

Dual-specificity MAP kinase phosphatases (MKPs) and cancer

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Abstract There are ten mitogen-activated protein kinase (MAPK) phosphatases (MKPs) that act as negative regulators of MAPK activity in mammalian cells and these can be subdivided into three groups. The first comprises DUSP1/MKP-1, DUSP2/PAC1, DUSP4/MKP-2 and DUSP5/hVH-3, which are inducible nuclear phosphatases. With the exception of DUSP5, these MKPs display a rather broad specificity for inactivation of the ERK, p38 and JNK MAP kinases. The second group contains three closely related ERK-specific and cytoplasmic MKPs encoded by DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4. The final group consists of three MKPs DUSP8/hVH-5, DUSP10/MKP-5 and DUSP16/MKP-7 all of which preferentially inactivate the stress-activated p38 and JNK MAP kinases. Abnormal MAPK signalling will have important consequences for processes critical to the development and progression of human cancer. In addition, MAPK signalling also plays a key role in determining the response of tumour cells to conventional cancer therapies. The emerging roles of the dual-specificity MKPs in the regulation of MAPK activities in normal tissues has highlighted the possible pathophysiological consequences of either loss (or gain) of function of these enzymes as part of the oncogenic process. This review summarises the current evidence implicating the dual-specificity MKPs in the initiation and development of cancer and also on the outcome of treatment.

Keywords MAP kinase · DUSP · MAP kinase phosphatase · Cancer

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Abbreviations

ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
BM	bone marrow
DUSP	dual-specificity protein phosphatase
ERK	extracellular-signal regulated kinase
JNK	c-Jun amino-terminal kinase
MAPK	mitogen-activated protein kinase
MEF	mouse embryo fibroblast
MKK or MEK	MAPK kinase
MKKK or MEKK	MAPK kinase kinase
MKP	MAP kinase phosphatase
NSCLC	non-small cell lung cancer
SCA	serous ovarian carcinoma
SAGE	serial analysis of gene expression
SBT	serous borderline ovarian tumour

1 Introduction

1.1 Mitogen-activated protein kinases and cancer

Mitogen-activated protein kinase (MAPK) pathways constitute a highly conserved family of kinase modules that serve to relay information from extracellular signals to the effectors that control diverse cellular processes such as proliferation, differentiation, migration and apoptosis (reviewed in [1–5]). MAPKs are activated by phosphorylation on both the threonine and tyrosine residues of a conserved signature T-X-Y motif within the activation loop of the kinase. This is mediated by a dual-specificity MAPK kinase (MKK or MEK), which is in turn regulated by phosphorylation on serine/threonine residues by a MAPK kinase kinase (MKKK or MEKK [6]). Three major groups of MAPK have been characterised in mammalian cells

based on sequence similarity, differential regulation by agonists and substrate specificity. These are the classical p42 and p44 MAPKs otherwise known as extracellular signal-regulated kinases ERK2 and ERK1 respectively, the c-Jun amino-terminal kinases JNK1, 2 and 3 and the four p38 MAPKs (α , β , δ and γ) [7].

Because MAPK activities impinge on many of the processes involved in the initiation and genesis of cancer, abnormalities in MAPK signalling pathways have been implicated in a wide range of human malignancies. The classical ERK pathway has long been associated with the ability of cancer cells to grow independently of normal proliferation signals and is deregulated in approximately 30% of human tumours (Fig. 1). Oncogenic abnormalities are found in upstream components of the ERK MAPK signalling pathways and include the over-expression or activating mutation of receptor tyrosine kinases, activating mutations in the Ras GTPase and mutations in the serine/threonine MAPK kinase kinase B-raf (reviewed in [8]).

With respect to the stress activated MAP kinases their role in cancer can be complex and, in the case of JNK, somewhat controversial (Fig. 1). For instance, there is evidence implicating JNK activity in Ras-induced cell transformation *in vitro* (reviewed in [9]). However, tumorigenesis studies using Ras-transformed fibroblasts derived from mice lacking JNK1 and 2 indicate that loss of JNK does not prevent tumour formation on injection of these cells into nude mice. Furthermore, lung metastases were actually larger when JNK was ablated indicating that JNK acts as a tumour suppressor *in vivo* [10]. Studies of fibroblasts derived from p38 α knockout mice also support a role for this MAPK as a tumour suppressor. These cells are more susceptible to H-RasV12-induced transformation, show decreased levels of apoptosis and form tumours more readily when injected into nude mice [11]. Furthermore, the deletion of p38 α in adult mice leads to an immature and hyperproliferative lung epithelium that is highly sensitized

to K-Ras(G12V)-induced tumourigenesis [12]. The tumour suppressive function of p38 MAPK could be mediated in a number of different ways. The p38 pathway may be involved in the activation of p53 and p53-mediated apoptosis and recent work has also implicated p38 in the promotion of cellular senescence as a means of evading oncogene-induced transformation [13, 14]. Finally, because of the key role that these MAPK pathways play in mediating stress-induced apoptosis, both p38 and JNK signalling are important determinants of cellular responses to conventional cancer therapies including chemotherapy and radiation (Fig. 1).

A major determinant of the biological outcome of MAPK signalling is the duration and magnitude of kinase activation [15]. This reflects a balance between the activities of upstream activators and various negative regulatory mechanisms, which oppose pathway activation. It is now clear that a major point of control occurs at the level of the MAPK itself through the activities of specific MAPK phosphatases. The requirement for phosphorylation on both threonine and tyrosine residues in order to activate the MAPK means that dephosphorylation of either residue is sufficient for kinase inactivation. This can be achieved by serine/threonine phosphatases, tyrosine specific phosphatases or by dual-specificity phosphatases and studies in a wide variety of model organisms from yeast to man have demonstrated that all three major classes of protein phosphatase can perform this task *in vivo* [16, 17]. However, by far the largest group of protein phosphatases that act to specifically regulate the phosphorylation and activity of mammalian MAPKs are the dual-specificity MAPK phosphatases (MKPs)

1.2 Dual-specificity MAPK phosphatases

The MKPs constitute a distinct subgroup of ten catalytically active enzymes within the larger family of cysteine-dependent dual-specificity protein phosphatases (DUSPs) [18]. They share a common structure that comprises a C-terminal catalytic domain, which shares sequence similarity with the prototypic VH-1 DUSP encoded by vaccinia virus, and an N-terminal non-catalytic domain. This latter region contains both a conserved cluster of basic amino acid residues involved in MAPK recognition known as the kinase interaction motif (KIM) and also sequences which determine the subcellular localisation of these enzymes [19, 20]. On the basis of sequence similarity, gene structure, substrate specificity and subcellular localisation the ten MKPs can be subdivided into three distinct groups (Table 1). The first of these contains DUSP1/MKP-1, DUSP2/PAC-1, DUSP4/MKP-2 and DUSP5/hVH-3. All of these proteins are encoded by highly inducible genes, which are rapidly up-regulated in response to both mitogenic and/or stress stimuli at the transcriptional level.

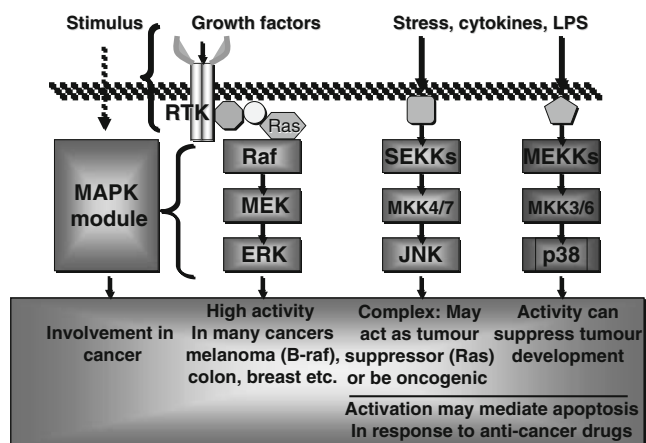


Fig. 1 MAPK signalling cascades and cancer

Table 1 Nomenclature, properties and physiological functions for dual-specificity MKPs

Gene/MKP	Trivial names	Chromosomal localisation	Subcellular localisation	Substrate specificity	Physiological function(s)
DUSP1/MKP-1	CL100, erp, 3CH134, hVH1	5q34	Nuclear	JNK, p38, ERK	Negative regulator of immune function. Protects mice from lethal endotoxic shock. Plays a key role in metabolic homeostasis and mediates resistance to cellular stress in mouse fibroblasts
DUSP4/MKP-2	Typ1, Sty8, hVH2	8p12-p11	Nuclear	JNK, p38, ERK	Unknown
DUSP2/None	PAC-1	2q11	Nuclear	ERK, p38	Positive regulator of inflammatory responses. Knockout mice are resistant to immune inflammation
DUSP5/None	hVH3, B23	10q25	Nuclear	ERK	Unknown
DUSP6/MKP-3	Pyst1, rVH6	12q22-q23	Cytoplasmic	ERK	Negative feedback regulator of ERK2 downstream of FGFR signalling
DUSP7/MKP-X	Pyst2, B59	3p21	Cytoplasmic	ERK	Unknown
DUSP9/MKP-4	Pyst3	Xq28	Cytoplasmic	ERK>p38	Essential for placental development and function (labyrinth formation)
DUSP8/None	M3/6, hVH5, HB5	11p15.5	Cytoplasmic/ nuclear	JNK, p38	Unknown
DUSP10/MKP-5		1q41	Cytoplasmic/ nuclear	JNK, p38	Functions in innate and adaptive immunity
DUSP16/MKP-7		12p12	Cytoplasmic/ nuclear	JNK, p38	Unknown

They are all nuclear proteins and with the exception of DUSP5/hVH-3, which appears to specifically target the ERK1/2 MAPKs, they exhibit broad substrate specificity and can inactivate ERK, JNK and p38 MAPKs. The second group of MKPs consists of DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4. All three proteins are cytoplasmic enzymes and exhibit a degree of selectivity towards the classical ERK1/2 MAPKs. This is most marked in DUSP6/MKP-3, which has little or no activity towards either JNK or p38 when over-expressed in mammalian cells. The final group of MKPs comprises DUSP8/hVH-5, DUSP10/MKP-5 and DUSP16/MKP-7. These proteins are found in both the cytoplasm and the nucleus and selectively dephosphorylate the stress-activated MAPKs p38 and JNK, while showing little or no activity towards ERK1/2.

Despite a fairly detailed knowledge of the biochemical properties and catalytic mechanism employed by the MKPs [20, 21], our knowledge of their physiological functions is somewhat less complete. However, recent gene targeting experiments in mice have been instrumental in revealing a diverse range of physiological functions for these enzymes [22]. DUSP1/MKP-1 plays an essential role in regulating cytokine biosynthesis in response to bacterial lipopolysaccharide (LPS) and also mediates, at least in part, the anti-inflammatory response to glucocorticoids [23–26]. In addition, DUSP1/MKP-1 plays a key role in metabolic homeostasis, as mice lacking this gene are resistant to obesity induced by a high fat diet [27].

Interestingly, the effects of DUSP1/MKP-1 deletion on metabolic control appear to result from the deregulation of more than one MAPK, indicating that this enzyme acts as an integration point in the control of these signalling pathways. DUSP10/MKP-5 also plays a significant role in mediating innate and adaptive immunity, primarily by regulating the JNK pathway in immune effector cells [28]. In contrast, DUSP2/PAC-1 is a positive regulator of inflammatory responses as DUSP2/PAC-1 knockout mice are resistant in experimental models of immune arthritis [29]. As was the case with DUSP1/MKP-1 these effects stem from complex changes in the activities of more than one MAPK pathway. Of the cytoplasmic MKPs so far analysed in this way, DUSP6/MKP-3 is responsible for regulating ERK activity in response to fibroblast growth factor (FGF) signalling during early mouse development and DUSP9/MKP-4 plays an essential role in the regulation of MAPK activity in extraembryonic tissues [30–32].

Given the diverse range of physiological endpoints which are regulated by the MKPs it would be surprising if they were not involved in the regulation of MAPK activities during processes of direct relevance to the initiation and development of human cancer. The evidence linking MKP function to the process of cell transformation and tumour development is summarised below. In addition the involvement of MKPs in mediating resistance to conventional cancer therapies is also discussed.

2 MAP kinase phosphatases and cancer

2.1 DUSP1/MKP-1

Soon after the characterisation of DUSP1/MKP-1 as a mitogen and stress inducible MAP kinase phosphatase, the levels of DUSP1/MKP-1 mRNA and protein were investigated in human cancers. These initial studies revealed significant over-expression of DUSP1/MKP-1 in a range of human epithelial tumours including prostate, colon and bladder [33]. Interestingly, over-expression was seen only in the early phases of disease with levels of DUSP1/MKP-1 expression falling progressively in tumours of higher histological grade and in metastases (Table 2). This pattern of expression was reinforced by the results of a serial analysis of gene expression (SAGE) study in which DUSP1/MKP-1 was identified as one of 20 transcripts which showed the largest decreases in level in colorectal cancer when compared with normal tissue [34]. No loss of chromosome *5q34-ter*, which contains the DUSP1 locus [35], was detected in tumours, nor were any mutations found within the catalytic domain of DUSP1/MKP-1 [33]. The mechanisms by which DUSP1/MKP-1 levels are altered in tumours may be varied and complex. The

DUSP1/MKP-1 gene is a transcriptional target of the p53 tumour suppressor [36] and is also up-regulated in response to a variety of cellular stress conditions including oxidative stress and DNA-damaging agents [37]. Interestingly, DUSP1/MKP-1 is also up-regulated in response to hypoxia at levels found in solid tumours, suggesting that it may play a key role in the regulation of MAPK activities within the tumour microenvironment [38].

With respect to prostate cancer, increased DUSP1/MKP-1 expression was also seen in the Noble rat model of sex hormone-induced prostatic dysplasia and carcinoma [39]. Furthermore, the increased levels of DUSP1/MKP-1 expression observed in human prostate cancer were inversely correlated with both JNK activity and markers of apoptosis, indicating that MKP-1 might be anti-apoptotic in these tumours via its activity towards JNK [40, 41]. Consistent with this idea, human DU145 prostatic cancer cells that over-express DUSP1/MKP-1 are resistant to Fas ligand-induced mitochondrial perturbation and apoptosis [42].

DUSP1/MKP-1 levels were also studied in primary ovarian tumours where moderate to strong expression of DUSP1/MKP-1 was detected in 57.6% of invasive ovarian carcinomas and MKP-1 expression was a prognostic marker for shorter progression-free survival (Table 2) [43].

Table 2 Summary of changes in expression observed for individual MKPs in human cancers

Gene/MKP	Cancer	Nature of change	Correlation with clinical outcome	Reference
DUSP1/MKP-1	Colon	Over-expression in early stages of disease	ND	[33, 34]
	Prostate	but expression is lost as tumour becomes		
	Bladder	more aggressive/invasive		
	Prostate	Increased expression levels of DUSP1/MKP-1	ND	[40, 41]
	Ovarian	showed inverse correlation with JNK activity and apoptotic markers		
	Ovarian	Moderate to strong expression seen in about 60% of invasive ovarian carcinomas	DUSP1/MKP-1 expression correlated with shorter progression-free survival	[43]
	Breast	Significant expression in poorly differentiated and late stage tumours. Overexpression correlated with lower JNK activity	ND	[33, 44]
	NSCLC	Elevated expression levels. Bias towards nuclear as opposed to nuclear/cytoplasmic localisation?	ND	[48, 49]
DUSP4/MKP-2	Ovarian	Expressed in serous borderline tumours (SBT) but not serous carcinoma (SCA). Association with more benign phenotype of the former?	ND	[52]
	Breast	Co-expression with DUSP1/MKP-1	ND	[44]
DUSP2/None	Ovarian	Expression in serous carcinoma	Associated with poor outcome in terms of overall survival	[54]
	Acute leukemia	Expression associated with elevated levels of ERK activation	ND	[55]
DUSP6/MKP-3	Pancreas	Over-expression in dysplastic tissue and carcinoma in situ but down regulation in invasive carcinoma	ND	[56, 61–63]
DUSP7/MKP-X	Leukemias	Elevated levels in acute myeloid leukaemia and acute lymphoblastic leukemia	ND	[65–67]

In contrast to the loss of DUSP1/MKP-1 expression noted in prostate, colon and bladder cancer, breast carcinomas showed significant MKP-1 expression even when poorly differentiated or in the late stages of the disease [33]. As was the case in prostate cancer, high levels of MKP-1 correlated with reduced JNK activity suggesting that therapeutic intervention to reduce the expression or activity of DUSP1/MKP-1 might enable the expression of the pro-apoptotic signals from JNK in malignant cells [44]. In support of this idea the over-expression of MKP-1 in breast carcinoma cell lines reduced JNK activity, caspase activation and DNA fragmentation and enhanced cell viability after treatment with alkylating agents (mechlorethamine), anthracyclines (doxorubicin), and microtubule inhibitors (paclitaxel) [45]. In contrast, reduction of DUSP1/MKP-1 levels using a small interfering RNA enhanced sensitivity to these drugs, and this was associated with increased JNK activity.

The idea that DUSP1/MKP-1 might mediate chemoresistance in cancer cells first came to prominence with the realisation that the stress-activated p38 and JNK MAPKs were *bona fide* substrates for this MKP. Preliminary studies found that over-expression of DUSP1/MKP-1 enhanced cellular resistance to cisplatin and that this correlated with reduced levels of JNK rather than p38 activity [46]. Confirmation of a key role for DUSP1/MKP-1 in mediating resistance to this drug came with the observation that mouse embryo fibroblasts (MEFs) derived from DUSP1/MKP-1 knockout mice were sensitive to cisplatin and that cisplatin-induced cell death could be inhibited by blocking JNK but not ERK and p38 activities in these cells [47]. Another human tumour which shows elevated levels of DUSP1/MKP-1 expression is non-small-cell lung cancer (NSCLC) [48, 49]. Cisplatin is often used to treat this disease, but often the late stage at which this cancer is diagnosed coupled with drug resistance results in a very poor prognosis with median survival of approximately 1 year. Analysis of NSCLC tumour tissues found strong nuclear staining for DUSP1/MKP-1 whereas in normal bronchial epithelium this protein was localized in the cytoplasm as well as the nucleus. In NSCLC cell lines DUSP1/MKP-1 was constitutively expressed and siRNA-mediated down-regulation of the gene caused a tenfold increase in sensitivity to cisplatin [50]. Xenograft experiments in nude mice using these siRNA-expressing cell lines also resulted in tumours that displayed slower growth rates and increased susceptibility to cisplatin. Taken together, these studies indicate that at least in a subset of human cancers, MKP-1 expression may mediate chemoresistance. Given the poor response to conventional chemotherapy in NSCLC and the ability of MKP-1 to mediate resistance to novel anticancer agents such as proteasome inhibitors [51], DUSP1/MKP-1 may be an important target as part of future strategies to improve tumour cell response to chemotherapy.

2.2 Other inducible nuclear MKPs and cancer

Compared with DUSP1/MKP-1, relatively little is known about the involvement of the other inducible nuclear MKPs in human malignancy. The most closely related enzyme to DUSP1/MKP-1 is DUSP4/MKP-2 and there is some evidence that this phosphatase is over-expressed in serous borderline tumours (SBT) of the ovary (Table 2). Compared with serous carcinomas (SCAs) these tumours present a more benign phenotype with a lack of stromal invasion. The expression levels of DUSP4/MKP-2 were found to be much higher in SBT when compared with SCAs and it was speculated that this may play some role in the more benign behaviour of SBT via the suppression of ERK-dependent events associated with degradation of the extracellular matrix [52]. MEK-dependent over-expression of DUSP4/MKP-2 has also been reported in pancreatic cancer cell lines harbouring activating mutations in K-Ras [53] and also in breast cancer where it appears to be co-expressed with DUSP1/MKP-1 [44]. Of the remaining members of this family, expression of DUSP2/PAC-1 has been observed in serous carcinomas of the ovary and its presence was associated with a poor outcome in terms of overall survival [54]. Loss of DUSP2/PAC-1 expression has also been associated with elevated levels of ERK activation in acute leukemias (Table 2) [55].

2.3 Cytoplasmic ERK-specific MKPs and cancer

Of the three cytoplasmic ERK-specific phosphatases encoded by DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4, the most widely studied and best characterized is DUSP6/MKP-3. The first hint that DUSP6/MKP-3 might be involved in the pathogenesis of human cancer came from studies of its possible role in pancreatic cancers associated with frequent allelic loss at 12q21. Although no gene mutations were detected, levels of DUSP6/MKP-3 mRNA were significantly reduced in a number of pancreatic cancer cell lines [56]. The vast majority (around 90%) of human pancreatic tumours carry activating mutations in K-Ras. Interestingly, DUSP6/MKP-3 was identified as one of only three genes which are uniquely expressed in myeloma cells harbouring a constitutively active mutant N-ras gene and is also overexpressed in human breast epithelial cells stably expressing H-ras and in human melanoma cell lines with potent activating mutations in B-raf [57–59]. These studies suggest that the over-expression of DUSP6/MKP-3 seen in response to activated Ras might represent a compensatory increase in the negative feedback control of the ERK1/2 MAPK pathway, which lies downstream of these activated oncogenes. In support of this, the tetracycline-induced expression of a functional fusion protein between DUSP6/MKP-3 and green fluores-

cent protein (GFP) in H-ras transformed fibroblasts following injection into nude mice resulted in a large delay in tumour emergence and growth as compared to the untreated control group [60]. In pancreatic cancer, the subsequent loss of DUSP6/MKP-3 expression might synergise with K-ras resulting in increased activation of ERK1/2 MAP kinase and thus contribute to the development and expression of the full malignant and invasive phenotype. An initial immunohistochemical study of primary pancreatic cancer tissues detected up-regulation in mildly as well as severely dysplastic/in situ carcinoma cells and down-regulation in invasive carcinoma, especially in the poorly differentiated type (Table 2). Furthermore, the reintroduction of active DUSP6/MKP-3 into cultured pancreatic cancer cells resulted in suppression of cell growth and increased levels of apoptosis [61].

A more extensive study of the expression of DUSP6/MKP-3 in pancreatic intraepithelial neoplasia and/or intraductal papillary-mucinous neoplasms, both of which are considered to be precursor lesions of invasive carcinoma of the pancreas, revealed that the expression of DUSP6/MKP-3 was selectively abrogated in invasive ductal carcinoma cells [62]. In contrast, expression was retained in the majority of precursor lesions. Interestingly, most of the intraductal adenoma/borderline lesions that had lost DUSP6/MKP-3 expression also harboured mutations of K-ras2. These results strongly suggest that loss of DUSP6/MKP-3 is associated exclusively with progression from pancreatic intraepithelial neoplasia to invasive ductal carcinoma. Loss of DUSP6/MKP-3 expression was found to correlate with methylation of CpG sequences in intron 1 of the DUSP6/MKP-3 gene in both pancreatic cell lines and in five of eight cases of pancreatic cancer, four of which were poorly differentiated adenocarcinoma [63]. This suggests that hypermethylation with modification of histone deacetylation play an important role in transcriptional suppression of DUSP6/MKP-3 in this disease. The recent generation of mice lacking the murine DUSP6/MKP-3 gene [30] should allow a systematic evaluation of its potential role in the development and progression of pre-neoplastic pancreatic lesions by intercrossing these animals with recently established murine models of pancreatic cancer, which employ tissue specific expression of mutant K-ras in the pancreatic epithelium [64].

With regard to the other members of this subfamily of cytoplasmic MKPs, much less is known about either their normal physiological functions or any potential roles in cell transformation and cancer. A micro-array study identified DUSP7/MKP-X (Pyst2) as over-expressed in acute leukemia [65] and this result was subsequently confirmed by RT-PCR and Northern blot analysis [66]. A more extensive study showed that DUSP7/MKP-X mRNA and protein were highly expressed in leukocytes obtained from acute

myeloid leukemia (AML) patients. High levels of mRNA were also expressed in bone marrow (BM) and peripheral leukocytes from nine AML and acute lymphoblastic leukemia (ALL) patients (Table 2). In contrast, BM from healthy individuals expressed very low levels of DUSP7/MKP-X [67]. While these studies point towards a link with abnormal MAPK signalling in these acute leukemias it is not yet clear if this observation has any pathological or prognostic significance.

DUSP9/MKP-4 the third member of this group of MKPs is a cytosolic MKP which, in contrast to DUSP6/MKP-3, shows a more relaxed substrate specificity displaying significant activity towards stress-activated MAPKs in addition to the classical ERK1/2 MAPKs [68, 69]. Gene targeting experiments in mice have recently shown that DUSP9/MKP-4 plays an essential role in placental development and function but its role in adult animals is as yet unclear [32]. In recent study DUSP9/MKP-4 was identified in an Affymetrix GeneChip analysis as an mRNA which was down-regulated in a non-Ras model of epithelial carcinogenesis. Loss of DUSP9/MKP-4 was also observed in tumours independently generated by the application of chemical or physical carcinogens in mice [70]. Interestingly, reconstitution experiments in which DUSP9/MKP-4 was re-expressed led to increased levels of tumour cell death. In contrast, expression of the related ERK-specific phosphatase DUSP6/MKP-3 did not increase levels of tumour cell apoptosis. Finally, lentiviral mediated expression of DUSP9/MKP-4 in tumourigenic cells led to almost total suppression of tumour formation following sub-cutaneous injection into BALB/c neonatal mice when compared with uninfected cells and conditional (tetracycline-inducible) expression of MKP-4 in established NSCLC cell line induced tumours also caused tumour stasis [70]. Taken together these results are suggestive of a tumour suppressor function for DUSP9/MKP-4 and indicate that its activity towards p38 and JNK in addition to inactivation of ERK may be responsible for this effect. Due to the embryonic lethality of the DUSP9/MKP-4 gene knockout, confirmation of this role *in vivo* will require carcinogenesis studies in conjunction with conditional loss of DUSP9/MKP-4 activity in adult tissues. This study should also prompt an extensive examination of the levels of DUSP9/MKP-4 mRNA and protein in human skin tumours.

3 Conclusions and perspectives

MAPKs play diverse and sometimes apparently contradictory roles in the initiation and development of human cancers. In the case of the protein phosphatases that negatively regulate MAPKs, there is increasing evidence that these enzymes may be abnormally regulated in certain

tumours. However, whether either over-expression or loss of expression is a cause of, or actually contributes to, the malignant phenotype rather than simply being a consequence of cell transformation is as yet unclear. The increasing availability of genetic knockout models for individual MKPs should allow a direct test in cases where loss of expression is associated with the development of certain cancers. These animals can also be used as experimental models to dissect out the roles of MKP in mediating the resistance of tumours to chemical and physical agents used in both conventional and novel cancer therapies. The next few years should bring significant advances in our understanding of how abnormalities in the regulation of MAPK pathways can be exploited as novel therapeutic targets in the war against cancer.

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