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Review

The dual-specificity MAP kinase phosphatases: critical roles in development and cancer

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Bermudez O, Pagès G, Gimond C. The dual-specificity MAP kinase phosphatases: critical roles in development and cancer. *Am J Physiol Cell Physiol* 299: C189–C202, 2010. First published May 12, 2010; doi:10.1152/ajpcell.00347.2009.—Intracellular signaling by mitogen-activated protein (MAP) kinases (MAPK) is involved in many cellular responses and in the regulation of various physiological and pathological conditions. Tight control of the localization and duration of extracellular-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), or p38 MAPK activity is thus a fundamental aspect of cell biology. Several members of the dual-specificity phosphatase (DUSPs) family are able to dephosphorylate MAPK isoforms with different specificity, cellular, and tissue localization. Understanding how these phosphatases are themselves regulated during development or in physiological and pathological conditions is therefore fundamental. Over the years, gene deletion and knockdown studies have completed initial in vitro studies and shed a new light on the global and specific roles of DUSPs in vivo. Whereas DUSP1, DUSP2, and DUSP10 appear as crucial players in the regulation of immune responses, other members of the family, like the ERK-specific DUSP6, were shown to play a major role in development. Recent findings on the involvement of DUSPs in cancer progression and resistance will also be discussed.

DUSP6; phosphorylation; phenotype

THE MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) family includes the extracellular-regulated kinases (ERKs) and the stress-activated protein kinases (SAPKs) p38 and c-Jun NH_2-terminal kinase (JNK). Their activation by various stimuli including growth factors, cytokines, integrin ligands, or stress conditions regulates major cellular responses such as proliferation, differentiation, survival, migration, or production of soluble factors such as inflammatory molecules (68). The tight control of MAPK signaling is determinant for defining the outcome of their activation and depends on factors that affect their spatiotemporal activity, including density and rates of internalization of cell surface receptors, desensitization of these receptors, formation of scaffold complexes containing the MAPKs and their partners, subcellular relocalization, and equilibrium between activated MAPKs and the phosphatases that extinguish their signal. Although MAPKs are dephosphorylated by broad-specificity phosphatases at early time points following their activation, they are also the targets of specific inhibitory molecules, the MAPKs phosphatases (MKPs), which belong to the dual-specificity phosphatase (DUSP) family. These phosphatases dephosphorylate both threonine and tyrosine residues within the substrates they target (101). The purpose of this review is to give a view of the current knowledge of the role of MKPs in cell biology, with an emphasis on DUSP6/MKP-3, a cytoplasmic, ERK-specific phosphatase that appears to play a crucial role in development and various pathologies and notably in the regulation of signaling by major growth factors/morphogens, i.e., members of the fibroblast growth factor (FGF) family.

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A Common Structure

MKPs share a conserved structure with a noncatalytic NH_2-terminal domain and a COOH-terminal catalytic extremity. The NH_2-terminal domain of all DUSPs has two regions of homology with the Cdc25 cell cycle regulatory phosphatase (designated CH_2 domains). The catalytic domain, which is more conserved than the NH_2-terminal within DUSPs, presents an active site sequence related to the prototypic VH-1 phosphatase encoded by the vaccinia virus. The catalytic domain contains a highly conserved sequence DX_{26} (V/L)*X*(V/I)HC*X*AG(I/V)-SRS*X*T(I/V)*XX*AY(L/I)M, where *X* is any amino acid.

Despite their common structure, MKPs have specific properties concerning their substrates and subcellular localization. Specificity of MKPs toward one or the other MAPKs relies on sequences in the NH_2-terminal domain of MKPs, known as the kinase interaction motif (KIM). Although each MKP targets different subsets of MAPKs, there is an overlap between their specificities, possible due to gene duplication and gene divergence. Based on these features, they can be divided into three different subclasses (summarized in Table 1).

Inducible nuclear MKPs: DUSP1/MKP-1, DUSP2/PAC-1, DUSP4/MKP-2, and DUSP5. The genes coding for these phosphatases have four exons and introns with highly conserved positions, suggesting that they arose from a common ancestral gene. They were shown to dephosphorylate ERKs,

Table 1. *MAP kinase phosphatases of the DUSP family*

Class	Other Names	Subcellular Localization	Substrate Preference		
			ERK1/2	p38	JNK
Class I					
DUSP1	MKP-1, CL100, erp, 3CH134, hVH1	Nuclear	X	X	X
DUSP2	PAC-1	Nuclear	X	X	
DUSP4	MKP-2, Typ1, Sty8, hVH2	Nuclear	X	X	X
DUSP5	hVH-3, B23	Nuclear	X		
Class II					
DUSP6	MKP-3, Pyst1, rVH6	Cytoplasmic	X		
DUSP7	MKP-X, Pyst2, B59	Cytoplasmic	X		
DUSP9	MKP-4, Pyst3	Cytoplasmic	X	x	
Class III					
DUSP8	M3/6, hVH5, HB5	Cytoplasmic/nuclear		X	X
DUSP10	MKP-5	Cytoplasmic/nuclear		X	X
DUSP16	MKP-7	Cytoplasmic/nuclear		X	X

MAP, mitogen-activated protein; DUSP, dual-specification phosphatase; *Class I,* inducible nuclear MAP kinase phosphatases (MKPs); *Class II,* cytoplasmic extracellular-regulated kinase (ERK)-specific MKPs; *Class II,* MKPs that inactivate stress p38 and c-Jun NH_2 terminal kinase (JNK) MAP kinases; X, preferred substrate; x, some detectable activity toward this kinase.

JNK, and p38 MAPKs to the same extent. Interestingly, DUSP1 may be the phosphatase responsible for VEGF-induced histone H3 dephosphorylation (64).

Cytoplasmic ERK-specific MKPs: DUSP6/MKP-3, DUSP7/MKP-X, and DUSP9/MKP-4. The genes of these phosphatases have four exons. DUSP6 seems to be highly selective for ERK1/2 as it fails to bind to or to inactivate ERK5 (1), although previous reports had first suggested the opposite (56, 114). In agreement with the results of Arkell et al. (1), invalidation of the *dusp6* gene in the mouse was recently reported to have no effect on ERK5 signaling (86). DUSP6 shuttles between the nucleus and the cytosol but exhibits a cytoplasmic localization dependent on leucin-rich nuclear export signal located in its NH_2-terminal domain and distinct from the KIM domain (59). It was proposed that DUSP6 constitutes an anchor for inactive ERK in the cytosol (12) and even participates to the transport of dephosphorylated ERKs from the nucleus to the cytosol (59).

MKPs that inactivate p38 and JNK MAPKs. DUSP8, DUSP10/MKP-5, and DUSP16/MKP-7 are cytoplasmic and nuclear. Six exons are present in the genes coding for these DUSPs.

DUSPs appear to be evolutionary conserved with 16 homologs identified in the budding yeast, six in *Drosophila melanogaster* and up to 26 in *Caenorhabditis elegans* according to its genome sequence (126). Moreover, there is a certain degree of tissue specificity in DUSP expression in mammals.

Atypical DUSPs

Some of the DUSPs phosphatases have common characteristics with the MKPs but share more similiarities with the VH1 (vaccinia virus open reading frame H1) phosphatase. In addition, as they are phylogenetically distinct form classical protein tyrosine phosphatases (PTPs) and MKPs, they are considered as "atypical DUSPs". Most of these 16 atypical DUSPs do not have the NH_2-terminal CH_2 domain present in MKPs but contain the DUSP catalytic domain. These DUSPs can target the MAPK but can also have other type of substrates, although this issue is controversial. Thus additional studies appear to be necessary to confirm targets and characteristics of the action of these atypical DUSPs in vivo. They have been recently reviewed by Paterson et al. (101).

DUSP Regulation

Transcriptional regulation and mRNA stability. MKPs are subjected to tight regulation. First of all, many MKPs are expressed at low levels in resting, nonstimulated cells and are induced as early response genes after activation of the MAPK pathways they target. Examples are DUSP1 and DUSP6, which we will take as an example.

In silico analysis of the *dusp6* promoter revealed the presence of putative regulatory sequences for Forkhead transcription factor, a downstream effector of the phosphatidylinositol 3-kinase (PI3K) signaling pathway and for the Ets family of transcription factors, well-known targets of ERKs (36). PI3K signaling was first suggested to be involved in FGF8-induced *dusp6* transcription in chick embryos (34, 60), but functional analysis of the promoter regions of *dusp6* failed to reproduce this finding in mouse cells (36). PI3K inhibition did not alter *dusp6* expression in mouse embryos either (122). In contrast, the ERK pathway consistently appears as a major regulator of *dusp6* mRNA expression, notably in a negative feed-back loop of FGF signaling (34, 122, 127). Luciferase reporter assays showed that the transcriptional activation of *dusp6* by FGF family members, which also takes place in *Drosophila* and *Xenopus* (48), occurs as early as 30 min after stimulation and involves an Ets2-dependent mechanism dependent on the intron 1 in the phosphatase gene (36, 44). A regulatory enhancer sequence, partially overlapping with the Ets2 binding site, was also described to play a role in the regulation of *dusp6* transcription in embryonic stem cells (144). Specific MEK/ERK activation through a ΔRaf:ER chimera was sufficient for inducing *dusp6* transcription (36) suggesting that other ERK-stimulating agonists may play a role in this regulation, such as PDGF (55) or EGF in *Drosophila* (48). Other motives in the murine *dusp6* promoter include consensus-binding sites for nuclear factor (NF)-κB and pre-B-cell leukemia transcription factor 1 (PBX1), the latter being a known target of retinoic acid. Finally, in the early zebrafish embryo, *dusp6* transcription is also regulated by maternal β-catenin and Tcf-3 (127).

Phosphatases may also be regulated at the level of mRNA stability. This is the case of *dusp1* mRNA, stabilized by HuR and NF90 (69) or degraded by the AU-rich elements (ARE)-binding protein tristetraprolin (37, 77). Recently, miR-29b microRNA was found to downregulate *dusp2* mRNA during osteoblast differentiation (75).

Posttranslational regulation: protein stability and catalytic activation. Another mechanism of regulation is the control of protein stability. Ubiquitination appears as a critical step for degradation of several DUSPs. The fate of DUSP1 following ERK activation is controversial. Whereas initial reports suggested that ERK-dependent DUSP1 phosphorylation stabilized the phosphatase (10), it was found to rather decrease the half-life of DUSP1 in a subsequent study (78). Reasons for these discrepancies are unclear but may be related to the conditions of ERK activation, i.e., transient versus persistent, depending on the reports.

In contrast, DUSP6 was consistently found to be degraded by phosphorylation (4, 55, 88). DUSP6 phosphorylation is induced by various growth factors and is MEK dependent (Fig. 1). Site-directed mutagenesis identified three serines (Ser159, Ser174, and Ser197) involved in this regulation. Although DUSP6 is a substrate for ERK in vitro (88), it may also be phosphorylated upon specific stimulation of another pathway involved in cell growth and proliferation, the mammalian target of rapamycin (mTOR) pathway. This latter phosphorylation also reduces the half-life of the phosphatase, suggesting that both signaling pathways may be interconnected and possibly collaborate through DUSP6 (4). Finally, DUSP6 was also shown to interact with and to be phosphorylated by protein kinase CK2a/casein kinase 2, another ubiquitous kinase involved in the regulation of cell proliferation and survival (19).

Another way to regulate these phosphatases is to regulate their catalytic activity. Some of DUSPs are in an active ready conformation (DUSP3, DUSP5, and DUSP10), whereas others require the binding of their substrate for the induction of an active catalytic site conformation (DUSP1, DUSP6, DUSP9, and DUSP2). The dephosphorylation of MAPKs by MKPs occurs in two main steps, starting with the dephosphorylation of the MAPK tyrosine residue followed by the threonine residue, leading to its complete inactivation (39). This interaction involves a KIM in the phosphatases and a common docking site (CDS) within the kinases. The KIM is a conserved positively charged sequence in the amino terminal part of DUSPs that interacts with the negatively charged CDS of the kinases. The CDS of MAPKs allows not only an interaction with their phosphatases but also with other kinases and with their substrate (125). The KIM sequence could determine the specificity of the binding of one DUSP to ERK or p38 or JNK; this specificity can be really precise as mutation of the KIM domain in DUSP1 prevents binding to ERK and p38 but had no effect on binding and inactivation of JNK (120). Another interaction site, the ED domain (named like this because it contains a glutamic residue E followed by an aspartic residue D) in ERK2 and p38 MAPK also participates in the interaction between the kinases and DUSPs. Another docking region in DUSPs, rich in hydrophobic residues, has been identified, lying between the KIM and the Arg72/74. This region seems to be involved in the specific interaction between DUSP1 and JNK.

The molecular basis of DUSP6 catalytic activation has been extensively studied and was shown to depend on its interaction with ERK (17), mainly through the KIM domain in the NH_2-terminus of the phosphatase, although it involves weaker interactions with its COOH-terminal domain (38, 96, 98). However, activation of DUSP6 does not require ERK kinase activity (17) but relies instead on allosteric modifications and stabilization of the catalytic "general acid" loop, a characteristic of protein-tyrosine phosphatase catalysis (40).

Recently, another mode of posttranslational regulation was identified for DUSP1, the acetylation of which potentiates its association with p38 and the dephosphorylation of this MAPK (18).

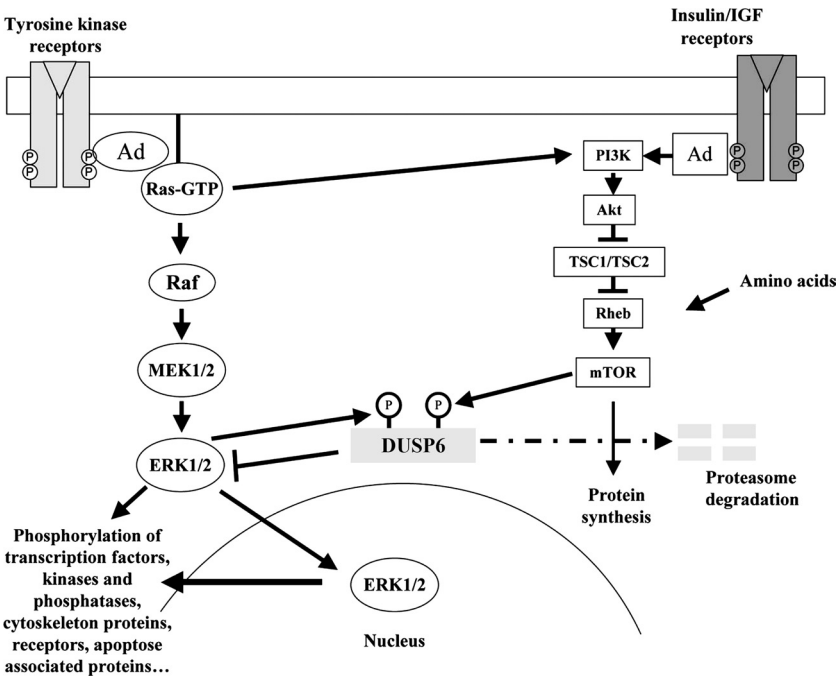

Fig. 1. Dual-specificity phosphatase (DUSP6) phosphorylation by signaling pathways. The cytosolic extracellular-regulated kinase (ERK)1/2-specific DUSP6 phosphatase is phosphorylated upon activation of the MEK/ERK and phosphatidylinositol 3-kinase (PI3K)/mammalian target or rapamycin (mTOR) pathways by various agonists, leading to the degradation of DUSP6 by the proteasome.

DUSPs in Embryonic Development

Table 2 summarizes the effects of *dusp* gene invalidation or transgenic expression in various model organisms. The obtained phenotypes are often a global response of the model organism to the deletion or the overexpression of such genes and not necessarily a description of the role of these genes in vivo. For example, invalidation of any phosphatase gene may result in the upregulation of other members of the family or in overactivation of given MAPKs through the inactivation of other MAPKs isoforms as a result of a cross-talk between signaling pathways. This, of course, complicates the interpretation of the DUSP knockout phenotypes and should be taken into account. Nevertheless, various degrees of specificity occur in the phenotypes obtained.

Although DUSP1 phosphatase targets the principal MAPKs (ERK1/2, JNK, and p38), invalidating its gene does not result in any obvious or lethal phenotype during development, suggesting compensation by other members of the DUSP family. However, if this may hold true for development, subsequent

Table 2. *Role of DUSPs in development, organogenesis, and the immune system*

DUSP	Animal Model	Phenotype
DUSP1	Mice	DUSP1 knockout, developmental stages
		No overt phenotypic or histological abnormalities (33).
		dusp −/− MEFs display increased sensitivity to apoptosis mediated by p38 and JNK MAPKs (138, 148).
		DUSP1 knockout: muscle and adipose tissues
		Activated p38 MAPK, JNK1, and ERK levels are elevated in skeletal muscle and white adipose tissue, compared with levels in similarly treated wild-type mice (139).
		dusp1-null mice are lean and resistant to diet-induced obesity due to increased energy expenditure (139).
		DUSP1 knockout: immune and inflammatory responses
		Greater innate immune response to LPS, increased cytokines levels in serum (112, 148).
		Peritoneal macrophages from *dusp1*-null mice have prolonged phosphorylated p38MAPK and JNK in response to LPS compared with wild-type mice (146).
		dusp1 Knockout mice have a greater severity and incidence of arthritis in a chicken type II collagen-mediated model of rheumatoid arthritis (112).
		DUSP1 transgenic expression in the heart
		Reduced activation of p38, JNK et ERK1/2.
		Reduced development of the myocardium.
		Decrease in the hypertrophic response to aortic banding and catecholamine infusion (13).
DUSP2	Mice	DUSP2 knockout: immune and inflammatory responses
		LPS-stimulated DUSP2 null macrophages produce lower levels of proinflammatory cytokines and chemokines when compared with wild-type cells.
		DUSP2-deficient macrophages and mast cells have decreased phosphorylation of ERK and p38 and decreased transcriptional activation of transcription factors NFAT-AP-1 and Elk1.
		JNK activity is increased in DUSP2-deficient macrophages and mast cells.
		Reduced inflammatory responses in a model of rheumatoid arthritis (53).
DUSP4	Zebrafish	Antisense morpholino oligonucleotides
		During late gastrulation, loss of *sox17* expression.
		Loss of foregut and pancreatic endoderm, necrosis of head tissues at later stages of development (11).
DUSP5	Zebrafish	Expression during embryonic development
		dusp5 is expressed in angioblasts in the embryonic zebrafish and is essential for vascular development in vivo (102).
		apoptosis of endothelial cells in vitro.
		DUSP5 and SNRK-1 target a common signaling pathway responsible for maintaining angioblast populations along the lateral plate mesoderm (102).
	Mice	Transgenic expression of DUSP5 in the lymphoid compartment
		Blocks thymocyte development at the double positive stage, defective response to IL2, autoimmune symptoms (67).
DUSP6	Drosophila	Loss of function mutants
		Role of the phosphatase in photoreceptor cell differentiation, wing vein formation and oogenesis (48).
	Zebrafish	Morpholino oligonucleotides and overexpression
		Alteration in axial patterning of the zebrafish embryo: a dorsalized phenotype is obtained after injection of the oligonucleotides while the overexpression of DUSP6 induces the ventralisation of the embryo (127).
		Chemical inhibitor of DUSP6
		Increased heart size (92).
	Chick	siRNA
		DUSP6 siRNA injection induces apoptosis in the mesenchyme of the chick limbs (60).
	Mice	DUSP6 Knockout: development
		Perinatal death of some but not all animals (incomplete penetrance).
		Dwarfism, premature fusion of the cranial sutures (craniosynostoses), defects in the middle ear bones and the otic capsule, leading to hearing loss (73).
		Increased basal ERK activity in *dusp6* in the heart, spleen, kidney, brain and mouse embryonic fibroblasts (86).
		DUSP6 knockout: heart
		Overproliferation of cardiac myocyte progenitors, cardiac hypertrophy (86).
DUSP9	Mice	DUSP9 Knockout: development
		Die in utero from placental insufficiency, secondary to a failure of labyrinth development (27).
DUSP10	Mice	DUSP10 Knockout: immune and inflammatory responses
		Elevated production of pro-inflammatory cytokine by macrophages. After secondary infection with LCMV (Lymphocytic Choriomeningitis Virus), enhanced production of cytokine and enhanced CD4+ and CD8+ T cell responses (143).

See text for more details.

studies revealed a fundamental role of DUSP1 in the regulation of many cellular responses, including cell survival in various stress conditions. If initial studies had not identified any effect of DUSP1 gene deletion in ES cells and mouse embryonic fibroblasts (33), later works have clearly established a role for this phosphatase in protecting cells from apoptosis mediated by the stress MAPKs p38 and JNK (138, 148). Invalidating *dusp1* prolonged activity of p38, JNK, or both, depending on the nature of the stress signal. No effect on ERK signaling was reported, whether the cells were stimulated or not by stress signals. However, deletion of DUSP1 increased basal levels of all three types of MAPKs in muscle, adipose, and heart tissues (13, 139). This latter work also provided evidence for a role of DUSP1 in metabolic regulation, *dusp1* knockout mice being resistant to high-fat diet-induced obesity (139). Finally, DUSP1 plays an important role in the immune response and onset of inflammation that will be discussed below.

In contrast, other DUSPs were found to play critical roles in development. Loss of function studies revealed that DUSP4 is essential for early development and endoderm specification in the zebrafish (11), whereas DUSP5 was recently described to control angioblast populations in the lateral plate mesoderm, although it may rather control the activity of the serine-threonine kinase Snrk-1 in this particular setting (102).

Regarding specific ERK phosphatases, DUSP9 was the first to be silenced in mice. *Dusp9* null mice die in utero because of placental insufficiency (27). Surprisingly, no change in ERK1/2 or in p38 MAPK activity, the two targets of this phosphatase, was detected in their placental tissues, although it is difficult to rule out a transient effect of *dusp9* deletion that may remain unseen at earlier or later time points. Tetraploid rescue showed that DUSP9 is dispensable for normal embryogenesis.

In the following paragraphs we will focus on the role of DUSP6 in development and its relationship.

DUSP6 in development and organogenesis. Gene invalidation or overexpression studies in *Drosophila*, zebrafish, *Xenopus*, or chick embryos have suggested that DUSP6 plays a major role in embryonic development. Injection of antisense morpholino oligonucleotides showed that it is required for proper axial patterning of the zebrafish embryo (127), whereas small interfering RNA (siRNA) studies identified a protective role for DUSP6 against apoptosis in the chick limb (60). Ectopic expression of DUSP6 in *Drosophila* suppresses photoreceptor cell differentiation and wing vein formation, whereas, conversely, hypomorphs of DUSP6 exhibit ectopic differentiation of both cell types. This strongly suggests that proper activation of the ERK pathway is required for these differentiation events. DUSP6 was also found to be necessary for oogenesis in *Drosophila* (48, 62).

In the mouse, *dusp6* invalidation does not strongly affect embryonic development but results in perinatal death of some but not all animals, indicating that the severity of the phenotype varies due to penetrance phenomenon (73). This rather mild phenotype may be the result of compensation by other DUSPs, such as DUSP7 and DUSP9, which share some areas of expression in developing animals (limb buds, branchial arches; 129). Nevertheless, *dusp6*−/− mice present many abnormalities, including dwarfism and premature fusions of the cranial sutures, a condition known as cranyosynostoses, and found in animals expressing mutant forms of FGFR1 and FGFR2 receptors with a moderate constitutive activation (137, 147). However, at the histological level, the skeletal phenotype of *dusp6* knockout animals was even more similar to that of mice harboring an activating mutation of FGFR3. Indeed, the chondrocyte proliferation zone was disorganized in both *dusp6*−/− and FGFR3 mice, with no typical straight columns of cells (9), whereas FGFR2 mutant animals displayed reduced proliferation zones.

DUSP6 was also found to be a negative feedback modulator of FGF8 signaling in the mammalian brain isthmic organizer (35). In addition, *dusp6*−/− animals also present defects in the development of the ear, with variable abnormalities in middle ear bones and otic capsules, associated with hearing loss in surviving animals.

The relationship between DUSP6 and regulation of FGF signaling is also likely to explain the phenotype of DUSP6 overexpression or inactivation in chick and zebrafish embryos (60, 127). Smith et al. (123) proposed that tight control of FGF-induced ERK signaling by DUSP6 regulates the expression of the transcription factor scleraxis and the specification of rib progenitors in the developing chick somites. Very recently, Molina et al. (92) took advantage of a chemical inhibitor of DUSP6 to uncover a critical role of this phosphatase in restricting cardiac progenitors and controlling heart organ size in zebrafish, a process also involving FGF signaling. Indeed, this small allosteric inhibitor of DUSP6 caused an overactivation of FGF signaling and an expansion of the cardiac progenitor pools at the expense of the endothelial/hematopoietic lineage. This confirmed previous findings by Maillet et al. (86) in the mouse, who showed that the absence of DUSP6 results in greater proliferation of myocytes in the developing heart of knockout mice from another genetic background than the C57Bl/6 described by Li et al.(73) and not associated with postnatal mortality.

Interestingly, the invalidation of genes coding for other modulators of FGF signaling pathways produces phenotypes that resemble that of *dusp6*−/− mice. For example, knockout of *spry4*, a member of the sprouty family, leads to growth retardation, whereas invalidation of *spry2* results in defects of ear development. However, it was the inner and not the middle ear that was affected in *spry2*−/− mice. Finally, gene invalidation of Spred-2, a sprouty-related protein, also resulted in dwarfism and bone growth plate defects (reviewed in Ref. 15).

DUSP6 and the FGF family: a special relationship? The phenotypes of *dusp6* knockout and transgenic animals thus appear to be a consequence of an improper feedback regulation of FGF signaling. Although FGFs elicit various signaling pathways in their target cells, several lines of evidences indicate that, as expected, dysregulation of the MEK/ERK pathway is responsible for these defects. First, activating mutations in either MEK1 or MEK2, two direct upstream activators of ERKs, also caused the cranyosynostosis phenotype observed in *dusp6* knockout and mice with activated FGFRs (110). Conversely, pharmacological inhibition of MEK was shown to inhibit cranyosynostosis in a mouse model of the Apert syndrome, expressing an activated FRGFR2. Analysis of ERK signaling at the cellular level in *dusp6*-null animal models indicated that developmental failures correlated with dysregulation of ERK signaling. Loss of DUSP6 increased the basal activity of ERK1/2 in multiple tissues and in mouse embryonic fibroblasts isolated from *dusp6*−/− mice. Effects on ERK

activity were paralleled by an accumulation of phospho-ERK in the nucleus and notably in the perinuclear region (86).

One consequence of unregulated activation of the MEK/ERK axis is increased cellular apoptosis and, consequently, one of the roles of DUSP6 could be to protect cells from apoptosis through appropriate control of ERK signaling. This is supported by RNA interference and overexpression studies performed in the chick limb in the context of FGF signaling (60). A modest prosurvival role for DUSP6 was also reported in Hela cells (84). However, the role of DUSP6 in this cellular response may depend on the context. Hence, invalidation of the *dusp6* gene partially protects mouse embryonic fibroblasts from various apoptotic signals (86). The absence of DUSP6 was also observed to protect the heart of *dusp6*−/− mice from chronical hypoxia, resulting from the permanent ligation of the left coronary artery (86). Reciprocally, a previous study by the same group showed that overexpressing DUSP6 in the heart of transgenic mice promoted heart failure (104). In this context it is interesting to note the findings by Lips et al. (80) who showed that ERK2 signaling is required to protect the myocardium from ischemia-reperfusion injury in vivo. However, since Maillet et al. found that *dusp6* deletion was not sufficient to reduce the infarct size following such an acute injury, this may indicate that ERK signal is necessary, but perhaps not sufficient, in this process. The protective effect of ERK was due to inhibition of apoptosis, likely through the formation of a complex of ERK and protein kinase Cε, causing the phosphorylation and the inactivation of the pro-apoptotic protein Bad in the mitochondria. ERK-mediated phosphorylation of p90 ribosomal S6 kinase (RSK) is another way of inhibiting Bad in the heart (reviewed in Ref. 14). Studies in other cellular systems have evidenced additional mechanisms for the protective role of ERKs against apoptosis-inducing stimuli. For example, ERK activity is directly involved in the maintenance of mitochondrial membrane potential. Through the phosphorylation and inhibition of GSK-3, ERKs prevent the formation of large conductance channels known as mitochondrial permeability transition pores, which play an important role in the release of pro-apototic molecules and the disruption of the respiratory chain (54, 93). Although it is difficult to generalize the role of the MEK/ERK signaling in the regulation of cell death, it remains that its proper control by phosphatases, and among them DUSP6, is critical.

Many agonists other than FGF activate ERKs during development and in adult tissues. If some of them, including EGF, NGF, HGF, VEGF, and PDGF, induce the transcription of *dusp6* (45, 48, 55, 131), few data emerged concerning the regulation of their respective signaling pathways by DUSP6 in vivo. The emergence of FGF-related phenotypes in *dusp6*−/− animal models might first be due to the prevalence of the FGF family in fundamental developmental processes, especially at early stages of development. Alternatively, other growth factors may induce the transcription of additional members of the DUSP family, which may compensate for the lack of DUSP6 in knock-out models as far as the specific cellular responses to these growth factors are concerned. This may be the case in embryonic lethal PDGF-B null embryos (79), as PDGF was shown to induce multiple *dusp* genes, including *dusp6* but also *dusp1* (55). FGF itself may be involved in the regulation other DUSPs, including DUSP7 and DUSP9, as shown in the developing mouse ear (129).

Another hypothesis for the preferential relationship between FGF and DUSP6 may rely on differential spatiotemporal activation of ERKs by different growth factors, displaying specific kinetics in which DUSP6 may play a specific role. It is well known for EGF versus NGF signaling in PC12 cells, for example, where NGF stimulates a long-lasting ERK phosphorylation leading to neural differentiation (58). FGF also induces a much more sustained phosphorylation of ERK than EGF in mouse embryonic fibroblasts (141), but the long-lasting signal is associated with proliferation in this cell type. In addition, different agonists may differentially influence the subcellular compartmentalization of ERKs. Nuclear accumulation of ERK upon EGF stimulation is transient in HeLa cells (20). But differential compartmentalization could also affect regulators of ERK signaling, such as phosphatases. If the apparent subcellular localization of DUSP6 is cytoplasmic, several subcompartments exist in the cytoplasm, including MAPK scaffolding complexes, which may contain different molecules depending on the agonist stimulus (66). For example, the long-lasting activation of ERK by NGF in neural cells lies on its ability to induce the phosphorylation of the scaffold protein FRS2, responsible for the stabilization of a signaling complex, including the NGF receptor Crk, the small G-protein Rap-1, and B-Raf (58). In this respect, DUSP6 may perhaps preferentially associate with certain types of scaffolds, namely those involved in the regulation of FGF signaling. Experiments showing that the invalidation of *dusp6* had no effect on the regulation of ERKs by α-adrenergic agonists (86), favored the hypothesis that DUSP6 involvement in ERK regulation may depend on the nature of the agonist and on the duration of the ERK signal (89).

Finally, although other growth factors are able to induce *dusp6*, there might be also a special, preferential relationship between FGF and DUSP6 at the level of transcription in some circumstances. Transcriptional activation of *dusp6* by FGF might be favored and precisely regulated by specific FGF signaling pathways (31) and through FGF-specific responsive elements in the regulatory regions of the *dusp6* gene. The promoter and intronic sequences of the collagenase syndecan-1 or osteocalcin genes contain multipartite FGF-responsive elements composed of sites for Ets1 but also AP1 and T-cell factor-lymphoid enhancer factor (TCF-LEF) families of transcription factors. In this context, FGFs were suggested to elaborate transcriptional activation signals that functionally differ from those of other RTK agonists (52, 97, 105). Functional binding sites for both Ets and TCF-LEF families are found in the *dusp6* promoter (36, 127), and their juxtaposition and combination at specific locations in the regulatory regions of the *dusp6* gene may play a role in the regulation of FGF response in vivo. Alternatively, FGF signaling may also regulate the access of transcription factors to promoter regions of *dusp6* through specific epigenetic mechanisms and modifications of the chromatin, as described previously for other genes (31).

DUSP6 in pathologies. As mentioned previously, animal models suggest that DUSP6 may be negatively involved in some cardiac pathologies. But changes in DUSP6 expression have been detected in several other pathological conditions, including neuronal diseases. While DUSP6 is downregulated in familial amyloidotic polyneuropathy (94), its expression is increased in human neocortical epileptic foci (106). Deregula-

tion of this phosphatase might also be involved in other neuronal pathologies. DUSP6 was recently found to block dynamin-dependent internalization of dopamine transporter, leading to a reinforcement of the transporter activity (95). This effect was mediated through the inhibition of dynamin-dependent internalization. However, it was not mimicked by pharmacological inhibition of MAPKs, suggesting that DUSP6 regulates internalization through a yet unidentified MEK/ERK-independent mechanism (95). At the enzymatic level, DUSP6 oxidation and catalytic inhibition by reactive oxygen species (ROS) might also be involved in oxidative stress in neurons, a characteristic of diverse neuropathological conditions including stroke, Parkinson's disease, and Alzheimer's disease (70).

DUSPs and Regulation of Immune Response

DUSP1 involvement in the immune system has been extensively reviewed elsewhere recently (25, 74), and we will therefore only briefly summarize these findings.

Null animals for *dusp1* have an increased incidence and severity of induced autoimmune arthritis and are more sensitive to lethal endotoxic shock (24, 50, 112, 146). Concordant with these phenotype effects, a more important production of cytokines was found in macrophages from these animals. Higher p38 and JNK MAPK activities were detected in these cells in response to bacterial lipopolysaccharide LPS, whereas ERK signaling was not significantly modified. DUSP1 thus appears as a major modulator of the systemic and local innate immune response.

This is also the case for another member of the family, i.e., DUSP10, which preferentially dephosphorylates JNK and p38 MAPK. Like in *dusp1* null mice, *dusp10*$-/-$ macrophages produce more pro-inflammatory cytokines when exposed to LPS. In addition, *dusp10* KO mice present increased resistance to experimental autoimmune encephalitis but show little difference in primary response to infection with lymphocytic choriomeningitis virus (LCV) when compared with wild-type animals. Nevertheless, a second exposure to LCV provokes immune-mediated lethality among *dusp10* null mice, probably due to higher serum levels of tumor necrosis factor (TNF) after virus exposure (143).

If the absence of DUSP1 and DUSP10 causes exaggerated inflammation, *dusp2* knockout mice were found on the contrary to have reduced inflammation in an autoimmune model of rheumatoid arthritis. Macrophages from these knock-out mice produce lower levels of proinflammatory cytokines and chemokines after LPS stimulation than their wild-type counterparts. It may seem surprising that DUSP2, which dephosphorylates p38 and ERK in vitro just like DUSP1, may cause effects opposite to that of this latter phosphatase. At the molecular level, *dusp2* invalidation induces an increase in JNK activation concomitant with a decrease in ERK and p38 activities (53). When JNK inhibitors are used, ERK activity can be rescued, suggesting a negative cross-talk between the two MAPKs. Thus one role of DUSP2 would be to strengthen ERK activity by inhibiting JNK. Why ERK activity is not decreased in *dusp1*$-/-$ mice is not clear, and other unidentified partners specific to each phosphatase may be involved in this differential regulation.

DUSPs and Cancer

MAPKs are implicated in important events involved in cell proliferation, survival, and migration, often deregulated in cancers. Oncogenic abnormalities have often been found in upstream components of the MEK/ERK pathway, and p38 MAPK and JNK may also play a role as tumor suppressors in some conditions (reviewed in Ref. 61). In addition, MAPK are also involved in the response of tumor cells to anti-cancer treatments. DUSPs may thus have a determinant role in cancer induction and progression. Evidences about the correlation of altered expression of some DUSPs in cancer are accumulating over the years and suggest that these phosphatases can act as tumor regulators according to the cancer type and to the state of progression. Variations in expression may have several causes, one of them being loss of heterozygosity, as shown for DUSP4, DUSP6, DUSP7, DUSP10, and DUSP16 (101).

Table 3 summarizes the dysregulation of DUSPs expression in many human cancers. Many tumors express high levels of DUSP1, but this increase is often specific to certain stages of tumor progression. In human epithelial tumors including prostate, colon, and bladder, DUSP1 is overexpressed during early phases of cancer and progressively diminishes during tumor progression. For example, DUSP1 expression is increased in benign prostate hyperplasia and high-grade prostate intraepithelial neoplasia, but its expression is lost in later stages of this cancer. Increased levels of DUSP1 in human prostate cancer correlated with a decrease in apoptosis (85). Experiments performed in nude mice showed that the downregulation of DUSP1 expression leads to a reduction of tumorigenicity of pancreatic cancer cells (76). But DUSP1 is also upregulated by hypoxia, a hallmark of many grown tumors (8), and is also increased in invasive stages of certain cancers. Hence, an increase in DUSP1 expression was detected in 60% of invasive ovarian carcinomas and was found to be a prognostic marker for short progression-free survival (32). DUSP1 is also more specifically expressed in poorly differentiated and late stages of breast cancer, where it correlated with reduced JNK activity but also with caspase activation and DNA fragmentation (121). DUSP1 is also expressed at higher levels in NSCLC cell lines. Finally, siRNA studies revealed that DUSP1 enhances resistance of breast cancer, osteosarcoma, and non-small cell lung carcinoma cell lines to cisplatin, doxorubicin, or paclitaxel by inhibiting JNK activity (28, 113, 121, 134, 135). Thus DUSP1 could be considered as an interesting target for improving cancer treatment and treating drug resistance cases in those cases where it is overexpressed and where its downregulation increases apoptosis. However, it should be noted that in other cancer types, such as hepatocellular carcinoma, DUSP1 may have an opposite role in tumor progression, since its expression is decreased rather than upregulated (128).

Among the other inducible nuclear phosphatases, DUSP4 is also overexpressed in early stages of ovarian tumors, in pancreatic cancer cells with K-ras mutations, in hepatomas, and breast cancer (reviewed in Ref. 61). However, its expression levels are decreased in lung tumors harboring EGFR mutations (26).

The potential role of DUSP2 also depends on the cellular context as loss of its expression in acute leukemias correlates

Table 3. *Expression of DUSP1, 2, 4, 6, 7, and 9 in human cancers*

DUSP	Cancer	Levels of Expression of Phosphatase, Relation of the Expression With Stage of Cancer Progression, Resistance and Other Findings
DUSP1	Human epithelial tumors: prostate, colon and bladder	Overexpression of DUSP1 found only in the early phases of human epithelial tumors. DUSP1 expression decreases in tumors with higher histological grade and metastasis (83).
	Prostate	Expression of DUSP1 is increased in benign prostate hyperplasia and high-grade-prostate intraepithelial neoplasia but its expression is lost in later stages of this cancer.
		DUSP1 expression is lower in hormone-refractory prostate carcinomas, compared to benign prostate hyperplasia or untreated prostate carcinomas (108).
	Gastric	DUSP1 is overexpressed in gastric adenocarcinoma (3).
	Pancreas	Downregulation of DUSP1 decreases tumorigenicity of pancreatic cancer cells in nude mice (76).
	Ovary	Moderate to strong expression of DUSP1 in 60% of invasive ovarian carcinomas.
		DUSP1 expression is a prognostic marker for shorter progression-free survival (32).
	Breast	DUSP1 expression is increased in malignant versus nonmalignant samples of breast cancers.
		High levels of DUSP-1 correlated with a decrease in JNK activity in breast cancer cells (134)
	Non-small-cell lung cancer (NSCLC)	Increased levels of DUSP1 in NSCLC cells compared with normal lung cells
		Higher expression of DUSP1 in NSCLC than in small cell lung cancer cells (130).
	Liver	The expression of DUSP1 is decreased in tumor cells compared with normal hepatocytes (128).
		The decrease in DUSP1 expression correlates with serum α-fetoprotein levels and tumor size (128).
	Chemoresistance	Treatment with cisplatin produces a high expression of DUSP1 which correlates with resistance to cisplastin and reduced levels of JNK.
		In NSCLC cell lines, siRNA mediated downregulation of *dusp1* caused an increase in sensitivity to cisplatin and an increase in JNK and p38 activation upon cisplastin treatment. In nude mice, *dusp1* siRNA xenografts displayed slower growth rates and increased susceptibility to cisplatin (22).
		Overexpression of DUSP1 inhibits Fas ligand-induced apoptosis in human prostate DU145 cells (124).
		Overexpression of DUSP1 decreases proteasome inhibitor-mediated apoptosis in human mammary epithelial and breast carcinoma cell lines, which correlates with decreased pJNK levels (121).
	Hypoxia	Upregulation of DUSP1 in hypoxia (5, 81, 82, 91, 115).
DUSP2	Serous carcinoma of the ovary	Presence of DUSP2 was associated with a poor outcome in overall survival (47).
	Acute Leukemias	Loss of DUSP2 expression has been associated with elevated levels of ERK activation in acute leukemias (63).
DUSP4	Ovary	DUSP4 is overexpressed in serous borderline tumors, compared with serous carcinomas (119).
	Pancreas	High expression of DUSP4 in pancreas cancer cells lines harboring a K-ras mutation and with elevated Raf/MEK activities (142).
	Breast cancer	High levels of DUSP4 in breast cancer cells (134).
DUSP6	Pancreas	Loss of DUSP6 expression correlates with methylation of CpG sequences in intron 1 of *dusp6* gene in both pancreatic cell lines and in some pancreatic cancer tissues (140).
		Increased DUSP6 expression in mildly and severely dysplastic/in situ carcinoma cells but DUSP6 expression is downregulated in invasive ductal carcinoma.
		In cultured pancreatic cancer cells, the reintroduction of DUSP6 in cells having reduced levels of DUSP6 results in a decrease of ERK signaling, suppression of cell growth and increased apoptosis (43).
	Myeloma	DUSP6 is overexpressed in myeloma with a constitutively active form of N-ras (29).
	Melanoma	DUSP6 is overexpressed in melanoma cell lines having an activating B-raf form (6).
	Glioma	High expression of DUSP6 in glioma expressing a constitutively active EGFR, EGFRvIII (107).
	Keratinocytes and breast cancer	High levels of DUSP6 in keratinocytes and breast cancer cells expressing oncogenic H-ras (136).
		High expression of DUSP6 in patients displaying tamoxifen resistance and in cells grown in culture in the chronic presence of the drug (30).
	Lung	In primary lung cancers, DUSP6 expression levels decreased in advanced histological grade of the tumor. DUSP6 restoration in lung cancer cells suppresses cellular growth (100).
		siRNA of DUSP6 in lung cancer cells increases ERK 1/2 activity and cellular proliferation. Overexpression of DUSP6 synergizes with EGFR inhibitor treatment in an EGFR-mutant cancer cell line (145).
	Ovarian cancer	Downregulation of DUSP6 in primary human cells of ovarian cancer, associated with high ERK activity (21).
		DUSP6 knockdown increases the resistance of ovarian cancer cells to cisplatin and forced expression of DUSP6 reduces ERK activity and resensitizes cells to cisplastin-induced cell death in vitro and in vivo (21).
	Hypoxia	DUSP6 expression is increased in tissues of animals exposed to hypoxia (91), in melanoma cells cultured in 3D spheroids (46) and in arterial cells (87).
	Tumorogenicity in Mice	Expression of an inducible fusion protein between DUSP6 and GFP in H-ras transformed fibroblasts injected in mice provokes a delay in the apparition of tumors and in tumor growth (88).
DUSP7	Acute leukemia	DUSP7 protein and mRNA are high in leukocytes from acute myeloid leukemia patients (71, 72)
DUSP9	Mice	In a clonal model of epidermal carcinogenesis that lacks Ras mutation, DUSP9 expression is downregulated at initiation and lost at malignant conversion. Reexpression of DUSP9 in malignant tumor cells provokes cell death and tumor suppression (82).

See text for more details.

with high levels of ERK activity (63), whereas high levels of DUSP2 expression have been associated with poor outcome of overall survival in serous carcinomas of the ovary (47).

Among cytoplasmic phosphatases, DUSP7 is overexpressed in acute leukemia (71, 72), especially in leukocytes from myeloid leukemia (AML) patients, but it is not clear if these observations can have a prognosis signification. In contrast, studies on a non-Ras model of epithelial carcinogenesis suggested that DUSP9 might be a tumor suppressor (82). Indeed, DUSP9 expression levels decrease in tumors and its reexpres-

sion results in cell death and suppression of tumor formation in subcutaneously injected mice (82).

DUSP6 in cancer. DUSP6 has been the subject of many studies related with cancer progression and resistance.

Pancreatic cancer is frequently associated with loss of heterozygosity in several chromosomal regions, including 12q21-q23.1 among others. *Dusp6* gene is located in this region and is often underexpressed in pancreatic cancer tissues and cell lines, as a result of hypermethylation of its promoter (140). The reintroduction of DUSP6 in these cells through adenoviral infection results in downregulation of ERK signaling, suppression of cell growth, and increased apoptosis. DUSP6 expression is exquisitely regulated in pancreatic cancer progression; while it is upregulated in intraepithelial neoplasia compared with normal cancer cells, it is often totally abrogated when tumor progresses towards the invasive ductal carcinoma state. This finding is reminiscent of those observed for DUSP1 expression, as mentioned above. Moreover, some intraductal papillary-mucinous adenoma/borderline neoplasms exhibit concomitant altered expression of DUSP6 and mutations in KRAS2 (43). This suggests that the downregulation or absence of DUSP6 synergizes with gain-of-function KRAS mutations occurring in a vast majority of pancreatic cancer cells and contributes to hyperactivation of ERK signaling.

Very recently, downregulation of DUSP6 expression was also observed in lung cancer, and restoration of its expression resulted in suppressed tumor growth (100).

In contrast, DUSP6 appear to be upregulated in other types of cancers harboring activating mutations in the Ras-MAPK pathway. B-raf is frequently mutated in melanomas but also in benign melanocytic nevi. The V600E mutant accounts for over 90% of these B-raf mutations and results in the overactivation of MEK and ERK in melanocytes. Other melanomas exhibit Q61R N-ras mutations, mainly in sun-exposed sites. Both mutations are mutually exclusive that suggests that N-ras and B-raf act linearly in the signaling pathway. DUSP6 levels were increased in cell lines from both types of melanoma (6). High levels of DUSP6 were also reported in keratinocytes and breast cancer cells expressing oncogenic H-ras (136). DUSP6 was also found to be upregulated by BCR/ABL in chronic myeloid leukemia (49) and in glioma expressing a constitutively active EGFR, EGFRvIII (107). It is likely that overactivation of the MEK/ERK axis in these cell types results in transcriptional activation of the *dusp6* gene.

Thus data obtained with different tumor types indicate that mutations leading to MEK/ERK activation do not necessarily predict a specific fate for DUSP6 expression levels. A parallel may perhaps be drawn with the finding that activating mutations in the Ras/Raf/MEK/ERK axis does not always result in global ERK overactivation (51) and that ERK activity levels is not correlated with cancer aggressiveness.

Regulation of DUSP6 expression and activity by hypoxia. During development and pathological conditions including cancer, cells can be exposed to changes in oxygen levels from hyperoxia to hypoxic conditions, leading to adaptations in cell metabolism and gene expression. In hypoxic conditions, the transcription factor HIF (hypoxia inducible factor) controls the expression of over 100 genes, including those coding for angiogenic factors such as VEGF, carbonic anhydrase IX, or Bnip3, a member of the BH3-only protein family of cell death factors. In arterial endothelial cells cultured in hypoxia, DUSP6 was found to be upregulated in the same order of magnitude as VEGF (87). In addition, the expression of DUSP6 was also found to be 9.2-fold higher in tumor spheroids of NA8 melanoma than in their two-dimensional cultured counterparts (46). This is probably related to the formation of a hypoxic gradient within the spheroids, which also triggers the induction of typical hypoxic genes such as carbonic anhydrase IX.

In endothelial cells, effect of hypoxia on DUSP6 induction was mimicked by adenoviral expression of a constitutively active form of HIF-1α (87). It is not clear, however, whether the effects of hypoxia on DUSP6 expression are direct and mediated through putative HIF-responsive elements in regulatory regions of the *dusp6* gene. Several studies on cells of neural origin suggest an indirect mechanism through nitric oxide (NO) synthase, a downstream target of HIF (41, 42, 91).

Surprisingly, the increase in *dusp6* mRNA caused by hypoxia in neurons is concomitant with an increase in ERK activity. This might be explained by the inhibition of DUSP6 enzymatic activity by NO, through the modification of a critical cysteine residue in the catalytic domain of the phosphatase. This cysteine may also be a target for other products generated by hypoxic conditions, including mitochondrial ROS. TNF-α-induced ROS convert the catalytic cysteine 293 of the phosphatase to sulfenic acid and causes its enzymatic inactivation (57). Other cysteine residues were shown to be oxidized by mild treatment with H_2O_2 (116), but their oxidation did not have any overt effect on DUSP6 catalytic activity (57). Moreover, ROS also have a negative effect on the stability of the DUSP6 protein in ovarian carcinoma, by stimulating its ubiquitination and subsequent degradation by the proteasome (21). However, effects of NO and ROS might be cell-type specific: hence, in endothelial cells, NO was found to downregulate DUSP6 not only at the protein level but also at the mRNA level (111).

Finally, it is interesting to note that DUSP6 activity is also decreased in lung macrophages exposed to hyperoxia, resulting in ERK overactivation and cell survival (99).

DUSP6 and resistance to chemotherapy. Recent data show that DUSP6 can also be involved in the resistance of tumors to drug treatment. A downregulation of DUSP6 at the protein and mRNA level has been detected in primary human cells of ovarian cancer and is associated with high ERK activity (21). Further DUSP6 knockdown by siRNA increases the resistance of these cells to cisplatin, a DNA cross-linker and an apoptosis inducer. On the contrary, forced expression of DUSP6 reduces ERK activity and resensitizes them to cisplastin-induced cell death in vitro and in vivo (21).

Estrogen receptor-α (ER-α) is expressed in 70% of breast cancers and is classically targeted with anti-estrogen therapies, such as tamoxifen, which competes with estrogen for ER and causes cell cycle arrest. Unfortunately, tamoxifen resistance is not a rare phenomenon. Although many mechanisms may play a role in tumor escape, DUSP6 is highly expressed in patients displaying tamoxifen resistance, and in cells grown in culture in the chronic presence of the drug (30). Transfection of nonresistant MCF-7 cells with DUSP6 provided a growth advantage in conditions where tamoxifen was added to the culture. Accordingly, the growth of Ishikawa cells, which express high levels of endogenous DUSP6 and are resistant to tamoxifen, is affected upon siRNA-mediated DUSP6 knock-

down in tamoxifen culture conditions. Similar results were obtained with tumors grown in nude mice, where DUSP6-overexpressing cells show higher growth rates than controls in tamoxifen-treated animals. However, in the same study, Ciu et al. (30) have shown that tamoxifen also blocks DUSP6 catalytic activity through ROS production. Hence, it is difficult to reconcile these data with the dependence on DUSP6 for cell resistance to tamoxifen. This may be due to an exquisite but necessary regulation of DUSP6 at both the transcriptional and catalytic level, leading to the best conditions allowing resistance, or to a potential role of DUSP6 independent of its enzymatic activity.

DUSPs as targets for therapies? Altogether these findings show that DUSPs/MKPs phosphatases may play a role in both cancer progression and cancer resistance. Therefore, DUSPs are rational targets for novel therapeutics. However, depending on the cases studied, each of them may have either a tumor-promoting or, on the contrary, a tumor-suppressing effect. It is therefore difficult to predict in which cells these molecules may be used as a target against tumor progression, unless tissue samples are analyzed for expression before potential treatment. In this respect, one may draw a parallel with MAPK signaling in tumors. Although ERKs seem to play a critical role in the proliferation of both untransformed and transformed cells, although oncogenic abnormalities are often found in the signaling pathways regulating ERKs, the magnitude of ERK signaling varies tremendously among different tumors and does not allow any prediction concerning their growth or malignant properties (51). Whereas the growth of many cancer cells is prevented by MEK inhibitors both in vitro and in vivo (65, 117), persistent activation of ERK can also lead to cell-cycle arrest or cell death not only in nontransformed cells (16, 103) but also in lung cancer (109). The role of JNK and p38 MAPKs in cancer initiation and progression is also very complex. For example, JNK1 and JNK2 seem to have opposite role in cell proliferation, and each of these kinases may be either pro- or anti-apoptotic depending on cell types and on the duration of the stimulus. Finally, gene invalidation of *jnk1* or *jnk2* increased or suppressed skin tumorigenesis in knockout mice, respectively (23, 118). p38 MAPKs also display different pro- versus anti-apoptotic properties depending on the context, block proliferation, and promote cell differentiation in some systems but favor invasion and metastasis in other instances. The existence of cross-talks between these MAPK pathways, where the inhibition of one type of MAPK may have an impact on the activity of another one, adds even more complexity to the picture (133). This complexity of signaling networks has produced surprising results in the search for inhibitors of another major signaling pathway involved in cell growth and proliferation, the mTOR pathway. While preventing tumor progression in some models, mTOR inhibition by rapamycin actually stimulates the prosurvival and promigration Akt signaling pathway in other tumor types, as a result of the loss of the feedback inhibition of mTOR on the insulin receptor (90). Those findings proved the need for identifying predictive markers of sensitivity to specific pharmacological treatments and even the need for checking the inhibitor effects on the tumor tissue signaling status during the first phases of these potential treatments.

Despite these difficulties and because some DUSPs are upregulated in many cancers and involved in tumor resistance to cytotoxic treatments, a novel interest for pharmacological DUSP inhibitors has developed recently (2, 92, 132). Many of these inhibitors induce the formation of ROS and some of them were shown to inhibit DUSP1 and DUSP6 and reverses resistance to paclitaxel (132). Whereas initial studies were performed in vitro, a recent work by Molina et al. (92) used chemical screen in zebrafish and identified (E)-2-benzydilene-3-(cyclohexilamino)-2,3-dihydro-1H-inden-1-one (BCI) as an allosteric inhibitor of DUSP6 that prevents the catalytic activation of the phosphatase induced by substrate binding. However, BCI is, like most of the others inhibitors, not specific to particular DUSPs. This may actually constitute a therapeutic advantage, as proved by the ever-growing testing of nonspecific tyrosine kinase inhibitors in clinical trials. Indeed, simultaneous blockade of several actors in cell signaling pathways is often preferred and sometimes delays resistance. It may be interesting to block several DUSPs simultaneously, such as DUSP1 and DUSP6, both involved in cancer resistance to cytotoxic agents, in tumor types where their expression is deregulated.

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REFERENCES

1. **Arkell RS, Dickinson RJ, Squires M, Hayat S, Keyse SM, Cook SJ.** DUSP6/MKP-3 inactivates ERK1/2 but fails to bind and inactivate ERK5. *Cell Signal* 20: 836–843, 2008.
2. **Arnold DM, Foster C, Huryn DM, Lazo JS, Johnston PA, Wipf P.** Synthesis and biological activity of a focused library of mitogen-activated protein kinase phosphatase inhibitors. *Chem Biol Drug Des* 69: 23–30, 2007.
3. **Bang YJ, Kwon JH, Kang SH, Kim JW, Yang YC.** Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. *Biochem Biophys Res Commun* 250: 43–47, 1998.
4. **Bermudez O, Marchetti S, Pages G, Gimond C.** Post-translational regulation of the ERK phosphatase DUSP6/MKP3 by the mTOR pathway. *Oncogene* 27: 3685–3691, 2008.
5. **Bernaudin M, Tang Y, Reilly M, Petit E, Sharp FR.** Brain genomic response following hypoxia and re-oxygenation in the neonatal rat. Identification of genes that might contribute to hypoxia-induced ischemic tolerance. *J Biol Chem* 277: 39728–39738, 2002.
6. **Bloethner S, Chen B, Hemminki K, Muller-Berghaus J, Ugurel S, Schadendorf D, Kumar R.** Effect of common B-RAF and N-RAS mutations on global gene expression in melanoma cell lines. *Carcinogenesis* 26: 1224–1232, 2005.
7. **Boutros T, Chevet E, Metrakos P.** Mitogen-activated protein (MAP) kinase/MAP kinase phosphatase regulation: roles in cell growth, death, and cancer. *Pharmacol Rev* 60: 261–310, 2008.
8. **Brahimi-Horn MC, Chiche J, Pouyssegur J.** Hypoxia and cancer. *J Mol Med* 85: 1301–1307, 2007.
9. **Brodie SG, Deng CX.** Mouse models orthologous to FGFR3-related skeletal dysplasias. *Pediatr Pathol Mol Med* 22: 87–103, 2003.
10. **Brondello JM, Pouyssegur J, McKenzie FR.** Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. *Science* 286: 2514–2517, 1999.
11. **Brown JL, Snir M, Noushmehr H, Kirby M, Hong SK, Elkahloun AG, Feldman B.** Transcriptional profiling of endogenous germ layer precursor cells identifies dusp4 as an essential gene in zebrafish endoderm specification. *Proc Natl Acad Sci USA* 105: 12337–12342, 2008.
12. **Brunet A, Roux D, Lenormand P, Dowd S, Keyse S, Pouyssegur J.** Nuclear translocation of p42/p44 mitogen-activated protein kinase is

required for growth factor-induced gene expression and cell cycle entry. *EMBO J* 18: 664–674, 1999.

13. **Bueno OF, De Windt LJ, Lim HW, Tymitz KM, Witt SA, Kimball TR, Molkentin JD.** The dual-specificity phosphatase MKP-1 limits the cardiac hypertrophic response in vitro and in vivo. *Circ Res* 88: 88–96, 2001.
14. **Bueno OF, Molkentin JD.** Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death. *Circ Res* 91: 776–781, 2002.
15. **Bundschu K, Walter U, Schuh K.** Getting a first clue about SPRED functions. *Bioessays* 29: 897–907, 2007.
16. **Cagnol S, Van Obberghen-Schilling E, Chambard JC.** Prolonged activation of ERK1,2 induces FADD-independent caspase 8 activation and cell death. *Apoptosis* 11: 337–346, 2006.
17. **Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, Chabert C, Boschert U, Arkinstall S.** Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* 280: 1262–1265, 1998.
18. **Cao W, Bao C, Padalko E, Lowenstein CJ.** Acetylation of mitogen-activated protein kinase phosphatase-1 inhibits Toll-like receptor signaling. *J Exp Med* 205: 1491–1503, 2008.
19. **Castelli M, Camps M, Gillieron C, Leroy D, Arkinstall S, Rommel C, Nichols A.** MAP kinase phosphatase 3 (MKP3) interacts with and is phosphorylated by protein kinase CK2alpha. *J Biol Chem* 279: 44731–44739, 2004.
20. **Caunt CJ, Rivers CA, Conway-Campbell BL, Norman MR, McArdle CA.** Epidermal growth factor receptor and protein kinase C signaling to ERK2: spatiotemporal regulation of ERK2 by dual specificity phosphatases. *J Biol Chem* 283: 6241–6252, 2008.
21. **Chan DW, Liu VW, Tsao GS, Yao KM, Furukawa T, Chan KK, Ngan HY.** Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. *Carcinogenesis* 29: 1742–1750, 2008.
22. **Chattopadhay S, Machado-Pinilla R, Manguan-Garcia C, Belda-Iniesta C, Moratilla C, Cejas P, Fresno-Vara JA, de Castro-Carpeno J, Casado E, Nistal M, Gonzalez-Barón M, Perona R.** MKP1/CL100 controls tumor growth and sensitivity to cisplatin in non-small-cell lung cancer. *Oncogene* 25: 3335–3345, 2006.
23. **Chen N, Nomura M, She QB, Ma WY, Bode AM, Wang L, Flavell RA, Dong Z.** Suppression of skin tumorigenesis in c-Jun NH(2)-terminal kinase-2-deficient mice. *Cancer Res* 61: 3908–3912, 2001.
24. **Chi H, Barry SP, Roth RJ, Wu JJ, Jones EA, Bennett AM, Flavell RA.** Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc Natl Acad Sci USA* 103: 2274–2279, 2006.
25. **Chi H, Flavell RA.** Acetylation of MKP-1 and the control of inflammation. *Sci Signal* 1: pe44, 2008.
26. **Chitale D, Gong Y, Taylor BS, Broderick S, Brennan C, Somwar R, Golas B, Wang L, Motoi N, Szoke J, Reinersman JM, Major J, Sander C, Seshan VE, Zakowski MF, Rusch V, Pao W, Gerald W, Ladanyi M.** An integrated genomic analysis of lung cancer reveals loss of DUSP4 in EGFR-mutant tumors. *Oncogene* 28: 2773–2783, 2009.
27. **Christie GR, Williams DJ, Macisaac F, Dickinson RJ, Rosewell I, Keyse SM.** The dual-specificity protein phosphatase DUSP9/MKP-4 is essential for placental function but is not required for normal embryonic development. *Mol Cell Biol* 25: 8323–8333, 2005.
28. **Cortes-Sempere M, Chattopadhyay S, Rovira A, Rodriguez-Fanjul V, Belda-Iniesta C, Tapia M, Cejas P, Machado-Pinilla R, Manguan-Garcia C, Sanchez-Perez I, Nistal M, Moratilla C, Castro-Carpeno JD, Gonzalez-Baron M, Albanell J, Perona R.** MKP1 repression is required for the chemosensitizing effects of NF-kappaB and PI3K inhibitors to cisplatin in non-small cell lung cancer. *Cancer Lett* 286: 206–216, 2009.
29. **Croonquist PA, Linden MA, Zhao F, Van Ness BG.** 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, 2003.
30. **Cui Y, Parra I, Zhang M, Hilsenbeck SG, Tsimelzon A, Furukawa T, Horii A, Zhang ZY, Nicholson RI, Fuqua SA.** Elevated expression of mitogen-activated protein kinase phosphatase 3 in breast tumors: a mechanism of tamoxifen resistance. *Cancer Res* 66: 5950–5959, 2006.
31. **Dailey L, Ambrosetti D, Mansukhani A, Basilico C.** Mechanisms underlying differential responses to FGF signaling. *Cytokine Growth Factor Rev* 16: 233–247, 2005.
32. **Denkert C, Schmitt WD, Berger S, Reles A, Pest S, Siegert A, Lichtenegger W, Dietel M, Hauptmann S.** Expression of mitogen-activated protein kinase phosphatase-1 (MKP-1) in primary human ovarian carcinoma. *Int J Cancer* 102: 507–513, 2002.
33. **Dorfman K, Carrasco D, Gruda M, Ryan C, Lira SA, Bravo R.** Disruption of the erp/mkp-1 gene does not affect mouse development: normal MAP kinase activity in ERP/MKP-1-deficient fibroblasts. *Oncogene* 13: 925–931, 1996.
34. **Eblaghie MC, Lunn JS, Dickinson RJ, Munsterberg AE, Sanz-Ezquerro JJ, Farrell ER, Mathers J, Keyse SM, Storey K, Tickle C.** Negative feedback regulation of FGF signaling levels by Pyst1/MKP3 in chick embryos. *Curr Biol* 13: 1009–1018, 2003.
35. **Echevarria D, Belo JA, Martinez S.** Modulation of Fgf8 activity during vertebrate brain development. *Brain Res Rev* 49: 150–157, 2005.
36. **Ekerot M, Stavridis MP, Delavaine L, Mitchell MP, Staples C, Owens DM, Keenan ID, Dickinson RJ, Storey KG, Keyse SM.** Negative-feedback regulation of FGF signalling by DUSP6/MKP-3 is driven by ERK1/2 and mediated by Ets factor binding to a conserved site within the DUSP6/MKP-3 gene promoter. *Biochem J* 412: 287–298, 2008.
37. **Emmons J, Townley-Tilson WH, Deleault KM, Skinner SJ, Gross RH, Whitfield ML, Brooks SA.** Identification of TTP mRNA targets in human dendritic cells reveals TTP as a critical regulator of dendritic cell maturation. *RNA* 14: 888–902, 2008.
38. **Farooq A, Chaturvedi G, Mujtaba S, Plotnikova O, Zeng L, Dhalluin C, Ashton R, Zhou MM.** Solution structure of ERK2 binding domain of MAPK phosphatase MKP-3: structural insights into MKP-3 activation by ERK2. *Mol Cell* 7: 387–399, 2001.
39. **Farooq A, Zhou MM.** Structure and regulation of MAPK phosphatases. *Cell Signal* 16: 769–779, 2004.
40. **Fjeld CC, Rice AE, Kim Y, Gee KR, Denu JM.** Mechanistic basis for catalytic activation of mitogen-activated protein kinase phosphatase 3 by extracellular signal-regulated kinase. *J Biol Chem* 275: 6749–6757, 2000.
41. **Freeman RK.** Medical and legal implications for necessary requirements to diagnose damaging hypoxic-ischemic encephalopathy leading to later cerebral palsy. *Am J Obstet Gynecol* 199: 585–586, 2008.
42. **Freeman SE, Patil VV, Durham PL.** Nitric oxide-proton stimulation of trigeminal ganglion neurons increases mitogen-activated protein kinase and phosphatase expression in neurons and satellite glial cells. *Neuroscience* 157: 542–555, 2008.
43. **Furukawa T, Fujisaki R, Yoshida Y, Kanai N, Sunamura M, Abe T, Takeda K, Matsuno S, Horii A.** Distinct progression pathways involving the dysfunction of DUSP6/MKP-3 in pancreatic intraepithelial neoplasia and intraductal papillary-mucinous neoplasms of the pancreas. *Mod Pathol* 18: 1034–1042, 2005.
44. **Furukawa T, Tanji E, Xu S, Horii A.** Feedback regulation of DUSP6 transcription responding to MAPK1 via ETS2 in human cells. *Biochem Biophys Res Commun* 377: 317–320, 2008.
45. **Gerritsen ME, Tomlinson JE, Zlot C, Ziman M, Hwang S.** Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. *Br J Pharmacol* 140: 595–610, 2003.
46. **Ghosh S, Spagnoli GC, Martin I, Ploegert S, Demougin P, Heberer M, Reschner A.** Three-dimensional culture of melanoma cells profoundly affects gene expression profile: a high density oligonucleotide array study. *J Cell Physiol* 204: 522–531, 2005.
47. **Givant-Horwitz V, Davidson B, Goderstad JM, Nesland JM, Trope CG, Reich R.** The PAC-1 dual specificity phosphatase predicts poor outcome in serous ovarian carcinoma. *Gynecol Oncol* 93: 517–523, 2004.
48. **Gomez AR, Lopez-Varea A, Molnar C, de la Calle-Mustienes E, Ruiz-Gomez M, Gomez-Skarmeta JL, de Celis JF.** Conserved cross-interactions in Drosophila and Xenopus between Ras/MAPK signaling and the dual-specificity phosphatase MKP3. *Dev Dyn* 232: 695–708, 2005.
49. **Hakansson P, Nilsson B, Andersson A, Lassen C, Gullberg U, Fioretos T.** Gene expression analysis of BCR/ABL1-dependent transcriptional response reveals enrichment for genes involved in negative feedback regulation. *Genes Chromosomes Cancer* 47: 267–275, 2008.
50. **Hammer M, Mages J, Dietrich H, Servatius A, Howells N, Cato AC, Lang R.** Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J Exp Med* 203: 15–20, 2006.

51. **Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, Shimada Y, Ari-i S, Wada H, Fujimoto J, Kohno M.** Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* 18: 813–822, 1999.
52. **Jaakkola P, Maatta A, Jalkanen M.** The activation and composition of FiRE (an FGF-inducible response element) differ in a cell type- and growth factor-specific manner. *Oncogene* 17: 1279–1286, 1998.
53. **Jeffrey KL, Brummer T, Rolph MS, Liu SM, Callejas NA, Grumont RJ, Gillieron C, Mackay F, Grey S, Camps M, Rommel C, Gerondakis SD, Mackay CR.** Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nat Immunol* 7: 274–283, 2006.
54. **Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, Sollott SJ.** Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest* 113: 1535–1549, 2004.
55. **Jurek A, Amagasaki K, Gembarska A, Heldin CH, Lennartsson J.** Negative and positive regulation of MAPK phosphatase 3 controls platelet-derived growth factor-induced Erk activation. *J Biol Chem* 284: 4626–4634, 2009.
56. **Kamakura S, Moriguchi T, Nishida E.** Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. *J Biol Chem* 274: 26563–26571, 1999.
57. **Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M.** Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120: 649–661, 2005.
58. **Kao S, Jaiswal RK, Kolch W, Landreth GE.** Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth factor and nerve growth factor in PC12 cells. *J Biol Chem* 276: 18169–18177, 2001.
59. **Karlsson M, Mathers J, Dickinson RJ, Mandl M, Keyse SM.** Both nuclear-cytoplasmic shuttling of the dual specificity phosphatase MKP-3 and its ability to anchor MAP kinase in the cytoplasm are mediated by a conserved nuclear export signal. *J Biol Chem* 279: 41882–41891, 2004.
60. **Kawakami Y, Rodriguez-Leon J, Koth CM, Buscher D, Itoh T, Raya A, Ng JK, Esteban CR, Takahashi S, Henrique D, Schwarz MF, Asahara H, and Izpisua Belmonte JC.** MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb. *Nat Cell Biol* 5: 513–519, 2003.
61. **Keyse SM.** Dual-specificity MAP kinase phosphatases (MKPs) and cancer. *Cancer Metastasis Rev* 27: 253–261, 2008.
62. **Kim M, Cha GH, Kim S, Lee JH, Park J, Koh H, Choi KY, Chung J.** MKP-3 has essential roles as a negative regulator of the Ras/mitogen-activated protein kinase pathway during Drosophila development. *Mol Cell Biol* 24: 573–583, 2004.
63. **Kim SC, Hahn JS, Min YH, Yoo NC, Ko YW, Lee WJ.** 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, 1999.
64. **Kinney CM, Chandrasekharan UM, Yang L, Shen J, Kinter M, McDermott MS, DiCorleto PE.** Histone H3 as a novel substrate for MAP kinase phosphatase-1. *Am J Physiol Cell Physiol* 296: C242–C249, 2009.
65. **Kohno M, Pouyssegur J.** Targeting the ERK signaling pathway in cancer therapy. *Ann Med* 38: 200–211, 2006.
66. **Kolch W.** Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol* 6: 827–837, 2005.
67. **Kovanen PE, Bernard J, Al-Shami A, Liu C, Bollenbahcer-Reilley J, Young L, Pise-Masison C, Spolski R, Leonard WJ.** T-cell development and function are modulated by dual specificity phosphatase DUSP5. *J Biol Chem* 283: 17362–17369, 2008.
68. **Krishna M, Narang H.** The complexity of mitogen-activated protein kinases (MAPKs) made simple. *Cell Mol Life Sci* 65: 3525–3544, 2008.
69. **Kuwano Y, Kim HH, Abdelmohsen K, Pullmann R Jr, Martindale JL, Yang X, Gorospe M.** MKP-1 mRNA stabilization and translational control by RNA-binding proteins HuR and NF90. *Mol Cell Biol* 28: 4562–4575, 2008.
70. **Levinthal DJ, Defranco DB.** Reversible oxidation of ERK-directed protein phosphatases drives oxidative toxicity in neurons. *J Biol Chem* 280: 5875–5883, 2005.
71. **Levy-Nissenbaum O, Sagi-Assif O, Raanani P, Avigdor A, Ben-Bassat I, Witz IP.** cDNA microarray analysis reveals an overexpression of the dual-specificity MAPK phosphatase PYST2 in acute leukemia. *Methods Enzymol* 366: 103–113, 2003.
72. **Levy-Nissenbaum O, Sagi-Assif O, Raanani P, Avigdor A, Ben-Bassat I, Witz IP.** Overexpression of the dual-specificity MAPK phosphatase PYST2 in acute leukemia. *Cancer Lett* 199: 185–192, 2003.
73. **Li C, Scott DA, Hatch E, Tian X, Mansour SL.** Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development. *Development* 134: 167–176, 2007.
74. **Li L, Chen SF, Liu Y.** MAP kinase phosphatase-1, a critical negative regulator of the innate immune response. *Int J Clin Exp Med* 2: 48–67, 2009.
75. **Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB.** Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem* 284: 15676–15684, 2009.
76. **Liao Q, Guo J, Kleeff J, Zimmermann A, Buchler MW, Korc M, Friess H.** Down-regulation of the dual-specificity phosphatase MKP-1 suppresses tumorigenicity of pancreatic cancer cells. *Gastroenterology* 124: 1830–1845, 2003.
77. **Lin NY, Lin CT, Chen YL, Chang CJ.** Regulation of tristetraprolin during differentiation of 3T3–L1 preadipocytes. *FEBS J* 274: 867–878, 2007.
78. **Lin YW, Chuang SM, Yang JL.** ERK1/2 achieves sustained activation by stimulating MAPK phosphatase-1 degradation via the ubiquitin-proteasome pathway. *J Biol Chem* 278: 21534–21541, 2003.
79. **Lindahl P, Johansson BR, Leveen P, Betsholtz C.** Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 277: 242–245, 1997.
80. **Lips DJ, Bueno OF, Wilkins BJ, Purcell NH, Kaiser RA, Lorenz JN, Voisin L, Saba-El-Leil M. K, Meloche S, Pouyssegur J, Pages G, De Windt LJ, Doevendans PA, and Molkentin JD.** MEK1-ERK2 signaling pathway protects myocardium from ischemic injury in vivo. *Circulation* 109: 1938–1941, 2004.
81. **Liu C, Shi Y, Han Z, Pan Y, Liu N, Han S, Chen Y, Lan M, Qiao T, Fan D.** Suppression of the dual-specificity phosphatase MKP-1 enhances HIF-1 transactivation and increases expression of EPO. *Biochem Biophys Res Commun* 312: 780–786, 2003.
82. **Liu Y, Lagowski J, Sundholm A, Sundberg A, and Kulesz-Martin M.** Microtubule disruption and tumour suppression by mitogen-activated protein kinase phosphatase 4. *Cancer Res* 67: 10711–10719, 2007.
83. **Loda M, Capodieci P, Mishra R, Yao H, Corless C, Grigioni W, Wang Y, Magi-Galluzzi C, Stork PJ.** Expression of mitogen-activated protein kinase phosphase-1 in the early phases of human epithelial carcinogenesis. *Am J Pathol* 149: 1553–1564, 1996.
84. **MacKeigan JP, Murphy LO, Blenis J.** Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nat Cell Biol* 7: 591–600, 2005.
85. **Magi-Galluzzi C, Montironi R, Cangi MG, Wishnow K, Loda M.** Mitogen-activated protein kinases and apoptosis in PIN. *Virchows Arch* 432: 407–413, 1998.
86. **Maillet M, Purcell NH, Sargent MA, York AJ, Bueno OF, Molkentin JD.** DUSP6 (MKP3) null mice show enhanced ERK1/2 phosphorylation at baseline and increased myocyte proliferation in the heart affecting disease susceptibility. *J Biol Chem* 283: 31246–31255, 2008.
87. **Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL.** Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 105: 659–669, 2005.
88. **Marchetti S, Gimond C, Chambard JC, Touboul T, Roux D, Pouyssegur J, Pages G.** Extracellular signal-regulated kinases phosphorylate mitogen-activated protein kinase phosphatase 3/DUSP6 at serines 159 and 197, two sites critical for its proteasomal degradation. *Mol Cell Biol* 25: 854–864, 2005.
89. **Meloche S, Pouyssegur J.** The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* 26: 3227–3239, 2007.
90. **Meric-Bernstam F and Gonzalez-Angulo AM.** Targeting the mTOR signaling network for cancer therapy. *J Clin Oncol* 27: 2278–2287, 2009.
91. **Mishra OP and Delivoria-Papadopoulos M.** Effect of hypoxia on the expression and activity of mitogen-activated protein (MAP) kinase-phosphatase-1 (MKP-1) and MKP-3 in neuronal nuclei of newborn piglets: the role of nitric oxide. *Neuroscience* 129: 665–673, 2004.

92. **Molina G, Vogt A, Bakan A, Dai W, de Oliveira PQ, Znosko W, Smithgall TE, Bahar I, Lazo JS, Day BW, Tsang M.** Zebrafish chemical screening reveals an inhibitor of Dusp6 that expands cardiac cell lineages. *Nat Chem Biol* 5: 680–687, 2009.
93. **Monick MM, Powers LS, Barrett CW, Hinde S, Ashare A, Groskreutz DJ, Nyunoya T, Coleman M, Spitz DR, Hunninghake GW.** Constitutive ERK MAPK activity regulates macrophage ATP production and mitochondrial integrity. *J Immunol* 180: 7485–7496, 2008.
94. **Monteiro FA, Sousa MM, Cardoso I, do Amaral JB, Guimaraes A, Saraiva MJ.** Activation of ERK1/2 MAP kinases in familial amyloidotic polyneuropathy. *J Neurochem* 97: 151–161, 2006.
95. **Mortensen OV, Larsen MB, Prasad BM, Amara SG.** Genetic complementation screen identifies a mitogen-activated protein kinase phosphatase, MKP3, as a regulator of dopamine transporter trafficking. *Mol Biol Cell* 19: 2818–2829, 2008.
96. **Muda M, Theodosiou A, Gillieron C, Smith A, Chabert C, Camps M, Boschert U, Rodrigues N, Davies K, Ashworth A, Arkinstall S.** The mitogen-activated protein kinase phosphatase-3 N-terminal noncatalytic region is responsible for tight substrate binding and enzymatic specificity. *J Biol Chem* 273: 9323–9329, 1998.
97. **Newberry EP, Boudreaux JM, Towler DA.** The rat osteocalcin fibroblast growth factor (FGF)-responsive element: an okadaic acid-sensitive, FGF-selective transcriptional response motif. *Mol Endocrinol* 10: 1029–1040, 1996.
98. **Nichols A, Camps M, Gillieron C, Chabert C, Brunet A, Wilsbacher J, Cobb M, Pouyssegur J, Shaw JP, Arkinstall S.** Substrate recognition domains within extracellular signal-regulated kinase mediate binding and catalytic activation of mitogen-activated protein kinase phosphatase-3. *J Biol Chem* 275: 24613–24621, 2000.
99. **Nyunoya T, Monick MM, Powers LS, Yarovinsky TO, Hunninghake GW.** Macrophages survive hyperoxia via prolonged ERK activation due to phosphatase down-regulation. *J Biol Chem* 280: 26295–26302, 2005.
100. **Okudela K, Yazawa T, Woo T, Sakaeda M, Ishii J, Mitsui H, Shimoyamada H, Sato H, Tajiri M, Ogawa N, Masuda M, Takahashi T, Sugimura H, Kitamura H.** Down-regulation of DUSP6 expression in lung cancer: its mechanism and potential role in carcinogenesis. *Am J Pathol* 175: 867–881, 2009.
101. **Patterson KI, Brummer T, O'Brien PM, Daly RJ.** Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem J* 418: 475–489, 2009.
102. **Pramanik K, Chun CZ, Garnaas MK, Samant GV, Li K, Horswill MA, North PE, Ramchandran R.** Dusp-5 and Snrk-1 coordinately function during vascular development and disease. *Blood* 113: 1184–1191, 2009.
103. **Pumiglia KM, Decker SJ.** Cell cycle arrest mediated by the MEK/mitogen-activated protein kinase pathway. *Proc Natl Acad Sci USA* 94: 448–452, 1997.
104. **Purcell NH, Wilkins BJ, York A, Saba -El-Leil MK, Meloche S, Robbins J, and Molkentin JD.** Genetic inhibition of cardiac ERK1/2 promotes stress-induced apoptosis and heart failure but has no effect on hypertrophy in vivo. *Proc Natl Acad Sci USA* 104: 14074–14079, 2007.
105. **Pursiheimo JP, Saari J, Jalkanen M, Salmivirta M.** Cooperation of protein kinase A and Ras/ERK signaling pathways is required for AP-1-mediated activation of fibroblast growth factor-inducible response element (FiRE). *J Biol Chem* 277: 25344–25355, 2002.
106. **Rakhade SN, Yao B, Ahmed S, Asano E, Beaumont TL, Shah AK, Draghici S, Krauss R, Chugani HT, Sood S, Loeb JA.** A common pattern of persistent gene activation in human neocortical epileptic foci. *Ann Neurol* 58: 736–747, 2005.
107. **Ramnarain DB, Park S, Lee DY, Hatanpaa KJ, Scoggin SO, Otu H, Libermann TA, Raisanen JM, Ashfaq R, Wong ET, Wu J, Elliott R, Habib AA.** Differential gene expression analysis reveals generation of an autocrine loop by a mutant epidermal growth factor receptor in glioma cells. *Cancer Res* 66: 867–874, 2006.
108. **Rauhala HE, Porkka KP, Tolonen TT, Martikainen PM, Tammela TL, Visakorpi T.** Dual-specificity phosphatase 1 and serum/glucocorticoid-regulated kinase are downregulated in prostate cancer. *Int J Cancer* 117: 738–745, 2005.
109. **Ravi RK, Weber E, McMahon M, Williams JR, Baylin S, Mal A, Harter ML, Dillehay LE, Claudio PP, Giordano A, Nelkin BD, Mabry M.** Activated Raf-1 causes growth arrest in human small cell lung cancer cells. *J Clin Invest* 101: 153–159, 1998.
110. **Rodriguez-Viciana P, Tetsu O, Tidyman WE, Estep AL, Conger BA, Cruz MS, McCormick F, Rauen KA.** Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. *Science* 311: 1287–1290, 2006.
111. **Rossig L, Haendeler J, Hermann C, Malchow P, Urbich C, Zeiher AM, Dimmeler S.** Nitric oxide down-regulates MKP-3 mRNA levels: involvement in endothelial cell protection from apoptosis. *J Biol Chem* 275: 25502–25507, 2000.
112. **Salojin KV, Owusu IB, Millerchip KA, Potter M, Platt KA, Oravecz T.** Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J Immunol* 176: 1899–1907, 2006.
113. **Sanchez-Perez I, Martinez-Gomariz M, Williams D, Keyse SM, Perona R.** CL100/MKP-1 modulates JNK activation and apoptosis in response to cisplatin. *Oncogene* 19: 5142–5152, 2000.
114. **Sarkozi R, Miller B, Pollack V, Feifel E, Mayer G, Sorokin A, Schramek H.** ERK1/2-driven and MKP-mediated inhibition of EGF-induced ERK5 signaling in human proximal tubular cells. *J Cell Physiol* 211: 88–100, 2007.
115. **Seta KA, Kim R, Kim HW, Millhorn DE, and Beitner-Johnson D.** Hypoxia-induced regulation of MAPK phosphatase-1 as identified by subtractive suppression hybridization and cDNA microarray analysis. *J Biol Chem* 276: 44405–44412, 2001.
116. **Seth D, Rudolph J.** Redox regulation of MAP kinase phosphatase 3. *Biochemistry* 45: 8476–8487, 2006.
117. **Sharma A, Tran MA, Liang S, Sharma AK, Amin S, Smith CD, Dong C, Robertson GP.** Targeting mitogen-activated protein kinase/extracellular signal-regulated kinase kinase in the mutant (V600E) B-Raf signaling cascade effectively inhibits melanoma lung metastases. *Cancer Res* 66: 8200–8209, 2006.
118. **She QB, Chen N, Bode AM, Flavell RA, Dong Z.** 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, 2002.
119. **Sieben NL, Oosting J, Flanagan AM, Roemen GM, Kolkman-Uliee SM, van Eijk R, Cornelisse CJ, Fleuren GJ, van Engeland M.** Differential gene expression in ovarian tumors reveals Dusp4 and Serpina5 are key regulators for benign behaviour of serous borderline tumors. *J Clin Oncol* 23: 7257–7264, 2005.
120. **Slack DN, Seternes OM, Gabrielsen M, Keyse SM.** Distinct binding determinants for ERK2/p38alpha and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J Biol Chem* 276: 16491–16500, 2001.
121. **Small GW, Shi YY, Higgins LS, Orlowski RZ.** Mitogen-activated protein kinase phosphatase-1 is a mediator of breast cancer chemoresistance. *Cancer Res* 67: 4459–4466, 2007.
122. **Smith TG, Karlsson M, Lunn JS, Eblaghie MC, Keenan ID, Farrell ER, Tickle C, Storey KG, Keyse SM.** Negative feedback predominates over cross-regulation to control ERK MAPK activity in response to FGF signalling in embryos. *FEBS Lett* 580: 4242–4245, 2006.
123. **Smith TG, Sweetman D, Patterson M, Keyse SM, Munsterberg A.** Feedback interactions between MKP3 and ERK MAP kinase control scleraxis expression and the specification of rib progenitors in the developing chick somite. *Development* 132: 1305–1314, 2005.
124. **Srikanth S, Franklin CC, Duke RC, Kraft RS.** Human DU145 prostate cancer cells overexpressing mitogen-activated protein kinase phosphatase-1 are resistant to Fas ligand-induced mitochondrial perturbations and cellular apoptosis. *Mol Cell Biochem* 199: 169–178, 1999.
125. **Tanoue T, Adachi M, Moriguchi T, Nishida E.** A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat Cell Biol* 2: 110–116, 2000.
126. **Theodosiou A, Ashworth A.** MAP kinase phosphatases. *Genome Biol* 3: S3009, 2002.
127. **Tsang M, Maegawa S, Kiang A, Habas R, Weinberg E, Dawid IB.** A role for MKP3 in axial patterning of the zebrafish embryo. *Development* 131: 2769–2779, 2004.
128. **Tsujita E, Taketomi A, Gion T, Kuroda Y, Endo K, Watanabe A, Nakashima H, Aishima S, Kohnoe S, Maehara Y.** Suppressed MKP-1 is an independent predictor of outcome in patients with hepatocellular carcinoma. *Oncology* 69: 342–347, 2005.
129. **Urness LD, Li C, Wang X, Mansour SL.** Expression of ERK signaling inhibitors Dusp6, Dusp7, and Dusp9 during mouse ear development. *Dev Dyn* 237: 163–169, 2008.
130. **Vicent S, Garavoa M, López-Picazo JM, Lozano MD, Toledo G, Thunnissen FB, Manzano RG, Montuenga LM.** 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, 2004.

131. **Vician L, Basconcillo R, Herschman HR.** Identification of genes preferentially induced by nerve growth factor versus epidermal growth factor in PC12 pheochromocytoma cells by means of representational difference analysis. *J Neurosci Res* 50: 32–43, 1997.
132. **Vogt A, McDonald PR, Tamewitz A, Sikorski RP, Wipf P, Skoko JJ, 3rd, Lazo JS.** 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, 2008.
133. **Wagner EF, Nebreda AR.** Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 8: 537–549, 2009.
134. **Wang HY, Cheng Z, Malbon CC.** Overexpression of mitogen-activated protein kinase phosphatases MKP1, MKP2 in human breast cancer. *Cancer Lett* 191: 223–237, 2003.
135. **Wang Z, Zhou JY, Kanakapalli D, Buck S, Wu GS, Ravindranath Y.** High level of mitogen-activated protein kinase phosphatase-1 expression is associated with cisplatin resistance in osteosarcoma. *Pediatr Blood Cancer* 51: 754–759, 2008.
136. **Warmka JK, Mauro LJ, Wattenberg EV.** Mitogen-activated protein kinase phosphatase-3 is a tumor promoter target in initiated cells that express oncogenic Ras. *J Biol Chem* 279: 33085–33092, 2004.
137. **Wilkie AO.** Bad bones, absent smell, selfish testes: the pleiotropic consequences of human FGF receptor mutations. *Cytokine Growth Factor Rev* 16: 187–203, 2005.
138. **Wu JJ, Bennett AM.** Essential role for mitogen-activated protein (MAP) kinase phosphatase-1 in stress-responsive MAP kinase and cell survival signalling. *J Biol Chem* 280: 16461–6466, 2005.
139. **Wu JJ, Roth RJ, Anderson EJ, Hong EG, Lee MK, Choi CS, Neufer PD, Shulman GI, Kim JK, Bennett AM.** Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. *Cell Metab* 4: 61–73, 2006.
140. **Xu S, Furukawa T, Kanai N, Sunamura M, Horii A.** Abrogation of DUSP6 by hypermethylation in human pancreatic cancer. *J Hum Genet* 50: 159–167, 2005.
141. **Yamada S, Taketomi T, Yoshimura A.** Model analysis of difference between EGF pathway and FGF pathway. *Biochem Biophys Res Commun* 314: 1113–1120, 2004.
142. **Yip-Schneider MT, Lin A, Marshall MS.** 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, 2001.
143. **Zhang Y, Blattman JN, Kennedy NJ, Duong J, Nguyen T, Wang Y, Davis RJ, Greenberg PD, Flavell RA, Dong C.** Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. *Nature* 430: 793–797, 2004.
144. **Zhang J, Nomura J, Maruyama M, Nishimoto M, Muramatsu M, Okuda A.** Identification of an ES cell pluripotent state-specific DUSP6 enhancer. *Biochem Biophys Res Commun* 378: 319–323, 2009.
145. **Zhang Z, Kobayashi S, Borczuk AC, Leidner RS, LaFramboise T, Levine AD, Halmos B.** Dual specificity phosphatase 6 (DUSP6) is an ETS-regulated negative feedback mediator of oncogenic ERK-signaling in lung cancer cells. *Carcinogenesis* 31: 577–586, 2010.
146. **Zhao Q, Wang X, Nelin LD, Yao Y, Matta R, Manson ME, Baliga RS, Meng X, Smith CV, Bauer JA, Chang CH, Liu Y.** MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. *J Exp Med* 203: 131–140, 2006.
147. **Zhou YX, Xu X, Chen L, Li C, Brodie SG, Deng CX.** A Pro250Arg substitution in mouse Fgfr1 causes increased expression of Cbfa1 and premature fusion of calvarial sutures. *Hum Mol Genet* 9: 2001–2008, 2000.
148. **Zhou JY, Liu Y, Wu GS.** The role of mitogen-activated protein kinase phosphatase-1 in oxidative damage-induced cell death. *Cancer Res* 66: 4888–4894, 2006.