehara. 2005. Suppressed MKP-1 is an independent predictor of outcome in patients with hepatocellular carcinoma. Oncology 69: 342-347.

Givant-Horwitz, V., B. Davidson, J. M. Goderstad, J. M. Nesland, C. G. Trope, and R. Reich. 2004. The PAC-1 dual-specificity phosphatase predicts poor outcome in serous ovarian carcinoma. Gynecol. Oncol. 93: 517-523.

Kim, S. C., J. S. Hahn, Y. H. Min, N. C. Yoo, Y. W. Ko, and W. J. Lee. 1999. Constitutive activation of extracellular signal-regulated kinase in human acute leukemias; combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. Blood 93: 3893-3899.

Yokoyama, A., H. Karasuki, N. Urushibara, K. Nomoto, Y. Imai, K. Nakamura, Y. Mizuno, K. Ogawa, and K. Kikuchi. 1997. The characteristic gene expressions of MAPK phosphatases 1 and 2 in hepatocarcinogenesis, rat ascites hepatoma cells, and regenerating rat liver. Biochem. Biophys. Res. Commun. 239: 746-751.

Yip-Schneider, M. T., A. Lin, and M. S. Marshall. 2001. Pancreatic tumor cells with mutant K-ras suppress ERK activity by MEK-dependent induction of MAP kinase phosphatase-2. Biochem. Biophys. Res. Commun. 280: 992-997.

Sieben, N. L., J. Oosting, A. M. Flanagan, J. Prat, G. M. Roemen, S. M. Kolkman-Uljee, E. R. van, C. J. Cornelisse, G. J. Freulen, and E. M. van. 2005. Differential gene expression in ovarian tumors reveals Dusp 4 and Serpina 5 as key regulators for benign behavior of serous borderline tumors. J. Clin. Oncol. 23: 7257-7264.

Chitale, D., Y. Gong, B. S. Taylor, S. Broderick, C. Brennan, R. Somwar, B. Golas, L. Wang, N. Motoi, J. Zsako, J. Meiners, M. Major, C. Sander, V. E. Seshan, M. F. Zakowski, V. Rusch, W. Pao, W. Gerald, and M. Ladanyi. 2009. An integrated genomic analysis of lung cancer reveals loss of DUSP4 in EGFR-mutant tumors. Oncogene 28: 2773-2783.

Armes, J. E., F. Hammet, S. M. de, J. Ciciulla, S. J. Rannus, W. K. Soo, A. Mahoney, N. Yarovenaya, M. A. Henderson, K. Gish, A. M. Hutchins, G. R. Price, and D. J. Venter. 2004. Candidate tumor-suppressor genes on chromosome arm 8p in early-onset and high-grade breast cancers. Oncogene 23: 5697-5702.

Waha, A., J. Felsberg, W. Hartmann, K. A. von dem, M. Mikelsa, S. Joos, M. Wolter, A. Koch, P. S. Yan, E. Endl, O. D. Wiestler, G. Reifenberger, T. Pietsch, and A. Waha. 2010. Epigenetic downregulation of mitogen-activated protein kinase phosphatase-2 relieves its growth suppressive activity in glioma cells. Cancer Res. 70: 1689-1699.

Staeger, M. S., K. Muller, S. Kewitz, I. Vollkom, C. Mauz-Korholz, T. Bernig, and D. Korholz. 2014. Expression of dual-specificity phosphatase 5 pseudogene 1 (DUSP5P1) in tumor cells. PLoS One 9: e89577.

Furukawa, T., M. Sanamura, F. Motoi, S. Matsuno, and A. Horii. 2003. Potential tumor suppressive pathway involving DUSP6/MKP-3 in pancreatic cancer. Am. J. Pathol. 162: 1807-1815.

Furukawa, T., T. Yatsuka, E. M. Youssef, T. Abe, T. Yokoyama, S. Fukushima, T. Soeda, M. Hoshii, Y. Hayashi, M. Sanamura, M. Kobari, and A. Horii. 1998. Genomic analysis of DUSP6, a dual specificity MAP kinase phosphatase 2 relieves its growth suppressive activity in glioma cells. Cancer Res. 70: 1689-1699.

Okuda, K., T. Yazawa, T. Woo, M. Sakaeeda, J. Ishii, H. Mitsuji, H. Shimoyama, H. Sato, M. Taji, N. Ogawa, M. Masuda, T. Takahashi, H. Sugimura, and H. Kitamura. 2009. Down-regulation of DUSP6 expression in lung cancer: its mechanism and potential role in carcinogenesis. Am. J. Pathol. 175: 867-881.

Warmka, J. K., L. J. Maaro, and E. V. Wattenberg. 2004. Mitogen-activated protein kinase phosphatase-3 is a tumor promoter target in initiated cells that express oncopgenic Ras. J. Biol. Chem. 279: 33085-33092.

Chan, D. W., V. W. Liu, G. S. Tsao, K. M. Yao, T. Furukawa, K. K. Chan, and H. Y. Ngan. 2008. Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. Cancer Metastasis Rev. 27: 7257-7264.

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66. Cui, Y., I. Parra, M. Zhang, S. G. Hilsenbeck, A. Tsimelzon, T. Furukawa, A. Horii, Z. Y. Zhang, R. I. Nicholson, and S. A. Fuqua. 2006. Elevated expression of mitogen-activated protein kinase phosphatase 3 in breast tumors: a mechanism of tamoxifen resistance. *Cancer Res.* 66: 5990-5995.

67. Wong, V. C., H. Chen, J. M. Ko, K. W. Chan, Y. P. Chan, S. Law, D. Chua, D. L. Kwong, H. L. Lung, G. Srivastava, J. C. Tang, S. W. Tsao, E. R. Zabarovsky, E. J. Stanbridge, and M. L. Lung. 2012. Tumor suppressor dual-specificity phosphatase 6 (DUSP6) impairs cell invasion and epithelial-mesenchymal transition (EMT)-associated phenotype. *Int. J. Cancer* 130: 83-95.

68. Levy-Nissenbaum, O., O. Sagi-Assif, D. Kapon, S. Hantisteanu, O. Nonn, L., D. Duong, and D. M. Peehl. 2007. Chemopreventive and anti-inflammatory activities of curcumin and other phytocompounds mediated by MAP kinase phosphatase-5 in prostate cells. *Cancer Res.* 67: 2644-2648.

69. Liu, Y., J. Lagowski, A. Sundholm, A. Sundberg, and M. Kulesz-Martín. 2007. Microtubule disruption and tumor suppression by mitogen-activated protein kinase phosphatase 4. *Cancer Res.* 67: 1071-1079.

70. Krishnam, A. V., J. Moreno, L. Nonn, S. Swami, D. M. Peehl, and D. Feldman. 2007. Calcitriol as a chemopreventive and therapeutic agent in prostate cancer: role of anti-inflammatory activity. *J. Bone Miner. Res.* 22 Suppl. 2: V74-V80.

71. Noun, L., D. Duong, and D. M. Peehl. 2007. Chompeopotent anti-inflammatory activities of curcumin and other phytochemicals mediated by MAP kinase phosphatase-5 in prostate cells. *Carcinogenesis* 28: 1188-1196.

72. Noun, L., L. Peng, D. Feldman, and D. M. Peehl. 2006. Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. *Cancer Res.* 66: 4516-4524.

73. Nomura, M., K. Shiiba, C. Katagiri, I. Kasugai, K. Masuda, I. Sato, M. Sato, Y. Kagukawa, E. Nomura, K. Hayashi, Y. Nakamura, T. Nagata, T. Otsuka, R. Kataoka, Y. Yamashita, M. Sato, N. Tanuma, and H. Shimizu. 2012. Novel function of MKP-5/DUSP10, a phosphatase of stress-activated kinases, as anti-angiogenic regulatory in colon carcinomas. *Oncol. Rep.* 28: 931-936.

74. Hoornaert, I., P. Marynen, J. Goris, R. Sciot, and M. Baens. 2003. MAPK phosphatase DUSP16/MKP-7, a candidate tumor suppressor for chromosome region 12p12-13, reduces BCR-ABL-induced transformation. *Oncogene* 22: 7728-7736.

75. Grepmeier, U., W. Dietmaier, J. Merk, P. J. Wild, E. C. Obermann, M. Pfeifer, F. Hofstaedter, A. Hartmann, and M. Woemckhaus. 2005. Deletions at chromosome 2q and 12p are early and frequent molecular alterations in bronchial epithelium and NSCLC of long-term smokers. *Int. J. Oncol.* 27: 481-488.

76. Kibel, A. S., J. Huagen, C. Guo, W. B. Isaacs, Y. Yan, K. J. Pienta, and P. J. Goodfellow. 2004. Expression mapping at 12p12-13 in advanced prostate carcinoma. *Int. J. Cancer* 109: 668-672.

77. Zaidi, S. K., C. R. Dowdy, A. J. van Wijnen, J. B. Lian, A. Raza, J. L. Stein, C. M. Croce, and G. S. Stein. 2009. Altered Runx1 subnuclear targeting enhances myeloid cell proliferation and blocks differentiation by activating a miR-24/MKP-7/MAPK network. *Cancer Res.* 69: 8249-8255.

78. Lee, S., N. Syed, J. Taylor, P. Smith, B. Griffin, M. Baens, M. Bai, K. Bourantas, J. Stebbing, K. Naresh, M. Nelson, M. Tuthill, M. Bower, E. Hatzimichael, and T. Crook. 2010. DUSP16 is an epigenetically regulated determinant of JNK signalling in Burkitt's lymphoma. *Br. J. Cancer* 103: 265-274.

79. Zhou, J. Y., Y. Liu, and G. S. Wu. 2006. The role of mitogen-activated protein kinase phosphatase-1 in oxidative damage-induced cell death. *Cancer Res.* 66: 4888-4894.

80. Shi, H., E. Bouda, F. Mercan, A. M. Le, R. J. Flach, L. Zhang, K. J. Tyner, B. B. Otwin, and A. M. Bennett. 2010. MAP kinase phosphatase-1 deficiency impairs skeletal muscle regeneration and exacerbates muscular dystrophy. *FASEB J.* 24: 2985-2997.

81. Chi, H., S. P. Barry, R. J. Roth, J. J. Wu, E. A. Jones, A. M. Bennett, and R. A. Flavell. 2006. Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc. Natl. Acad. Sci. U. S. A.* 103: 2274-2279.

82. Zhang, Y., J. M. Reynolds, S. H. Chang, N. Martin-Orozco, Y. Chung, R. I. Nuriya, and C. Dong. 2009. MKP-1 is necessary for T cell activation and function. *J. Biol. Chem.* 284: 30815-30824.

83. Boutros, T., E. Chevet, and P. Metrakos. 2008. Mitogen-activated protein (MAP) kinase/MAP kinase phosphatase regulation: roles in cell growth, death, and cancer. *Pharmacol. Rev.* 60: 261-310.

84. Magi-Galluzzi, C., R. Mishra, M. Fiorentino, R. Montironi, H. Yao, P. Capodiecchi, K. Wishnow, I. Kaplan, P. J. Stork, and M. Lod. 1997. Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and is inversely related to apoptosis. *Lab. Invest.* 76: 37-51.

85. Wang, H. Y., Z. Cheng, and C. C. Malton. 2003. Overexpression of mitogen-activated protein kinase phosphatases MKP1, MKP2 in human breast cancer. *Cancer Lett.* 191: 229-237.

86. Candas, D., C. L. Lu, M. Fan, Y. Y. Chuang, C. Sweeney, A. M. Borowsky, and J. J. Li. 2014. Mitochondrial MKP1 is a target of breast cancer chemoresistance. *Clin. Cancer Res.* 20: 44095-44107.

87. Rojo, F., I. Gonzalez-Navarrete, R. Bragado, A. Dalmases, E. Garcia-Gil, De la Cruz-Morcillo MA, M. Ortega-Muelas, E. Moragon, L. Lema, S. Serrano, A. Rovira, F. Rojo, J. L. Callejas-Valera, R. Pascual-Serra, J. Garcia-Cano, E. Garcia-Gil, De la Cruz-Morcillo MA, M. Ortega-Muelas, E. Moragon, L. Lema, S. Serrano, A. Rovira, F. Rojo.
91. Park, J., J. Lee, W. Kang, S. Chang, E. C. Shin, and C. Choi. 2013. TGF-betal and hypoxia-dependent expression of MKP-1 leads tumor resistance to death receptor-mediated cell death. Cell Death Dis. 4: e521.

92. Wang, J., J. Y. Zhou, and G. S. Wu. 2007. ERK-dependent MKP-1-mediated cisplatin resistance in human ovarian cancer cells. Cancer Res. 67: 11933-11941.

93. Wang, J., J. Y. Zhou, L. Zhang, and G. S. Wu. 2009. MKP-1-mediated cisplatin resistance in ovarian cancer cells. Cell Cycle 8: 3191-3198.

94. Small, G. W., Y. Y. Shi, L. S. Higgins, and R. Z. Orlowski. 2007. Mitogen-activated protein kinase phosphatase-1 is a mediator of breast cancer chemoresistance. Cancer Res. 67: 4459-4466.

95. Ma, G., Y. Pan, C. Zhou, R. Sun, J. Bai, P. Liu, Y. Ren, and J. He. 2015. Mitogen-activated protein kinase phosphatase 1 is involved in tumoxifen resistance in MCF7 cells. Oncol. Rep. 34: 2423-2430.

96. Lee, M., K. S. Young, J. Kim, H. S. Kim, S. M. Kim, and E. J. Kim. 2013. Mitogen-activated protein kinase phosphatase-1 inhibition and sustained extracellular signal-regulated kinase 1/2 activation in camptothecin-induced human colon cancer cell death. Cancer Biol. Ther. 14: 1007-1015.

97. Lawan, A., S. Al-Harthi, L. Cadalbert, A. G. McCluskey, M. Shweash, G. Grassia, A. Grant, M. Boyd, S. Currie, and R. Plevin. 2011. Deletion of the dual specific phosphatase-4 (DUSP-4) gene reveals an essential non-redundant role for MAP kinase phosphatase-2 (MKP-2) in proliferation and cell survival. J. Biol. Chem. 286: 12933-12943.

98. Hasegawa, T., A. Enomoto, T. Kato, K. Kawai, R. Miyamoto, M. Iijima, M. Ichihara, M. Ishida, N. Asai, Y. Murakumo, K. Ohara, Y. Niva, H. Hota, and M. Takahashi. 2008. Roles of induced expression of MAPK phosphatase-2 tumor in development in RET-MEN2A transgenic mice. Oncogene 27: 5684-5695.

99. Groschl, B., M. Bettstetter, C. Giedl, M. Woenschka, T. Edmonston, F. Hofstadter, and W. Dietmaier. 2013. Expression of the MAP kinase phosphatase DUSP4 is associated with microsatellite instability in colorectal cancer (CRC) and causes increased cell proliferation. Int. J. Cancer 132: 1537-1546.

100. Cagnol, S., and N. Rivard. 2013. Oncogenic KRAS and BRAF activation of the MEK/ERK signaling pathway promotes expression of dual-specificity phosphatase 4 (DUSP4/MKP2) resulting in nuclear ERK1/2 inhibition. Oncogene 32: 564-576.

101. Balko, J. M., R. S. Cook, D. B. Vaughit, M. G. Kuba, T. W. Miller, N. E. Bholia, M. E. Sanders, N. M. Granja-Ingram, J. J. Smith, I. M. Meszoezy, J. Salter, M. Dowsett, K. Stenke-Hale, A. M. Gonzalez-Angulo, G. B. Mills, J. A. Pinto, H. L. Gomez, and C. L. Arteaga. 2012. Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. Nat. Med. 18: 1052-1059.

102. Venter, D. J., S. J. Ramus, F. M. Hammet, S. M. de, A. M. Hatchingis, V. Petrovic, G. Price, and J. E. Armes. 2005. Complex CGH alterations on chromosome arm 8p at candidate tumor suppressor gene loci in breast cancer cell lines. Cancer Genet. Cytogenet. 160: 134-140.

103. Haagenson, K. K., J. W. Zhang, Z. Xu, M. P. Shekhar, and G. S. Wu. 2014. Functional analysis of MKP-1 and MKP-2 in breast cancer tamoxifen sensitivity. Oncotarget 5: 1101-1110.

104. Cadalbert, L., C. M. Sloss, P. Cameron, and R. Plevin. 2005. Conditional expression of MAP kinase phosphatase-2 protects against genotoxic stress-induced apoptosis by binding and selective dephosphorylation of nuclear activated c-jun N-terminal kinase. Cell. Signal. 17: 1254-1264.

105. Muda, M., U. Boschert, R. Dickinson, J. C. Martinou, I. Martinou, M. Camps, W. Schlegel, and S. Arkinstall. 1996. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. J. Biol. Chem. 271: 4319-4326.

106. Bloethner, S., B. Chen, K. Hennminki, J. Muller-Berghaus, S. Uigurel, D. Schadendorf, and R. Kumar. 2005. Effect of common B-RAF and N-RAS mutations on global gene expression in melanoma cell lines. Carcinogenesis 26: 1224-1232.

107. Croomquist, P. A., M. A. Linden, F. Zhao, and B. G. Van Ness. 2003. Gene profiling of a myeloma cell line reveals similarities and unique signatures among IL-6 response, N-ras-activating mutations, and coculture with bone marrow stromal cells. Blood 102: 2581-2592.

108. Warmka, J. K., L. J. Mauro, and E. V. Wattenberg. 2004. Mitogen-activated protein kinase phosphatase-3 is a tumor promoter target in initiated cells that express oncogenic Ras. J. Biol. Chem. 279: 33085-33092.

109. Zhang, Z., S. Kobayashi, A. C. Boreczuk, R. S. Leidner, T. Latriformboise, A. D. Levine, and B. Halmos. 2010. Dual specificity phosphatase 6 (DUSP6) is an ETS-regulated negative feedback mediator of oncogenic ERK signaling in lung cancer cells. Carcinogenesis 31: 577-586.

110. Ma, J., X. Yu, L. Guo, and S. H. Lu. 2013. DUSP6, a tumor suppressor, is involved in differentiation and apoptosis in esophageal squamous cell carcinoma. Oncol. Lett. 6: 1642-1646.

111. Bergholz, J., Y. Zhang, J. Wu, L. Meng, E. M. Walsh, A. Rai, M. Y. Sherman, and Z. X. Xiao. 2014. DeltaNp63alpha regulates Erk signaling via MKP3 to inhibit cancer metastasis. Oncogene 33: 212-224.

112. Luo, M. L., C. Gong, C. H. Chen, H. Hu, P. Huang, M. Zheng, Y. Yao, S. Wei, G. Wulf, J. Lieberman, X. Z. Zhou, E. Song, and K. P. Lu. 2015. The Rab2A GT-Pase promotes breast cancer stem cells and tumorigenesis via Erk signaling activation. Cell Rep. 11: 111-124.

113. Li, W., and D. W. Melton. 2012. Cisplatin regulates the MAPK kinase pathway to induce increased expression of DNA repair gene ERCC1 and increase melanoma chemoresistance. Oncogene 31: 2412-2422.

114. Messina, S., L. Frati, C. Leonetti, C. Zucchena, Z. E. Di, A. Calogero, and A. Porcellini. 2011. Dual-specificity phosphatase DUSP6 has tumor-promoting properties in human glioblastomas. Oncogene 30: 3813-3820.

115. Choi, B. K., C. H. Choi, H. L. Oh, and Y. K. Kim. 2004. Role of ERK activation in cisplatin-induced apoptosis in A172 human glioma cells. Neurotoxicology 25: 915-924.

116. Tanoue, T., T. Moriyagi, and E. Nishida. 1999. Molecular cloning of MKPs in Cancer.
ing and characterization of a novel dual specificity phosphatase, MKP-5. J. Biol. Chem. 274: 19949-19956.

117. Theodosiou, A., A. Smith, C. Gillieron, S. Arkinstall, and A. Ashworth. 1999. MKPS, a new member of the MAP kinase phosphatase family, which selectively dephosphorylates stress-activated kinases. Oncogene 18: 6981-6988.

118. James, S. J., H. Jiao, H. Y. Teh, H. Takahashi, C. W. Png, M. C. Phoon, Y. Suzuki, T. Sawasaki, H. Xiao, V. T. Chow, N. Yamamoto, J. M. Reynolds, R. A. Flavell, C. Dong, and Y. Zhang. 2015. MAPK Phosphatase 5 Expression Induced by Influenza and Other RNA Virus Infection Negatively Regulates IRF3 Activation and Type I Interferon Response. Cell Rep. 10: 1722-1734.

119. Zhang, Y., J. N. Blattman, N. J. Kennedy, J. Duong, T. Nguyen, Y. Wang, R. J. Davis, P. D. Greenberg, R. A. Flavell, and C. Dong. 2004. Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. Nature 430: 793-797.

120. Qian, F., J. Deng, N. Cheng, E. J. Welch, Y. Zhang, A. B. Malik, R. A. Flavell, C. Dong, and R. D. Ye. 2009. A non-redundant role for MKP5 in limiting ROS production and preventing LPS-induced vascular injury. EMBO J. 28: 2896-2907.

121. Zhang, Y., T. Nguyen, P. Tang, N. J. Kennedy, H. Jiao, M. Zhang, J. M. Reynolds, A. Jaeschke, N. Martin-Orozco, Y. Chung, W. M. He, C. Wang, W. Jia, B. Ge, R. J. Davis, R. A. Flavell, and C. Dong. 2015. Regulation of Adipose Tissue Inflammation and Insulin Resistance by MAPK Phosphatase 5. J. Biol. Chem. 290: 14875-14883.

122. Png, C. W., M. Weerasooriya, J. Guo, S. J. James, H. M. Poh, M. Osato, R. A. Flavell, C. Dong, H. Yang, and Y. Zhang. 2016. DUSP10 regulates intestinal epithelial cell growth and colorectal tumorigenesis. Oncogene 35: 206-217.

123. Song, M. K., Y. K. Park, and J. C. Ryu. 2013. Polycyclic aromatic hydrocarbon (PAH)-mediated upregulation of hepatic microRNA-181 family promotes cancer cell migration by targeting MAPK phosphatase-5, regulating the activation of p38 MAPK. Toxicol. Appl. Pharmacol. 273: 130-139.

124. He, G., L. Zhang, Q. Li, and L. Yang. 2014. miR-92a/DUSP10/JNK signalling axis promotes human pancreatic cancer cell proliferation. Biomed. Pharmacother. 68: 25-30.

125. Tanoue, T., T. Yamamoto, R. Maeda, and E. Nishida. 2001. A Novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 alpha and beta MAPKs. J. Biol. Chem. 276: 26629-26639.

126. Masuda, K., H. Shima, M. Watanabe, and K. Kikuchi. 2001. MKP-7, a novel mitogen-activated protein kinase phosphatase, functions as a shuttle protein. J. Biol. Chem. 276: 39002-39011.

127. Zhang, Y., K. C. Nallaparaju, X. Liu, H. Jiao, J. M. Reynolds, Z. X. Wang, and C. Dong. 2015. MAPK phosphatase 7 regulates T cell differentiation via inhibiting ERK-mediated IL-2 expression. J. Immunol. 194: 3088-3095.

128. Wei, X., W. Guo, S. Wu, L. Wang, P. Huang, J. Liu, and B. Fang. 2010. Oxidative stress in NSC-741909-induced apoptosis of cancer cells. J. Transl. Med. 8: 37.

129. Vogt, A., A. Tamewitz, J. Skoko, R. P. Sikorski, K. A. Giuliano, and J. S. Lazo. 2005. The benzo[c]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1. J. Biol. Chem. 280: 19078-19086.

130. Wang, Z., J. Y. Zhou, D. Kanakapalli, S. Buck, G. S. Wu, and Y. Ravindranathan. 2008. High level of mitogen-activated protein kinase phosphatase-1 expression is associated with cisplatin resistance in osteosarcoma. Pediatr. Blood Cancer 51: 754-759.

131. Arnold, D. M., C. Foster, D. M. Hutyn, J. S. Lazo, P. A. Johnston, and P. Wipf. 2007. Synthesis and biological activity of a focused library of mitogen-activated protein kinase phosphatase inhibitors. Chem. Biol. Drug Des. 69: 23-30.

132. Lazo, J. S., J. J. Skoko, S. Werner, B. Mitasev, A. Bakan, F. Koizumi, A. Yellow-Duke, I. Bahar, and K. M. Brummond. 2007. Structurally unique inhibitors of human mitogen-activated protein kinase phosphatase-1 identified in a pyrrole carboxamide library. J. Pharmacol. Exp. Ther. 322: 940-947.

133. Vogt, A., P. R. McDonald, A. Tamewitz, R. P. Sikorski, P. Wipf, J. J. Skoko, III, and J. S. Lazo. 2008. A cell-active inhibitor of mitogen-activated protein kinase phosphatases restores paclitaxel-induced apoptosis in dexamethasone-protected cancer cells. Mol. Cancer Ther. 7: 330-340.