Dual-specificity MAP kinase phosphatases in health and disease☆

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**Article Info**

**ABSTRACT**

It is well established that a family of dual-specificity MAP kinase phosphatases (MKPs) play key roles in the regulated dephosphorylation and inactivation of MAP kinase isoforms in mammalian cells and tissues. MKPs provide a mechanism of spatiotemporal control of these key signalling pathways, but can also mediate crosstalk between different MAP kinase cascades and facilitate interactions between MAP kinase pathways and other key signalling modules. As our knowledge of the regulation, substrate specificity and catalytic mechanisms of MKPs has matured, more recent work using genetic models has revealed key physiological functions for MKPs and also uncovered potentially important roles in regulating the pathophysiological outcome of signalling with relevance to human diseases. These include cancer, diabetes, inflammatory and neurodegenerative disorders. It is hoped that this understanding will reveal novel therapeutic targets and biomarkers for disease, thus contributing to more effective diagnosis and treatment for these debilitating and often fatal conditions.

1. Introduction

Mammalian dual-specificity MAP kinase (MAPK) phosphatases (MKPs) comprise a subfamily of 10 catalytically active enzymes with a conserved domain structure. This consists of an amino-terminal non-catalytic domain and a carboxyl-terminal catalytic domain. The former contains the kinase interaction motif (KIM), which determines the specific binding and thus substrate selectivity of the MKP for the different MAP kinase isoforms and can also contain nuclear localisation (NLS) or export (NES) signals, which determine the subcellular localisation of certain MKPs. The catalytic domain carries the highly conserved active site consensus sequence (HCKxR) that is characteristic of the larger protein tyrosine phosphatase (PTPase) superfamily. The regulation, structure, catalytic mechanism and substrate selectivity of the MKPs have been extensively reviewed [1–6]. Briefly, the 10 enzymes can be divided into three subgroups based on amino acid sequence homology, subcellular localisation and substrate specificity. These are the inducible nuclear MKPs, comprising DUSP1/MKP-1, DUSP2, DUSP4/MKP-2 and DUSP5, the cytoplasmic extracellular-signal regulated kinase (ERK)-specific MKPs DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4 and a group of three MKPs DUSP8, DUSP10/MKP-5 and DUSP16/MKP-7 that are found in both the cytoplasm and cell nucleus and are relatively selective in their ability to dephosphorylate the p38 and c-Jun amino terminal kinases (JNKs), having little or no activity towards the classical extracellular signal-regulated kinase (ERK) MAPKs (Fig. 1). Key features and characteristics of each of the 10 MKPs are also summarised (Table 1).

Our understanding of the physiological and pathophysiological roles for the MKPs has largely been driven by the generation of genetically engineered mouse (GEM) models in which individual MKPs have been deleted, either unconditionally, or in a tissue specific manner. This work, combined with studies in other model organisms, cell lines and observations in human cells and tissues has gradually revealed that MKPs play fundamental roles in the regulation of signalling events associated with normal development and homeostasis, but can also modulate a wide range of pathophysiological signalling outcomes with relevance to human disease. In this review we will detail the current level of understanding for each of the MKPs in turn, highlighting recent advances and future perspectives in the field.

2. The inducible nuclear MKPs

2.1. DUSP1/MKP-1

*DUSP1/MKP-1* was the first of the dual-specificity MKPs to be characterised and was initially discovered as a growth factor or stress-
inducible gene encoding a nuclear protein with homology to VH1, the prototypic dual-specificity protein phosphatase encoded by vaccinia virus [7,8]. Initially characterised as a phosphatase able to specifically dephosphorylate the threonine and tyrosine residues of the signature T-E-Y motif within the activation loop of the classical MAPK ERK2 in vitro and in vivo [9,10] it was later realised that DUSP1/MKP-1 was capable of dephosphorylating all three major classes of MAPK with a distinct preference for the JNK isoforms followed by p38 and ERK1/2 MAPKs with a distinct preference to development, fertility or lifespan and no evidence for de-regulated ERK signalling was found in DUSP1−/− mouse embryo fibroblasts (MEFs) [16]. The failure to detect changes in MAPK activity in cells lacking DUSP1/MKP-1 was probably due to an initial focus on the ERK1/2 pathway. Subsequent work in MEFs clearly showed that loss of DUSP1/MKP-1 caused a significant increase in the activities of the stress-induced JNK and p38 MAPKs and revealed that MEFs lacking MKP-1 are acutely sensitive to JNK-mediated apoptosis in response to a wide variety of cellular stresses including UV-radiation, ionising radiation, hydrogen peroxide, anisomycin and cisplatin [17–21]. Further experiments conducted using DUSP1−/− mice quickly led to the realisation that this phosphatase regulates a number of physiological and pathophysiological processes including immunity, metabolic homeostasis, cellular responses to anticancer drugs, muscle regeneration, and neuronal function.

### 2.1.1. DUSP1/MKP-1 in innate and adaptive immunity

Given the wide range of roles that MAPKs perform in the development and function of cells of the immune system [22–24] it was perhaps no surprise that amongst the first phenotypes detected in DUSP1−/− mice was a failure to regulate stress-activated JNK and p38 signalling in macrophages and dendritic cells (Fig. 2: Table 2). These cells are key mediators of the innate immune response in which the p38 and JNK MAPKs lie downstream of the toll-like receptors (TLRs), which are activated by a wide variety of pathogen-derived stimuli and act to regulate the expression of both pro and anti-inflammatory cytokines and chemokines [22]. Several groups demonstrated that loss of DUSP1/MKP-1 led to elevated JNK and p38 activities in macrophages exposed to the bacterial endotoxin lipopolysaccharide (LPS) [25–28]. This led to an initial increase in the expression of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6), interleukin-12 (IL-12) and interferon-gamma (IFN-γ) while, at later times, levels of the anti-inflammatory mediator interleukin-10 (IL-10) were increased [25]. These cellular effects were accompanied by

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**Table 1**

Classification and properties of the mammalian dual specificity MAP kinase phosphatases.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene/MKP</th>
<th>Subcellular localisation</th>
<th>Substrate specificity</th>
<th>Mouse models</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear, inducible MKPs</td>
<td>DUSP1/MKP-1</td>
<td>Nuclear</td>
<td>JNK, p38 &gt; ERK</td>
<td>UC, C</td>
<td>[16,56]</td>
</tr>
<tr>
<td></td>
<td>DUSP2</td>
<td>Nuclear</td>
<td>ERK, JNK</td>
<td>UC</td>
<td>[86,87]</td>
</tr>
<tr>
<td></td>
<td>DUSP4/MKP-2</td>
<td>Nuclear</td>
<td>ERK, JNK &gt; p38</td>
<td>UC</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>DUSP5</td>
<td>Nuclear</td>
<td>ERK</td>
<td>UC</td>
<td>[128]</td>
</tr>
<tr>
<td>Cytoplasmic ERK-selective MKPs</td>
<td>DUSP6/MKP-3</td>
<td>Cytoplasmic</td>
<td>ERK</td>
<td>UC</td>
<td>[149]</td>
</tr>
<tr>
<td></td>
<td>DUSP7/MKP-X</td>
<td>Cytoplasmic</td>
<td>ERK</td>
<td>UC*</td>
<td>IMPC</td>
</tr>
<tr>
<td></td>
<td>DUSP9/MKP-4</td>
<td>Cytoplasmic</td>
<td>ERK &gt; p38</td>
<td>UC, C</td>
<td>[175,183]</td>
</tr>
<tr>
<td>JNK/p38-selective MKPs</td>
<td>DUSP8</td>
<td>Cytoplasmic/nuclear</td>
<td>JNK, p38</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DUSP10/MKP-5</td>
<td>Cytoplasmic/nuclear</td>
<td>JNK, p38</td>
<td>UC</td>
<td>[185]</td>
</tr>
<tr>
<td></td>
<td>DUSP16/MKP-7</td>
<td>Cytoplasmic/nuclear</td>
<td>JNK, p38</td>
<td>UC*</td>
<td></td>
</tr>
</tbody>
</table>

pathological changes such as inflammatory tissue infiltration, hypotension and multiple organ failure, all of which are markers of the severe septic shock and increased mortality observed in LPS-injected DUSP1−/− mice when compared to wild type controls.

With respect to the above changes in cytokine expression, the regulation of gene transcription by MAPK-regulated transcription factors such as activator protein-1 (AP1), activating transcription factor 1 (ATF-1) and cAMP response element binding protein (CREB) by DUSP1/MKP-1 was an early focus [25,29]. However, a major mechanism by which cytokine expression is controlled is the signalling axis a prime candidate for therapeutic intervention.

Taken together, this work demonstrates that TLR mediated expression of DUSP1/MKP-1 is a key component of a pathway, which acts through regulation of MAPK-dependent transcription factors and TTP to negatively regulate pathological inflammatory responses, to engage the “off phase” of macrophage-mediated responses to pro-inflammatory stimuli and promote the resolution of inflammation. As such, any defects in this pathway would be expected to impede the latter process and contribute to a range of chronic inflammatory diseases, making the DUSP1/MKP-1-p38-MK2 signalling axis a prime candidate for therapeutic intervention.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene/MKP</th>
<th>Immunological phenotypes of MKP KO mice</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear, inducible MKPs</td>
<td>DUSP1/MKP-1</td>
<td>Increased pro-inflammatory cytokine production &amp; innate immune response LPS challenge.</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impaired resolution of inflammation.</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased adaptive immune response &amp; viral clearance.</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protection from autoimmunity encephalitis (EAE).</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased sensitivity to bacterial infections.</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exacerbates inflammatory phenotypes including: colitis, anaphylaxis and psoriasis.</td>
<td>[40-42]</td>
</tr>
<tr>
<td></td>
<td>DUSP2</td>
<td>Protection from experimentally-induced arthritis.</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased macrophage cytokine expression &amp; mast cell survival.</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased susceptibility to DSS-induced model of intestinal inflammation.</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>DUSP4/MKP-2</td>
<td>Altered T-cell balance, via the promotion of Th17 differentiation and inhibition of Treg generation.</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased susceptibility to Leishmania mexicana, Leishmania donovani &amp; Toxoplasma gondii infection.</td>
<td>[97-99]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant to LPS-induced endotoxic shock.</td>
<td>[100]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased CD4+ T-cell proliferation.</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protection from autoimmunity encephalitis (EAE).</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>DUSP5</td>
<td>Negatively regulates IL-33 mediated eosinophil survival.</td>
<td>[119]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant to helminth infection, due to enhanced eosinophil activity.</td>
<td>[119]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased susceptibility to DSS-induced intestinal inflammation.</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic ERK-selective MKPs</td>
<td>Decreased CD4+ T-cell proliferation, altered T-cell polarisation &amp; impaired Treg function.</td>
<td>[150]</td>
</tr>
<tr>
<td></td>
<td>DUSP6/MKP-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased IL-6 in response to LCMV infection.</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td>DUSP7/MKP-X</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DUSP9/MKP-4</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JNK/p38-selective MKPs</td>
<td>DUSP8</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>DUSP10/MKP-5</td>
<td>Impaired T cell expansion, but enhanced priming of T-cells by APCs.</td>
<td>[185]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protection from autoimmunity encephalitis (EAE).</td>
<td>[185]</td>
</tr>
<tr>
<td></td>
<td>DUSP16/MKP-7</td>
<td>Increased CD4+ T-cell proliferation &amp; a reduced Th17 cell population.</td>
<td>[196]</td>
</tr>
</tbody>
</table>

LCMV, lymphocytic choriomeningitis virus. DSS, dextran sodium sulfate.
While innate immunity comprises an acute, non-specific response to foreign antigens, adaptive or acquired immunity is highly specific to a particular antigenic stimulus and comprises a network of specialized, immune cells and processes that either eliminate pathogens or prevent their growth. In addition, by generating immunological memory, this response also provides long-lasting immunity against infection, which is the basis of vaccination, while an abnormal or maladaptive response can result in autoimmune disease. The workhorses of the system are the B and T lymphocytes, which mediate humoral (antibody-mediated) immunity and cell-mediated (cytotoxic or effector cell-mediated) responses. Despite the key role for ERK signalling in thymocytes and the observation that DUSP1/MKP-1 is expressed at varying levels during T cell development, mice lacking DUSP1/MKP-1 do not present with abnormalities in this process and the ratio of CD4+ to CD8+ T-cells following thymic maturation is in the normal range [33]. This possibly reflects either redundancy amongst ERK-specific phosphatases or the fact that ERK is not the preferred target for DUSP1/MKP-1.

However, in mature CD4+ T cells, loss of DUSP1/MKP-1 seems to impact T cell function with decreased activation and proliferation following exposure to phorbol 12-myristate 13-acetate (PMA) and ionomycin in CD4+ T cells, which showed reduced production of IL-17 and IFN-\(\gamma\) following injection of myelin oligodendrocyte glycoprotein (EAE) [47]. This can be opposed by a subset of specific histone deacetylases (HDACs 1–3) in mouse macrophages [39], suggesting one possible mechanism by which the canonical substrate selectivity of DUSP1/MKP-1 might be regulated in a cell type specific manner.

Finally, given its key role as a critical regulator of innate and adaptive immunity (Table 2), loss of DUSP1/MKP-1 was also found to exacerbate a range of inflammatory phenotypes in mouse models including experimental colitis [40], anaphylaxis [41] and psoriasis [42]. However, for reasons as yet unclear, loss of DUSP1/MKP-1 did not sensitize mice to the development of spontaneous age-dependent osteoarthritis, despite the involvement of inflammatory processes and mediators such as TNF-\(\alpha\) and interleukin-1\(\beta\) in this disease [43]. DUSP1/MKP-1 is also directly targeted by a range of immune modulators. Enhanced expression of DUSP1/MKP-1 underpins, at least in part, the anti-inflammatory activity of glucocorticoids [44,45] and is also observed in response to vitamin D [46] and transforming growth factor-beta (TGF\(\beta\)) [47] both of which are anti-inflammatory. In contrast, pro-inflammatory stimuli such as IFN-\(\gamma\) and interleukin-17A (IL-17A) suppress DUSP1/MKP-1 expression and thus increase signalling through the p38 and JNK MAPK pathways [48,49].

### 2.1.2. DUSP1/MKP-1 in metabolic homeostasis

The first indication that DUSP1/MKP-1 might play a role in regulating metabolic homeostasis came with the finding that DUSP1−/− mice were resistant to diet-induced obesity (Fig. 3) and that this reflected a higher level of energy expenditure, but not overall activity in the null mice [50]. Surprisingly, despite remaining lean on a high-fat diet (HFD), DUSP1−/− mice did become glucose intolerant (as would wild-type animals), while still being protected from hepatic steatosis. This phenotype correlated with increased levels of JNK, p38 and ERK activity in insulin responsive tissues. However, DUSP1−/− mice did not show abnormalities in insulin signalling or glucose homeostasis, despite an established role for JNK signalling in promoting insulin resistance [51]. This apparent contradiction was possibly due to the finding that loss of DUSP1/MKP-1 affects nuclear rather than cytoplasmic JNK activity and that the latter is responsible for JNK-dependent abnormalities in the response to insulin [50].

Subsequent work has revealed mechanistic aspects of the DUSP1−/− metabolic phenotype. Firstly, mice lacking DUSP1/MKP-1 are protected from the loss of oxidative (slow-twitch) myofibers in skeletal muscle. The overall effect of this is to favour oxidative over glycolytic metabolism and, because the latter consumes less energy, to protect against diet-induced obesity [52]. This effect seems to be secondary to increased p38 MAPK-mediated phosphorylation and stabilisation of

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**Image 50x770 to 546x788**

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**Listeria monocytogenes**
peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) thus increasing its activity as a regulator of mitochondrial biogenesis and energy expenditure. Experiments in which grossly obese leptin-resistant (db/db) mice were crossed with DUSP1−/− animals revealed that loss of DUSP1/MKP-1 protected against hepatic steatosis. By increasing MAPK-dependent phosphorylation of peroxisome proliferator-activated receptor-γ (PPARγ) at a site (Ser112) that negatively regulates its activity, loss of DUSP1/MKP-1 prevents the PPARγ-dependent expression of lipogenic genes, thus reducing lipid droplet formation in hepatocytes [53].

While this work sheds some light of the functions of DUSP1/MKP-1 in metabolic homeostasis a severe limitation of these studies is the use of a whole body DUSP1/MKP-1 knockout. Metabolic control is complex and subject to both central and peripheral regulation [54]. Furthermore, diet-induced obesity has an inflammatory component [55] and the role(s) of DUSP1/MKP-1 in regulating immune responses may also be a confounding factor. To begin to address this, a conditional DUSP1fl/fl mouse has now been employed to study the metabolic effects of DUSP1/MKP-1 deletion in specific tissues.

Liver specific knockout of DUSP1/MKP-1 (MKP-1-LKO) using alburnin-Cre (Alb-Cre) resulted in increased hepatic JNK and p38 activation. However, unlike DUSP1−/− mice MKP-1-LKO animals exhibited increased adiposity, fasting hyperglycaemia and hyperinsulinemia on a normal chow diet, indicating that hepatic DUSP1/MKP-1 regulates glucose homeostasis (Fig. 3). This was confirmed in subsequent experiments using hyperinsulinemic-euglycemic clamps, which demonstrated that MKP-1-LKO mice were hyperglycaemic, glucose intolerant and develop hepatic insulin resistance [56]. While DUSP1−/− mice were resistant to HFD-induced obesity MKP-1-LKO mice were more susceptible, but were still protected against hepatic steatosis. Furthermore, unlike DUSP1−/− mice, they showed decreased energy expenditure [56]. Mechanistically, the effects of DUSP1/MKP-1 deletion on glucose metabolism were found to be secondary to increased hepatic p38 and JNK mediated transcription of gluconeogenic genes, increased p38-dependent phosphorylation of cyclic AMP responsive element binding protein (CREB), which promotes gluconeogenesis through PGC1/PPARγ and decreased activation of Signal Transducer and Activator of Transcription 3 (STAT3), a negative regulator of gluconeogenesis [56]. The latter effect is probably an indirect result of the lower circulating levels of IL-6 in MKP-1-LKO mice, as this metabolic cytokine is a potent inducer of Janus kinase (JAK)-STAT signalling. Finally, the decreased energy expenditure observed in MKP-1-LKO mice may be related to reduced levels of IL-6 and fibroblast growth factor 21 (FGF21). Both factors promote energy expenditure, insulin sensitivity, fatty acid oxidation, and weight loss and their reduction would be expected to impair skeletal muscle oxidative capacity and thus increase susceptibility to diet-induced obesity [56].

Skeletal muscle plays a major role in the regulation of glucose metabolism and metabolic homeostasis. Following on from the liver-specific deletion of DUSP1/MKP-1, the effects of skeletal muscle specific loss of this phosphatase (MKP-1-MKO), using human α-skeletal actin (HSA-Cre), have now been studied. MKP-1-MKO mice show increased levels of p38 and JNK signalling in skeletal muscle and are significantly protected from HFD-induced weight gain (Fig. 3). As was the case in the MKP1+/− mice, the failure to gain weight was secondary to enhanced energy expenditure when compared with MKP-1fl/fl controls and no differences in either food intake, or activity between the two genotypes was observed [57]. Interestingly, MKP-1-MKO mice were also resistant to hepatic steatosis, which was consistent with lower levels of hepatic PPARγ and sterol regulatory element-binding protein 1c (SREBP-1c) expression. However, no changes in either p38 or JNK activity were detected in liver tissue. Glucose (GTT) and insulin tolerance (ITT) tests revealed that MKP-1-MKO mice on a HFD produced lower levels of circulating insulin and were insulin sensitive, indicating that they are protected from the development of insulin resistance. Biochemically, an unexpected role for increased PI3-kinase-Akt signalling resulting from microRNA-21 (miR-21) dependent down-regulation of phosphatase and tensin homolog (PTEN) in MKP-1-MKO was uncovered and this could contribute to the increased insulin sensitivity observed in MKP-1-MKO mice. Finally, consistent with the results of whole body deletion of
**Table 3**
The involvement of MKPs in metabolic homeostasis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene/MKP</th>
<th>Evidence implicating functions for MKPs in metabolic homeostasis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear, inducible MKPs</td>
<td>DUSP1/MKP-1</td>
<td>Whole Body KO:</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant to diet-induced obesity.</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protected from the loss of oxidative myofibers in skeletal muscle.</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protected against hepatic steatosis.</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased energy expenditure.</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver-specific KO:</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose intolerant &amp; susceptible to diet-induced obesity.</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protected against hepatic steatosis.</td>
<td>[56]</td>
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<tr>
<td></td>
<td></td>
<td>Decreased energy expenditure.</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skeletal muscle specific KO:</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant to diet-induced obesity.</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased energy expenditure &amp; oxidative myofibers.</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td>DUSP2</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DUSP4/MKP-2</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DUSP5</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic ERK-selective MKPs</td>
<td>DUSP6/MKP-3</td>
<td>DUSP6/MKP-3 KO mice are resistant to high fat diet (HFD)-induced obesity &amp; hepatoosteatosis.</td>
<td>[155,156]</td>
</tr>
<tr>
<td></td>
<td>DUSP7/MKP-X</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DUSP9/MKP-4</td>
<td>DUSP9 is implicated in regulation of the response to insulin and GWAS studies have identified DUSP9 as a susceptibility locus for the development of Type-2 diabetes, in multiple ethnicities.</td>
<td>[179,180,182]</td>
</tr>
<tr>
<td>JNK/p38-selective MKPs</td>
<td>DUSP8</td>
<td>N/A</td>
<td></td>
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<tr>
<td></td>
<td>DUSP10/MKP-5</td>
<td>N/A</td>
<td></td>
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<td></td>
<td>DUSP16/MKP-7</td>
<td>N/A</td>
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</tr>
</tbody>
</table>

**DUSP1/MKP-1**, the increased energy expenditure observed in MKP-1/MKO mice was secondary to an increase in the proportion of oxidative myofibers and was reflected in enhanced oxidative capacity and mitochondrial function in skeletal muscle [57].

Taken together, these results begin to unravel some of the complexity and tissue specific interplay of DUSP1/MKP-1 action in the regulation of metabolic homeostasis (Fig. 3; Table 3) and also emphasize the importance of compartmentalised nuclear regulation of p38 and JNK activities in mediating the phenotypes observed. The observation that DUSP1/MKP-1 is up-regulated in insulin-responsive tissues in response to a HFD in mice and also in obese humans indicates that it forms part of a key stress response that leads to decreased energy expenditure in skeletal muscle, thus contributing to weight gain and may also mediate at least some of the adverse consequences of this disease, including abnormalities in glucose metabolism and hepatosteatosis. The further use of conditional DUSP1/MKP-1 ablation will reveal the relative importance of MAPK regulation in distinct tissues by this phosphatase in energy homeostasis and, from the information gathered so far, MKP-1/DUSP1 continues to be a potential pharmacological target for the treatment of metabolic disease.

### 2.1.3. DUSP1/MKP-1 in cancer

Given the central importance of deregulated MAPK signalling in the initiation and progression of human cancers it is no great surprise that the involvement of MKPs in regulating various aspects of the cancer phenotype has been of widespread interest [58–60]. Disappointingly, given that DUSP1/MKP-1 was both the first MKP to be discovered and also the first to be deleted from the mouse genome, there are currently no published studies in which DUSP1/MKP-1 has been directly implicated in either tumour initiation or progression. It is hoped that the recent development of the conditional DUSP1/MKP-1 mouse (see 2.1.2.) will facilitate definitive experiments, particularly as this model avoids the potentially confounding effects of the immune and inflammatory abnormalities seen when DUSP1/MKP-1 is knocked out globally.

In contrast, over the 25 or so years since DUSP1/MKP-1 and its role in regulating MAP kinase signalling were discovered, there have been numerous publications reporting either increased or reduced expression of DUSP1/MKP-1 in a wide range of human tumours including breast, pancreas, gastric, ovary, lung, skin and prostate (Table 4). In addition, a number of studies have relied on ectopic overexpression of DUSP1/MKP-1 in normal and cancer cell lines to study its possible role in modulating oncopgenic signalling. These studies have been extensively reviewed elsewhere [60] and as they have often yielded equivocal or even contradictory information regarding the role of DUSP1/MKP-1 in cancer, it is not proposed to list or discuss them further here.

One aspect of cancer biology in which DUSP1/MKP-1 does appear to play an important role is in the response of normal and cancer cells to a range of chemical and physical insults including modalities used in cancer chemotherapy (Table 4). Soon after it became clear that the p38 and JNK MAPKs were the preferred substrates for DUSP1/MKP-1, it was observed that the overexpression of this phosphatase enhanced cellular resistance to both UV-radiation and the chemotherapeutic drug cisplatin and that this was related to the suppression of JNK-mediated apoptosis [61,62]. That DUSP1/MKP-1 played a crucial role in modulating sensitivity to these insults was confirmed when MEFs derived from DUSP1−/− mice were found to be sensitive to UV-radiation, cisplatin, hydrogen peroxide and anisomycin [17–20]. In normal cells, DUSP1/MKP-1 expression is induced by UV and cisplatin via activation of p38 MAPK, whereas it is the suppression of JNK activity by DUSP1/MKP-1 that modulates cell death. This indicates that DUSP1/MKP-1 mediated crosstalk between these two distinct MAPK pathways regulates cellular sensitivity [20].

Thus it is likely that elevated expression of DUSP1/MKP-1 in tumours may mediate chemoresistance and this is supported by studies in non-small cell lung cancer (NSCLC) where overexpression of DUSP1/MKP-1 is observed and patients become resistant to treatment with cisplatin. In NSCLC cancer cell lines where DUSP1/MKP-1 was constitutively expressed, siRNA knockdown increased cisplatin sensitivity some 10 fold, reduced the growth of these cell lines in nude mice and rendered the resulting tumours cisplatin sensitive [63]. In lung cancer patients dexamethasone is also often co-administered with cisplatin to ameliorate the undesirable side effects of treatment. However, glucocorticoids are known to upregulate DUSP1/MKP-1 expression [64] and not surprisingly dexamethasone effectively suppressed cisplatin-induced apoptosis in a lung adenocarcinoma cell line indicating that DUSP1/MKP-1 plays a key role in this potentially undesirable drug-drug interaction [65]. The role of DUSP1/MKP-1 in mediating resistance to chemotherapy appears not to be restricted to cisplatin, as the ability of this phosphatase to inhibit JNK-mediated apoptosis has also been
implicated in the resistance of pancreatic cancer cells to gemcitabine [66], multidrug resistance in glioblastoma [67], resistance to doxorubicin and taxanes in breast cancer [68] and resistance to the proteasome inhibitor bortezomib [69].

Finally, a recent paper has implicated DUSP1/MKP-1 in a growth factor-dependent pathway, which promotes intrinsic resistance to the tyrosine kinase inhibitors (TKI) used to treat chronic myeloid leukemias (CML). In mouse pro-B BaF3 cells engineered to express the breakpoint cluster region (BCR)-Abl tyrosine kinase fusion, which drives CML, Kesarwani et al. [70] found that DUSP1/MKP-1 along with FBJ osteosarcoma oncogene (Fos) were responsible for resistance to the Abl TKI inhibitor imatinib (Gleevec). Genetic deletion or pharmacological inhibition of Fos and DUSP1/MKP-1 eradicated minimal residual disease (MRD) in multiple in vivo models as well as in patient-derived mouse xenografts. Mechanistically, DUSP1/MKP-1 seems to influence TKI sensitivity via its ability to suppress p38 MAPK activity and modulate AP1-dependent transcriptional networks. The latter hypothesis is supported by the finding that SB202190, a specific p38 MAPK inhibitor, also conferred imatinib resistance. While these results are potentially exciting, some caution is necessary in the interpretation of the data. BCI (2-benzylidene-3-(cyclohexylamino)-1-Indanone hydrochloride), the “specific” DUSP1 inhibitor used to treat mice and reverse disease in a retroviral bone marrow transduction transplantation leukemogenesis model is both highly toxic and relatively non-specific [71].

### 2.1.4. DUSP1/MKP-1 function in other tissues

Given the key role that MAPK signalling plays in aspects of brain development and function it is unsurprising that MKPs have been implicated in the regulation of these processes. Indeed DUSP1/MKP-1 plays important roles in neural cell development, neuronal cell survival and death, glial cell function and events, which underpin learning and memory (reviewed in [72]). In terms of pathophysiology, an important observation was that DUSP1/MKP-1 levels were elevated in the hippocampal region of post-mortem brain from patients who had been diagnosed with major depressive disorder (MDD) [73]. MDD is characterised by chronic or episodic depression and carries a significant (2–7%) risk of suicide.

Duric et al. [73] found that DUSP1/MKP-1 was also elevated in the hippocampus of rats exposed to chronic unpredictable stress (CUS) an effect that was attenuated by treatment with the antidepressant drug fluoxetine (Prozac) a selective serotonin reuptake inhibitor. Furthermore, adenoviral-mediated expression of DUSP1/MKP-1 in the hippocampus caused anhedonia (an inability to experience pleasure), as assessed by a reduced preference for sucrose over water, and these animals displayed other surrogates of depressive behaviour or helplessness such as disturbed feeding and increased immobility in the forced swim test, all of which were also seen in the CUS exposed rats. Interestingly, all of the latter endpoints were suppressed in CUS exposed DUSP1−/− mice when compared to wild type controls [73].

Mechanistically, these changes were associated with a reduction in phospho-ERK1/2 levels in CUS exposed wild type mice, which was not observed in DUSP1−/− mice. A result, which led the authors to conclude that ERK was the relevant DUSP1/MKP-1 target. This finding is somewhat surprising in the light of our knowledge that JNK and p38, but not ERK, are the preferred substrates for this phosphatase [15] and also conflicts with a previous study in which a reduction in hippocampal phospho-JNK but not phospho-ERK was observed in rats exposed to CUS [74]. Finally, a recent study has identified similar changes in DUSP1/MKP-1 levels in the anterior cingulate cortex (ACC) of mice exposed to neuropathic pain and CUS, which were again reversed by fluoxetine [75]. While not shedding new light on the biochemical mechanisms involved, this latter study does implicate the regulation of MAPK signalling by DUSP1/MKP-1 in another brain region tightly associated with regulating mood-related functions.

With respect to neurodegenerative disorders, DUSP1/MKP-1 has been reported to mediate neuroprotective effects in both in vitro and in vivo models of Huntington’s disease through its ability to suppress polyglutamine-expanded huntingtin-induced activation of c-Jun N-terminal kinases (JNKs) and p38 MAPKs [76]. Finally, by suppressing p38 MAPK activity, DUSP1/MKP-1 has been reported to protect dopaminergic neurons from the toxic effects of 6-hydroxydopamine (6-OHDA) suggesting that strategies aimed at either increasing MKP-1 expression or activity might be a viable strategy in the treatment of Parkinson’s disease [77].

DUSP1/MKP-1 has also been implicated in muscle regeneration as DUSP1−/− mice are impaired in their ability to recover from...
experimental muscle injury and, when crossed into a mouse model of Duchenne's muscular dystrophy (the mdx dystrophin null), they display exacerbated muscular dystrophinopathy [78]. Interestingly, this is exactly the reciprocal of the phenotype observed after deletion of DUSP10/MKP-5 (see Section 4.2.2) [79]. More recently, the study of DUSP11/DUSP10−/− double knockout (DKO) mice revealed a severe impairment in muscle regeneration. Satellite cells, the precursors of muscle cells, were less proliferative and DKO mice had increased inflammation at sites of injury suggesting that the positive regulation of myogenesis by DUSP1/MKP-1 is dominant over negative regulation by DUSP10/MKP-5 [80]. Despite the fact that they share common substrates in JNK and p38 it is clear that these two MKPs regulate distinct signalling events. This may be related to the fact that while DUSP1/MKP-1 regulates nuclear MAPK activity, DUSP10/MKP-5 can impinge on cytosolic signalling and thus the two MKPs may regulate quite distinct sets of MAPK substrates.

2.2. DUSP2

DUSP2 (also known as PAC-1) was first identified as a mitogen-inducible gene in human T-cells and is most closely related to DUSP1/MKP-1 and DUSP4/MKP-2, sharing 71% and 68% amino acid identity, respectively [81]. Mainly expressed in hematopoietic tissue, DUSP2 transcription is induced by activation of the ERK1/2 signalling pathway [81,82]. When expressed in mammalian cells, DUSP2 favours dephosphorylation of ERK1/2 and p38 MAPKs, being less able to inactivate JNK [83]. Its lack of activity against JNK was later suggested to be a result of the relative inability of this MAPK to cause catalytic activation of DUSP2 when compared with ERK2 [84]. In a recent twist, DUSP2 was found to be unique amongst the 10 mammalian MKPs in being able to bind to and dephosphorylate the “atypical” MAPK kinases ERK3 and ERK4 [85]. In both ERK3 and 4 the classical T-X-Y motif in the activation loop is replaced by S-E-G, in which the serine residue is the sole phospho-acceptor and DUSP2 efficiently dephosphorylates this residue in cultured cells.

2.2.1. DUSP2 in innate and adaptive immunity

DUSP2 expression is restricted to thymus, spleen and lymph nodes. However, DUSP2−/− mice develop normally and show no abnormalities in the numbers of lymphocytes in blood and bone marrow. Granulocyte numbers and lymphoid tissue development are also normal, indicating that DUSP2 is not required for immune system development [86,87]. However, using the K/BxN model of inflammatory arthritis, wild type mice injected with arthritogenic K/BxN serum containing autoantibodies to glucose-6-phosphate isomerase (GPI) developed peripheral inflammatory arthritis within 2 days while DUSP2−/− mice were protected. Further analysis showed that DUSP2−/− animals had impaired effector responses such as inflammatory mediator production by macrophages and mast cells and decreased mast cell survival [86]. Taken together, these results demonstrate an unexpected role for DUSP2 as a positive mediator of inflammation. Puzzlingly, stimulated mast cells and macrophages lacking DUSP2 displayed decreased ERK1/2, and p38 MAPK phosphorylation and increased JNK phosphorylation, which is exactly the opposite of the result predicted by prior biochemical studies [11,84]. No compensatory changes in the expression of other MKPs was observed and the authors invoke pathway crosstalk, postulating that the increase in JNK activity on DUSP2 deletion resulted in suppression of ERK activity.

More recently, Lu et al., have studied the role of DUSP2 in T cell development and differentiation and found that loss of this phosphatase has a profound effect on the differentiation of naive T cells in vitro by favouring Th17 differentiation, while inhibiting the production of into Treg cells [87]. Using the dextran sodium sulfate (DSS)-induced model of intestinal inflammation and colitis, they further show that DUSP2−/− mice exhibit more severe disease when compared to wild type, as evidenced by increased mucosal hyperemia and colonic ulceration. Consistent with the in vitro results, this pathology is accompanied by higher levels of Th17 cells in DSS-treated DUSP2−/− colon and increased levels of pro-inflammatory cytokines including IL-6, IL-17, TNFα and interleukin-1beta (IL-1β) [87]. Mechanistically, while levels of phospho-ERK and phospho-p38 were higher in untreated DUSP2−/− colon compared to wild type, no differences were seen in DSS-treated colon from the two genotypes. However, higher levels of phosphorylated STAT3 were consistently seen in mice lacking DUSP2 and the authors hypothesise that this transcription factor is a direct DUSP2 substrate in vivo. However, as JAK/STAT signalling is potentely activated in response to IL-6 and this cytokine is overproduced in response to DUSP2 deletion some caution must be attached to this interpretation, particularly as DUSP2 (like DUSP6/MKP-3 see Section 3.1) undergoes catalytic activation by bound ERK2, implying that its full activity as a protein phosphatase is dependent on binding to a MAPK substrate [84].

Taken together, these results demonstrate that DUSP2 plays key roles in both the innate and adaptive immune systems, which have implications for the initiation and progression of pathology in murine models of human inflammatory disease (Table 2). However, at present it is unclear whether or not these relate to the direct activity of this phosphatase in modulating MAPK signalling or may involve other relevant targets. Clearly more work is required to reconcile the in vivo observations with precise molecular mechanism.

2.2.2. DUSP2 in cancer

Thus far DUSP2−/− mice have not been crossed into any of the commonly used murine cancer models and reports of the involvement of DUSP2 in cancer are relatively scant (Table 4). Down-regulation of DUSP2 has been reported in a number of solid tumours, where its expression level was inversely proportional to that of the hypoxia-inducible transcription factor HIF-1α and its loss seemed to mediate increased ERK activation and chemoresistance in cancer cell lines and to contribute to colon cancer “stemness” [88,89]. Given its expression in hematopoietic tissues, there are also a number of studies linking DUSP2 with blood cell cancers. Down-regulation of DUSP2 in acute myeloid leukemia (AML) is associated with constitutive ERK activation [90], while recent data from cancer genome sequencing of Diffuse Large B-cell lymphomas (DLBCL), the major form of non-Hodgkin’s lymphoma, reveals that DUSP2 is one of the most frequently mutated genes in this disease [91,92]. The observation that DUSP2 expression is highly inducible upon stimulation of B-cell lymphoma cell lines suggests that mutations in DUSP2 may have the potential to modify MAPK signalling in DLBCL. It will be vital to determine the effects of these mutations on the localisation or activity of DUSP2 in order to explore the possible contribution of this phosphatase to the initiation and/or progression of disease.

2.3. DUSP4/MKP-2

DUSP4/MKP-2 was amongst the very earliest of the MKPs to be characterised and is most closely related to DUSP1/MKP-1 [93–95], sharing 58.8% identity at the amino-acid sequence level. Although it is not as widely studied, DUSP4/MKP-2 shares many features with its nearest relative including transcriptional regulation in response to growth factors, an ability to dephosphorylate ERK, JNK and p38 MAPKs and regulation of DUSP4/MKP-2 protein stability by the phosphorylation of its C-terminus [11,36,96]. The generation of knockout mice has now advanced our knowledge of DUSP4/MKP-2 function in a number of areas.

2.3.1. DUSP4/MKP-2 in innate and adaptive immunity

The earliest reports using the DUSP4−/− mice centred on its possible function as a regulator of innate immunity and inflammation. BMDMs from DUSP4−/− mice showed increased levels of both JNK and p38 but not ERK signalling in response to LPS. This correlated with a potentiation of LPS-stimulated induction of the pro-inflammatory
cytokines, IL-6, IL-12Beta (IL-12p40), TNFα, and also cyclooxygenase-2 (COX-2) derived prostaglandin E2 (PGE2) production [97]. However, IL-10 was suppressed, as was inducible nitric oxide synthase (iNOS) expression while arginase-1 levels were increased. The reciprocal changes in iNOS/arginase-1 levels would tend to suppress nitric oxide (NO) production as arginase-1 competes with iNOS for the same substrate. Following infection with the intracellular parasite *Leishmania Mexicana*, mice lacking DUSP4/MKP-2 were found to be more susceptible to infection, with an increased parasite burden and lesion size and this was accompanied by a suppression of Th1 and/or increased Th2 responses. The reason for the increased susceptibility to *Leishmania Mexicana* infection was due to decreased iNOS and increased expression and function of arginase-1 rather than any modulation of cytokine synthesis [97]. Taken together these results suggest that DUSP4/MKP-2 does not display simple functional redundancy with respect to its near relative, but instead is protective against *Leishmania Mexicana* infection due to up-regulation of iNOS and suppression of arginase-1 expression, thus promoting NO-mediated parasite death. This mechanism was also found to account for the protective effects of DUSP4/MKP-2 against *Leishmania donovani* the causative agent of visceral leishmaniasis [98] and Toxoplasma gondii, which causes toxoplasmosis [99].

Differences between DUSP4/MKP-2 and DUSP1/MKP-1 were further underlined in studies of the response of DUSP4−/− mice to experimental LPS-induced sepsis. The first major surprise came with the discovery that, in contrast to mice lacking DUSP1/MKP-1, mice lacking DUSP4/MKP-2 were more resistant to endotoxic shock and also had lower levels of circulating IL-1β, IL-6, and TNFα [100]. Furthermore, LPS-stimulated BMDMs derived from DUSP4−/− mice produced significantly less TNFα and IL-10 when compared to wild type cells and this was associated with increased levels of phosphorylated (active) ERK, but decreased levels of phospho-JNK and p38 [100]. It is unclear why there is a discrepancy between these results in LPS-stimulated BMDMs and those obtained by Al-Mutairi et al. [97], but they went on to show that elevated ERK2 signalling led to induction of DUSP1/MKP-1 in the DUSP4−/− macrophages and that this MKP was responsible for the reduction in JNK and p38 signalling and reduced cytokine production [100]. This supports a model in which ERK-mediated crosstalk between MKP-2 and MKP-1 acts to regulate cytokine production in response to LPS, a view supported by the observation that siRNA mediated knockdown of DUSP1/MKP-1 increased the production of TNFα by DUSP4−/− BMDMs [100].

In the adaptive immune system deletion of DUSP4/MKP-2, like deletion of DUSP1/MKP-1 [33], does not affect thymocyte maturation and positive selection. Furthermore, no enhanced ERK, JNK, or p38 phosphorylation was observed in either activated or phosphorib-12-tryptase-13-acetate (PMA)-treated DUSP4−/− T cells [101]. However, CD4+, but not CD8+, T cells did show higher rates of proliferation without affecting differentiated Th1 and Th2 T-cell functions in vivo. The proliferative change in CD4+ T cells lacking DUSP4/MKP-2 was associated with increased STAT5 phosphorylation and interleukin 2 receptor alpha (CD25) expression [101]. Subsequent work showed that DUSP4/MKP-2 decreases both the transcriptional activity and stability of STAT5 and both in vitro and in vivo data showed that DUSP4/MKP-4 deletion enhanced iReg and reduced Th17 polarisation while DUSP4-deficient mice were somewhat more resistant to the induction of autoimmune encephalitis [102]. Finally, increased DUSP4/MKP-2 expression has been implicated in age-dependent defective adaptive immunity. Increased expression of DUSP4/MKP-2 in CD4+ memory T cells from older (> 65 years) individuals inhibits the ERK and JNK-dependent expression of CD40L and reduces the production of the cytokines interleukin-4 (IL-4) and interleukin-21 (IL-21) by follicular helper cells, thus impairing T cell-dependent B cell responses [103]. These results suggest that specific inhibition of DUSP4/MKP-2 activity might form part of a strategy to combat increased morbidity from infections in the elderly.

Taken together these studies clearly implicate DUSP4/MKP-2 in the regulation of innate and adaptive immunity (Table 2). However, despite assumptions that its close relationship with the prototypic MKP DUSP1/MKP-1 might indicate overlapping or identical functions this enzyme seems to play a distinct role in regulating immune function. In macrophages there is some debate as to the effects of DUSP4/MKP-2 deletion on the activity of specific MAPK isoforms with discordant results in LPS-treated BMDMs [97,100]. In T cells it seems to mediate its effects via modulation of STATs and, as no perturbation in MAPK signalling was observed in cells lacking DUSP4/MKP-2, this has been taken as evidence of a MAPK-independent function for this enzyme [101,102].

2.3.2. DUSP4/MKP-2 in cancer

Although less well studied than DUSP1/MKP-1, there have nevertheless been numerous reports of either increased or reduced expression of DUSP4/MKP-2 in a wide variety of human cancer cell lines and primary tumours including pancreatic, lung, ovarian, breast, liver, thyroid and colon (reviewed in [60,104]). However the majority of these studies have relied on either association and/or correlation with clinical outcome/tumour subtype or ectopic expression of this MKP in cancer cell lines. Thus far the DUSP4−/−/MKP-2 knockout mice have not been crossed into or utilised in any of the well-characterised murine cancer models and there is no direct evidence of a role for DUSP4/MKP-2 in the initiation or progression of tumours. Furthermore, the fact that DUSP4/MKP-2 has a proven role in immune regulation indicates that a conditionally targeted allele of DUSP4/MKP-2 would be required to avoid the confounding effects of deleting this MKP in immune cells on any cancer phenotypes observed.

Despite this there have been some recent reports that indicate a role for this MKP in human cancers (Table 4). DUSP4/MKP-4 was found to be epigenetically silenced in some 75% of > 200 cases of diffuse B cell lymphoma (DBCL) and a lack of DUSP4/MKP-2 was a negative prognostic factor in three independent cohorts of DBCL patients. Mechanistically, this cancer appears to be dependent on JNK signalling for continued survival and loss of DUSP4/MKP-2 contributes to cancer progression by augmenting JNK activity. This is consistent with the results of ectopic expression of DUSP4/MKP-2, which ablates JNK activity and induces apoptosis in DBCL cells while dominant negative interference with JNK also restricts survival [105]. There are also indications that DUSP2/MKP-4 is implicated in resistance to chemotherapy in both gastric cancer, where resistance to doxorubicin is associated with DUSP4/MKP-2 driven epithelial-mesenchymal transition (EMT) [106] and in Her2 positive breast cancer where DUSP4/MKP-2 is associated with resistance to the anti-Her2 humanised monoclonal antibody Trastuzumab and siRNA mediated silencing of DUSP4/MKP-2 re-sensitised breast cancer cell lines with an amplified Her2 oncogene to this agent [107].

2.3.3. DUSP4/MKP-2 in other tissues

DUSP4/MKP-2 is expressed at increasing levels during the process of neuronal differentiation in neural progenitors derived from retinoic acid (RA)-treated murine embryonic stem cells (mESCs) and lentiviral DUSP4/MKP-2 siRNA knockdown significantly retarded this process. Importantly, this phenotype could be rescued with siRNA-resistant wild type but not a catalytically inactive mutant of DUSP4/MKP-2 and loss of DUSP4/MKP-2 resulted in increased levels of phospho-ERK, but not JNK or p38 MAPKs, indicating that this is the relevant target. Overall this data indicates that DUSP4/MKP-2 plays a role in both the neural commitment of mESCs and neuronal differentiation and may point to a wider role for this MKP in brain function and pathology [108]. More recently direct evidence of a role for DUSP4/MKP-2 in the brain has come from a study of hippocampal neuronal excitability, synaptic plasticity and behaviour in DUSP4−/− mice. Long-term potentiation (LTP) was found to be impaired in MKP-2−/− mice and the frequency of excitatory postsynaptic current (EPSC) was also increased in both hippocampal slices and hippocampal cultures. Finally, whereas
locomotor activity and anxiety-like behaviour were normal in DUSP4−/− mice, hippocampal-dependent spatial reference and working memory were both somewhat impaired [109]. Surprisingly, given the established role of ERK signalling in LTP [110] no abnormalities in ERK signalling were observed in either DUSP4−/− brain tissue or in primary hippocampal cultures. However, JNK or p38 activation was not studied and the former also play a role in memory formation and synaptic plasticity [111].

2.4. DUSP5

DUSP5 was first identified as a growth factor and heat shock-inducible nuclear MKP and is closely related to both DUSP1/MKP-1 and DUSP2/MKP-4 [112-114]. Despite its early discovery and characterisation as an MKP, little attention was paid to DUSP5, presumably on the assumption that it would share many of the properties of its nearest relatives with respect to a broad activity towards ERK, JNK and 38 MAPKs. However, it was later shown that DUSP5 is unique amongst the four inducible nuclear MKPs in being absolutely specific for ERK1/2 [115]. Furthermore, growth factor-inducible expression of DUSP5 is mediated by ERK activity making it a classical negative feedback regulator of this signalling pathway [116] and DUSP5 binds tightly to its substrate and is able to anchor inactive ERK in the cell nucleus [115]. Together, these properties define DUSP5 as the nuclear counterpart of the inducible cytoplasmic ERK specific phosphatase DUSP6/MKP-3 (see Section 3.1).

2.4.1. DUSP5 in innate and adaptive immunity

An early indication that DUSP5 might play a role adaptive immunity came with the observation that it was highly induced following IL-2 stimulation of T-cells [117]. This idea was seemingly reinforced by the finding that transgenic expression of DUSP5 in lymphoid cells arrested thymocyte development at the CD4+/CD8+ (double positive) stage and caused autoimmune symptoms in these animals [118]. However, these results illustrate the limitations of overexpression experiments and probably reflect the function of ERK itself, rather than endogenous DUSP5 in regulating immune cell development. This has been confirmed by more recent experiments utilising DUSP5−/− mice where global deletion had no effect on innate or adaptive immune cell numbers in the bone marrow, spleen or lymph nodes under homeostatic conditions [119]. However, subjecting DUSP5−/− mice to acute immune challenges has revealed more subtle phenotypes that are modulated in a DUSP5-dependent manner (Table 2).

Thus DUSP5 has been shown to be highly expressed in eosinophils where it negatively regulates IL-33 mediated survival, via the suppression of interleukin-33 (IL-33)-induced ERK-activity and down-regulation of the anti-apoptotic protein B-cell lymphoma-extra large (BCLxL). Consequently, DUSP5−/− mice challenged by helminth infection display a more chronic and severe phenotype with decreased resistance to infections [120]. Furthermore, in vitro experiments in DUSP5−/− MEFs revealed an essential non-redundant function for this MKP in suppressing nuclear ERK activity following acute pathway stimulation. Loss of DUSP5−/− also provided the upregulation of a cohort of ERK-dependent genes including SerpinB2 in TPA stimulated MEFs [128]. SerpinB2 had previously been identified as a susceptibility gene in this model of skin carcinogenesis [128,130] and concomitant deletion of SerpinB2 reversed the sensitivity of DUSP5−/− mice to DMB/A/TPA-induced papilloma formation identifying DUSP5 as a bona fide tumour suppressor by virtue of its ability to suppress SerpinB2 expression in this animal model of Ras-induced cancer [128]. More recently, experiments using wild type and DUSP5−/− MEFs have demonstrated that DUSP5 function is dependent on the nature of the oncogenic driver. Thus while loss of this MKP in the context of mutant Ras is compatible with continued cell proliferation, its deletion in cells expressing mutant BrafV600E causes ERK-dependent cell cycle arrest and senescence and prevents cell transformation by this oncogene in vitro [131]. This latter study supports the idea that MKPs might either suppress or promote carcinogenesis depending on the oncogenic and tissue context (Fig. 4, Table 4) and it will be interesting to see the results of DUSP5 ablation in other, more clinically relevant, murine models of Ras- and Braf-driven cancer.

2.4.2. DUSP5 and cancer

The canonical Ras-ERK MAPK signalling pathway is frequently deregulated in human cancers with activating mutations found in upstream components of the pathway including receptor tyrosine kinases (RTKs), Ras GTPases, the MAPK kinase Braf and MAPK kinase (MEK) [121]. The observation that Braf is mutated in 40–60% of malignant melanomas and in tumours of the thyroid, colon and lung underscores the importance of the Ras-ERK pathway in malignant disease, making it an intense focus of anticancer drug discovery [122]. In common with the cytoplasmic ERK-specific phosphatase DUSP6/MKP-3 (see Section 3.1.3), elevated DUSP5 expression is observed in a range of Ras or Braf mutant cancer cells [123-125] where it is presumed to suppress oncogenic ERK activation. DUSP5 has also been reported to be subject to epigenetic silencing in gastric cancers and this correlated with poorer patient survival [126]. More recently, DUSP5 down-regulation and promoter hypermethylation has been identified in colorectal tumour samples and cell lines. However, DUSP5 knockdown in colorectal cancer cell lines displayed limited effects on phospho-ERK levels and did not increase proliferation. Furthermore, a transgenic mouse overexpressing DUSP5 in the intestinal epithelium displayed no alterations in ERK signalling, intestinal homeostasis or adenoma formation and the authors concluded that DUSP5 does not regulate intestinal development or tumourigenesis [127]. Although surprising, given the demonstrable effects of DUSP5 overexpression on ERK activation in vitro, these results should be interpreted with a degree of caution as the constitutive transgene used here cannot recapitulate the transcriptional dynamics inherent in feedback control exerted by endogenous DUSP5.

In contrast Rushworth et al. demonstrated that DUSP5 loss sensitised mice to HRasGermline-driven skin papilloma formation in the well-established DMBA/TPA (7,12-dimethylbenza[a]anthracene/12-O-tetradecanoylphorbol-13-acetate)-inducible skin carcinogenesis model. Furthermore, in vitro experiments in DUSP5−/− MEFs revealed an essential non-redundant function for this MKP in suppressing nuclear ERK activity following acute pathway stimulation. Loss of DUSP5−/− also provoked the upregulation of a cohort of ERK-dependent genes including SerpinB2 in TPA stimulated MEFs [128]. SerpinB2 had previously been identified as a susceptibility gene in this model of skin carcinogenesis [129,130] and concomitant deletion of SerpinB2 reversed the sensitivity of DUSP5−/− mice to DMBA/TPA-induced papilloma formation identifying DUSP5 as a bona fide tumour suppressor by virtue of its ability to suppress SerpinB2 expression in this animal model of Ras-induced cancer [128]. More recently, experiments using wild type and DUSP5−/− MEFs have demonstrated that DUSP5 function is dependent on the nature of the oncogenic driver. Thus while loss of this MKP in the context of mutant Ras is compatible with continued cell proliferation, its deletion in cells expressing mutant BrafV600E causes ERK-dependent cell cycle arrest and senescence and prevents cell transformation by this oncogene in vitro [131]. This latter study supports the idea that MKPs might either suppress or promote carcinogenesis depending on the oncogenic and tissue context (Fig. 4, Table 4) and it will be interesting to see the results of DUSP5 ablation in other, more clinically relevant, murine models of Ras- and Braf-driven cancer.

2.4.3. DUSP5 in other tissues

DUSP5 is implicated in cardiovascular development, where it is expressed in angioblasts and mature vasculature in zebrafish and DUSP5 knockdown increased the e5spp+ (ets1-related protein) angioblast population during early embryonic development. DUSP5 over-expression also antagonised the function of a serine threonine kinase, Srfk-1, which promotes angioblast development [132]. DUSP5 has also been shown to act as a regulator of cardiac fibroblast proliferation and cardiac hypertrophy. Ferguson et al., demonstrated that the anti-hypertrophic activity of class I histone deacetylase (HDAC) inhibitors is mediated by their ability to increase DUSP5 gene expression, thus inhibiting both ERK activity and cardiac myocyte proliferation. Ectopic
The cytoplasmic localisation of DUSP6/MKP-3 is mediated by a leucine-rich nuclear export signal (NES) within the amino terminal domain and the tight binding of ERK to the KIM also indicates a role for this MKP as a cytoplasmic anchor for inactive ERK [143]. Studies of the pattern of DUSP6/MKP-3 expression during early mouse development established a link with sites of fibroblast growth factor (FGF) signalling [144] and subsequent work in the chicken embryo, DUSP6/−/− mice and cultured cells established that DUSP6/MKP-3 is transcriptionally induced in response to FGF-mediated ERK signalling and acts as a classical negative feedback regulator of ERK activity during early development [145–149].

3.1.1. DUSP6/MKP-3 in immunity and inflammation

Relatively few studies have addressed a role for DUSP6/MKP-3 in immune regulation. DUSP6/−/− mice are reported to be indistinguishable from their wild type littermates in terms of the total number of cells and proportions of CD4+, CD8+, and CD4/CD8 double-positive cells in spleen, mesenchymal lymph nodes (MLN) and thymus [150]. However, T cell receptor (TCR) stimulation of DUSP6/−/− or CD4+ T cells resulted in higher levels of phospho-ERK1/2, but not of JNK or p38 and anti-CD3/28 stimulated CD4+ T cells harvested from spleen and MLN produced higher amounts of IFN-γ and lower amounts of IL-17A when compared to wild type controls. Activated DUSP6/−/− or CD4+ T cells also displayed increased proliferation in vitro, but this was accompanied by increased levels of activation-induced cell death (AICD), perhaps explaining why lymphoid cellularity remains unchanged. In DUSP6/−/− CD8+ T cells the expression of CD107a or lysosomal associated membrane protein 1; (LAMP-1) a marker of lymphocyte degranulation, was reduced suggesting that DUSP6/MKP-3 may also regulate the cytotoxic activity of CD8+ T cells. The changes in cytokine production by CD4+ T cells after DUSP6/MKP-3 deletion are suggestive of a role in T cell polarisation. When subjected to Th1 polarizing conditions a larger number of DUSP6/−/− CD4+ T cells produced IFN-γ, while under Th17 polarizing conditions DUSP6/−/− CD4+ T cells gave rise to fewer IL-17A producing cells. Taken together these results indicate that DUSP6/MKP-3 regulates the polarisation of CD4+ T cell subsets by inhibiting Th1 differentiation and favouring Th17 differentiation. Furthermore, to assess the function of DUSP6/−/− regulatory T cells (Treg) these were isolated and co-cultured with naïve CD4+ T cells from WT mice and stimulated with anti-CD3/CD28 for 72 h before assessing CD4+ T cell proliferation. DUSP6/−/− Treg cells consistently showed a lower capacity to inhibit the proliferation of naive CD4+ T cells indicating that DUSP6/MKP-3 is required for suppressive Treg function.

Finally, to assess DUSP6/MKP-3 function in vivo DUSP6/−/− mice were crossed with IL-10−/− mice and assessed for the development of intestinal colitis. The double knockout (DKO) mice consistently developed severe inflammation with epithelial crypt hyperplasia, loss of goblet cells, and immune cell infiltration into colonic connective tissue while DKO colonic explants produced increased levels of IFN-γ and TNFα, but lower levels of IL-17A when compared to IL-10−/− tissues. Satisfyingly, administration of PD0325901, a specific MEK inhibitor, both ameliorated and reversed the inflammatory phenotype seen in the DKO mice demonstrating that this is a direct result of increased levels of ERK1/2 signalling in the absence of DUSP6/MKP-3 [150].

In a recent study of the role of DUSP6 in endothelial cell inflammation Hsu et al. found that tail vein injection of TNFα caused elevated expression of Intercellular Adhesion Molecule 1 (ICAM1) in wild type but not DUSP6/−/− animals, suggesting that DUSP6 would modulate neutrophil recruitment and transendothelial migration at sites of acute inflammation. In agreement with this hypothesis, DUSP6/−/− animals injected intraperitoneally with either TNFα or LPS showed lower levels of pulmonary neutrophil infiltration and lung injury and adoptive transfer of wild type neutrophils into DUSP6/−/−...
mice revealed that the defect was intrinsic to endothelial cells [151]. Surprisingly, in vitro experiments in primary human umbilical vein endothelial cells (HUVECs) revealed that the role of DUSP6/MKP-3 in regulating TNFα-induced expression of ICAM1 involved activation of canonical nuclear factor (NF)-κB but was not dependent on its ability to dephosphorylate ERK MAPK [151].

Taken together, these studies indicate that DUSP6/MKP-3 may play complex and tissue specific roles in immune cell function and inflammatory processes (Table 2) and further work will be required to delineate the precise nature of the signalling events involved and the tissue specificity of DUSP6/MKP-3 functions.

3.1.2. DUSP6/MKP-3 in metabolic homeostasis

The first indication that DUSP6/MKP-3 might be involved in metabolic control was the finding that its expression was able to prevent the suppression of phosphoenolpyruvate carboxylase (PEPCK) gene expression by insulin. DUSP6/MKP-3 was also expressed in insulin-responsive tissues and expression levels were markedly elevated in the livers of insulin-resistant genetically obese (db/db) mice [152]. Subsequent work showed that expression of DUSP6/MKP-3 is also increased in the livers of HFD-induced obese mice and that adenosine-5′-monophosphate (AMP)-mediated DUSP6/MKP-3 expression in lean mice promotes gluconeogenesis and increased levels of fasting blood glucose. In contrast, shRNA knockdown of DUSP6/MKP-3 in both lean and obese mice resulted in decreased fasting blood glucose levels [153]. Mechanistically the transcriptional upregulation of gluconeogenic genes such as PEPCK that underpinned these effects was mediated by the dephosphorylation and nuclear translocation of Forkhead box protein O1 (FOXO1). Surprisingly, the effects of DUSP6/MKP-3 on FOXO1 were postulated to be direct, via protein-protein interaction and dephosphorylation of this transcription factor [154]. However, this finding is extremely difficult to reconcile with the known biochemical properties of DUSP6/MKP-3 and in particular the requirement for ERK2 binding to achieve catalytic activation of this phosphatase [141].

Experiments utilising DUSP6+/− mice to study metabolism have now been performed with the finding that mice lacking DUSP6/MKP-3 are somewhat protected from diet-induced obesity (Table 3). However, quite different conclusions were reached regarding the underlying mechanism. Feng et al. reported that DUSP6+/− mice are protected against both HFD-induced weight gain and hepatosteatosis and that these effects were accompanied by reduced liver triglyceride (TG) levels and adiposity. DUSP6−/− mice also exhibited increased energy expenditure, enhanced peripheral glucose disposal, and improved systemic insulin sensitivity [155]. Phosphoproteomic analyses in cultured Hepa1–6 cells +/+ versus −/− siRNAs targeting DUSP6/MKP-3 and comparing liver lysates from DUSP6−/− and DUSP6+/+ mice revealed significant increases in the phosphorylation of HDAC1 and 2. Pharmacological inhibition or combined knockdown of these enzymes in primary hepatocytes from DUSP6−/− mice was able to reverse the protective effects of DUSP6/MKP-3 deletion by raising the expression levels of several lipogenic genes, indicating that these enzymes may be the relevant in vivo targets [155].

Ruan et al. also reported protection against HFD-induced weight gain in DUSP6+/− mice together with improved glucose tolerance, increased insulin sensitivity and protection against hepatosteatosis [156]. However, faecal transplantation from HFD-fed DUSP6−/− mice into germ-free mice phenocopied this resistance and following studies of DUSP6/MKP-3-defendant changes in gut microbiota, intestinal barrier function and the gut transcriptome they concluded that DUSP6/MKP-3 loss protects the intestinal epithelial barrier from HFD-induced interruption and subsequent remodelling of the gut flora to maintain a lean-associated microflora. They conclude that DUSP6/MKP-3 regulates homeostasis between the gut epithelium, mucosal immunity and microbiota [156]. In contrast, a recent study observed comparable body weight, fat and lean mass in DUSP6−/− and DUSP6+/+ mice after 26 weeks on a HFD. However, glucose tolerance was somewhat abnormal in both lean and obese DUSP6−/− mice when compared to controls [157]. At present it is unclear why there is a discrepancy between the latter study and the previous two, but the finding that variations in the gut microbiota can have profound consequences for sensitivity to HFD coupled with differences in the genetic backgrounds used in these studies (pure C57Bl/6 J vs. mixed 129 x C57Bl/6 J) may account for this. As was the case for studies of DUSP1/MKP-1, the use of an unconditional (whole body) knockout of DUSP6/MKP-3 also makes the interpretation of these studies more difficult and further experiments using conditional ablation of DUSP6/MKP-3, will be required to address complexity and tissue specific interplay in the regulation of metabolic homeostasis by this phosphatase.

3.1.3. DUSP6/MKP-3 and cancer

Given the direct involvement of Ras/ERK signalling in human cancer a possible role for DUSP6/MKP-3 has been explored in some depth (Table 4). As was the case for the inducible nuclear ERK-specific phosphatase DUSP5, increased DUSP6/MKP-3 expression has been observed in primary tumours and cancer cell lines, which harbor mutations in either Ras or Braf, where its role as a negative feedback regulator of ERK activity has led to the suggestion that it may act as a tumour suppressor [125,158,159]. Thus DUSP6 expression is initially elevated and then epigenetically silenced during the progression of mutant Kras-driven pancreatic ductal adenocarcinoma, with the lowest levels detected in the most invasive and poorly differentiated tumours [160–162]. In a similar vein, loss of DUSP6/MKP-3 expression in mutant Kras-driven lung tumours is associated with increased disease severity and histological grade and loss of heterozygosity at the DUSP6 locus occurs in almost 20% of patients [163]. However, as is the case for many MKPs much of the mechanistic data obtained so far involves the reversal of cancer-associated phenotypes by ectopic expression of DUSP6/MKP-3 in cancer cell lines [161,163] and must therefore be treated with a degree of caution. No direct evidence of a tumour suppressor function for this MKP has yet been obtained by crossing DUSP6−/− mice into established murine cancer models. In contrast, two recent reports contain evidence that DUSP6/MKP-3 may actually be an oncogenic driver in certain human cancers.

Firstly, Shojaae et al., found that the acute oncogenic activation of BCR-Abl and NrasG12D in human pre-B cells was invariably lethal. However, any surviving cells were transformed and displayed increased expression of negative regulators of ERK signalling including DUSP6/MKP-3. Furthermore, this upregulation of DUSP6/MKP-3 was also seen in pre-B ALL cells, where it was driven by both Abl and ERK activity and high DUSP6/MKP-3 mRNA levels in patients with Philadelphia chromosome positive (Ph+) (BCR-Abl-driven) ALL was associated with shorter survival [164]. To gain mechanistic insight, Shojaae et al. transformed bone marrow-derived B cell lineage progenitor cells from DUSP6−/− mice and wild-type controls with BCR-Abl1. Interestingly, the survival of the DUSP6−/− cells was significantly reduced and conditional expression of NrasG12D in pre-B cells was only able to transform wild type but not DUSP6+/− cells. siRNA-mediated knockdown of DUSP6/MKP-3 in human pre-B ALL cells also reduced survival. All of these observations strongly indicate that pre-B ALL cells are dependent on DUSP6/MKP-3-mediated negative feedback control of ERK signalling for continued survival and growth [164]. In support of this conclusion, BCI, a compound identified as an allosteric inhibitor of DUSP6/MKP-3, caused a rapid increase in ERK activity in patient-derived Ph+ ALL cells. Mouse xenograft experiments using Ph+ ALL cells derived from patients after relapse during ongoing therapy with the Abl inhibitor Imatinib (Gleevec) showed these to be resistant to tyrosine kinase inhibition, but sensitive to treatment with BCI, indicating that this drug might be used to treat TKI-resistant Ph+ ALL [164]. While these results are very provocative, they must be treated with a degree of caution in that BCI, as mentioned previously in the context of its use as an inhibitor of DUSP1/MKP-1, is known to be relatively non-specific and displays considerable off-target toxicity [71].
In a recent genetic screen Wittig-Blaich et al., identified DUSP6/MKP-3 amongst a set of genes with growth suppressive properties consistent with tumour suppressor function. However, specifically in the context of mutant BrαF-driven melanoma they found that siRNA-mediated knockdown of DUSP6/MKP-3 caused apoptosis. They speculate that DUSP6/MKP-3 might be required to prevent BrαF-driven hyperactivation from triggering cell death via ERK1/2 downstream substrates and suggest that it might represent a synthetic lethal drug target in this subset of melanoma patients [165]. The idea that MKPs can intervene to prevent the engagement of tumour suppressive pathways has been suggested previously, mainly in the context of ERK-dependent oncogene-induced senescence (OIS) [166] and, as discussed previously, there is some support for differential outcomes in terms of cell proliferation and senescence after deletion of the inducible nuclear ERK-specific MKP DUSP5 in cells expressing either activated Ras or BrαF [131]. In support of the idea that DUSP6/MKP-3 may be pro-oncogenic in certain cancer types DUSP6/MKP-3 is upregulated in human glioblastoma cell lines and mouse xenograft experiments showed that tumours arising from glioblastoma cells expressing DUSP6/MKP-3 grew significantly faster than non-expressing controls [167]. The overexpression of DUSP6/MKP-3 in papillary thyroid carcinoma (PTC) cell lines is also associated with increased cell migration and invasion [168].

Finally, although DUSP1/MKP-1 has been widely studied as a modulator of drug responses in cancer chemotherapy (see Section 2.1.3) less attention has been paid to the ERK-specific MKPs. However, Phuchareon et al. recently identified the down-regulation of DUSP6/MKP-3 as a contributing factor to the reactivation of Ras-ERK signalling and drug resistance in epidermal growth factor receptor (EGFR) mutant lung cancer cell lines exposed to the TKI gefitinib (Iressa) and erlotinib (Tarceva) [169]. Resistance was mediated by increased ERK-dependent phosphorylation and degradation of the extra-long isoform of the pro-apoptotic B-cell lymphoma-2 (Bcl-2) family protein Bim (BimEL) thus promoting cancer cell survival. Interestingly, DUSP6/MKP-3 down-regulation has also been implicated in mediating reactivation of Ras-ERK signalling and drug resistance in lung cancer cell lines harbouring the echinoderm microtubule-associated protein like 4 -anaplastic lymphoma kinase (ELM4-ALK) fusion protein exposed to the TKI crizotinib (Xalkori) [170]. As one of these two driver mutations are present in almost 20% of non-small cell lung cancers (NSCLC), targeting ERK signalling in combination with the use of TKIs may be a viable strategy to forestall or prevent TKI resistance and loss of DUSP6/MKP-3 is worthy of wider study as a possible ERK-mediated drug resistance mechanism in other tumour types driven by abnormal tyrosine kinase activity.

In conclusion it is perhaps no surprise that DUSP6/MKP-3 is implicated in the regulation of oncogenic signalling through the Ras/ERK pathway and recent work indicates that the effects of altered expression levels may depend on both the oncogenic background the tissue(s) involved (Table 4). Further studies will be required, preferably using conditional deletion of this MKP in murine models to dissect out its precise role in carcinogenesis, particularly given its emerging role in immune regulation and inflammatory processes, both of which may have a bearing on tumour initiation and development.

3.2. DUSP7/MKP-X

DUSP7/MKP-X most closely related to DUSP6/MKP-3 and shares many properties, including cytoplasmic localisation, a high degree of substrate specificity for the ERK1/2 MAPKs and substrate-induced catalytic activity [12,137,171] but, in contrast to DUSP6/MKP-3, almost nothing is known about its physiological function(s) or association with human disease. However, DUSP7/MKP-X was identified in an siRNA based phenotypic screen for genes involved in meiotic progression in mouse oocytes [172] and more recent work has shown that DUSP7-depleted oocytes either fail or are significantly delayed in resuming meiosis and that cyclin-dependent kinase-1/cyclin B (Cdk1/CycB) activity drops below the critical level required to reinitiate meiosis. Once in meiosis DUSP7/MKP-X depleted oocytes also had severe chromosome alignment defects and progressed into anaphase prematurely [173]. These effects were judged to be secondary to a failure to dephosphorylate and inhibit protein kinase C isoforms, a prerequisite for the timely activation of Cdk1/CycB and are likely to be significant as both male and female mice lacking DUSP7/MKP-X have been reported as viable but infertile (http://www.mousephenotype.org/data/mouse/SGI:12387100). As failure to resume oocyte meiosis is a contributing factor to female infertility in humans it will be important to establish whether this signalling pathway is conserved.

3.3. DUSP9/MKP-4

MKP-4, the third member of this group of cytoplasmic phosphatases, is encoded by the X-linked DUSP9 gene and has properties in common with DUSP6/MKP-3 and DUSP7/MKP-X, although its substrate specificity is somewhat more relaxed with respect to binding and inactivation of JNK and p38 MAPKs [141,147,175]. DUSP9/MKP-4 is also somewhat unusual in that it is not transcriptionally regulated in response to either growth factor simulation or stress, but instead seems to be constitutively expressed and regulated via phosphorylation of a conserved serine residue adjacent to the KIM, which abrogates substrate binding [176]. Unconditional deletion of DUSP9 results in embryonic lethality due to placental insufficiency, but tetraploid rescue experiments demonstrated that it is otherwise dispensable for normal embryonic development [177]. Information about possible physiological roles for DUSP9 and links to human disease is relatively scant. Selective expression of DUSP9 expression in Plasmacytoid dendritic cells (pDCs), but not conventional dendritic cells (cDCs), suggested a possible role in determining this phenotype. However, conditional deletion of DUSP9 in pDCs did not increase ERK activation after TLR9 stimulation and only weakly affected IFN-β and IL-12Beta (IL-12p40) production by these cells indicating that this MKP is not essential for the high level production of IFN-β, which is characteristic of pDCs [178]. However, recent work now indicates a link between DUSP9/MKP-4 and metabolic homeostasis (Table 3).

In obese and insulin resistant mouse models DUSP9/MKP-4, protein levels are reported to be elevated in insulin responsive tissues and expression of DUSP9/MKP-4 caused increased expression of PEPCk. Overexpression of DUSP9/MKP-4 in adipocytes also blocked insulin-stimulated glucose uptake, again suggesting that this enzyme antagonises the effects of insulin in responsive cells and tissues [179]. However, in a stress-induced in vitro model of insulin resistance and following adeno-medi-ated overexpression of DUSP9/MKP-4 in the livers of genetically obese (ob/ob) mice Emanuelli et al. reported that DUSP9/MKP-4 expression improved glucose intolerance, decreased the expression of gluconeogenic genes and reduced hepatic steatosis [180]. Despite these apparently contradictory results, a recent study in which conditional liver-specific deletion of DUSP9/MKP-4 was used to study the response to a high fat diet has demonstrated that loss of DUSP9/MKP-4 in the liver sensitizes animals to hepatic steatosis and inflammatory responses and that DUSP9 deficiency aggravated high fat, high cholesterol (HFHC)-induced liver fibrosis [181]. In this context it will be interesting to compare the effects of whole body DUSP9 KO and also loss of this MKP in other insulin responsive tissues as studies of DUSP1/MKP-1 function in metabolic homeostasis have revealed that the liver phenotype may not be dominant over the effects of loss of function in other tissues [50,56,57]. Finally, a genetic variant mapping near to the DUSP9 gene locus has repeatedly been detected in genome-wide association studies (GWAS) as a risk factor for the development of Type-2 diabetes across different ethnicities in human populations, further reinforcing a link between DUSP9/MKP-4 and metabolic control [182–184]. The availability of a conditionally targeted allele for DUSP9 should greatly accelerate future work on the possible role of this MKP, both in metabolic disease and in other pathologies.
4. The JNK and p38 specific MKPs

4.1. DUSP8

Along with DUSP7/MKP-X, DUSP8 is probably the least studied of the 10 dual-specificity MKPs and there is virtually nothing known about its physiological function. Since its identification as a gene encoding an MKP with a translated complex trinucleotide repeat within its coding region and characterisation of its specificity for the inactivation of JNK and p38 MAPKs [138] fewer than 20 papers have been published on DUSP8 and although targeted mouse ES cells have been generated by the international mouse phenotyping consortium (IMPC), these have not yet been exploited to produce a mouse model.

4.2. DUSP10/MKP-5

DUSP10/MKP-5 was first characterised as a widely expressed JNK and p38 specific MKP, which when expressed in mammalian cells is found in both the cytoplasm and nucleus [185,186]. One unique feature of DUSP10/MKP-5 is that it carries an amino-terminal extension of unknown function, but which may carry signals that specify its subcellular localisation [185].

4.2.1. DUSP10/MKP-5 in immunity and inflammation

Along with DUSP1/MKP-1, DUSP10/MKP-5 was one of the first MKPs found to regulate both innate and adaptive immune function. While development of the myeloid and lymphoid lineages was normal in mice lacking DUSP10/MKP-5, the DUSP10/MKP-5 gene is inducible at the transcriptional level in response to TLR signalling and peritoneal macrophages lacking DUSP10/MKP-5 showed increased production of the pro-inflammatory cytokines IL-6 and TNFα. Consistent with this, LPS injected DUSP10−/− mice had higher serum levels of TNFα when compared to wild type controls. Mechanistically, JNK appeared to be the relevant DUSP10/MKP-5 substrate as elevated levels of phosphoJNK were observed in both macrophages and T cells derived from DUSP10−/− mice [187]. However, subsequent experiments have clearly shown that DUSP10/MKP5 does regulate p38 MAPK activity in both monocyte/macrophages [188] and neutrophils [189] indicating that both stress activated MAPK pathways are subject to negative regulation by DUSP10/MKP-5.

As discussed previously in relation to the function of DUSP1/MKP-1 [35], an important function of the innate immune system is mediated via professional antigen presenting cells (APC), which activate antigen specific T cells, thus bridging the innate and adaptive immune systems [34]. LPS treated APCs isolated from DUSP10−/− mice exhibited enhanced priming of ovalbumin-transgenic OT-I (CD4+) and OT-II (CD8+) T cells as assessed by increased levels of IL-2 production and T cell proliferation when compared to wild type APCs. Taken together, these results indicate that DUSP10/MKP-5 plays a non-redundant role as a negative regulator of the innate immune response [187].

In terms of T cell differentiation and function DUSP10−/− Th1 and Th2 cells showed increased JNK activation, but this was lost rapidly on anti-CD3 re-stimulation, indicating that DUSP10/MKP-5 is not the sole arbiter of JNK activity in these cells. Activation of CD4+ T cells from DUSP10−/− mice with anti-CD3 (with or without anti-CD28 co-stimulation) resulted in reduced proliferation when compared to wild type, indicating that DUSP10/MKP-5 is required for T cell expansion. However, IFN-γ production by activated Th1 and IL-4 production by activated Th2 cells lacking DUSP10/MKP-5 were increased, while activated effector CD8+ T cells from DUSP10−/− mice produced more IFN-γ and TNFα than wild type cells [187]. In concordance with these results, immunisation of DUSP10−/− mice with keyhole limpet haemocyanin (KLH) and re-stimulation ex vivo, resulted in reduced antigen-driven proliferation of splenic T cells but increased levels of IFN-γ and IL-4 production confirming the reciprocal role of DUSP10/MKP-5 in regulating of T-cell clonal expansion and effector T-cell cytokine expression [187]. Finally, the role of DUSP10/MKP-5 in susceptibility to infection with LCMV and to MOG-induced EAE was tested. While DUSP10−/− mice showed little difference in their initial primary T cell response to infection or in viral clearance, re-challenge caused immune-mediated death, probably as a result of the markedly elevated levels of serum TNFα produced by CD4+ and CD8+ T cells. In the EAE model DUSP10−/− mice exhibited a reduced number of CD4 T cells in the brain, which correlated with reduced incidence and severity of disease, indicating that this MKP plays a positive role in the generation and/or expansion of autoreactive T cells in this autoimmune disease model [187].

More recent work has explored the role of this MKP in regulating host responses to inflammatory stimuli using a mouse model of local Shwartzman reaction (LSR). LSR is a delayed vascular injury produced by sequential subcutaneous injection of LPS and TNFα and DUSP10−/− mice were found to be much more susceptible to this form of injury. Mechanistically this was the result of increased p38 MAPK activation in neutrophils and the production of greatly increased levels of superoxide anion by the NADPH oxidase complex, thus revealing an essential role for DUSP10/MKP-5 as a negative regulator of p38-mediated neutrophil reactive oxygen intermediate (ROI) production [189]. Interestingly, p38-mediated cytokine and ROS production by macrophages also accounts for the increased susceptibility of DUSP10−/− mice to endotoxin-induced acute lung injury following intratracheal injection of LPS, again reinforcing the role of this MKP in protection against inflammatory tissue injury [190].

Collectively these studies reveal that DUSP10/MKP-5 like DUSP1/MKP-1 plays an important role in regulating both innate and adaptive immune responses (Table 2) and reveals significant complexity and tissue specificity in its interactions with MAP kinase signalling. This is illustrated by the observation that while the defect in T cell expansion seen on loss of DUSP10/MKP-5 is responsible for protection against EAE, this deficit does not result in a reduction in the numbers of LCMV-reactive T cells following viral infection, probably because of the compensatory effects of DUSP10/MKP-5 loss in stimulating APC function.

4.2.2. DUSP10/MKP-5 function in other tissues

Shi et al. have reported a function for DUSP10/MKP-5 in regulating muscle stem cell function and muscle regeneration. DUSP10−/− mice had increased levels of p38 and JNK activation, muscle mass and muscle fibre size when compared to wild type animals. Furthermore, in response to muscle injury following injection of cardiotoxin, they display an enhanced regenerative response associated with early upregulation of JNK and later of p38 activity. Interestingly, when crossed into the mdx (dystrophin null) mouse model of Duchenne’s muscular dystrophy, the double knockout animals showed an amelioration of disease manifested by improved skeletal muscle morphology, a reduced number of degenerating muscle fibres and improved contractile function. Mechanistically, this was underpinned by increased muscle stem cell proliferation and differentiation, which were regulated by increased JNK-mediated expression of cyclin D and p38 mediated myogenesis respectively. Finally, despite its function as an immune regulator, these effects of DUSP10/MKP-5 loss appeared to be completely independent of alterations in immune cell infiltration into damaged muscle [79].

Interestingly, the effects of DUSP10/MKP-5 deletion on myogenesis appear to be due to two main effects. Firstly, deletion of DUSP10/MKP-5 increases MAPK-dependent phosphorylation of guanine nucleotide exchange factor for the Ras-related protein Rab-3A Rab3A (GRAB) at Serine 169, a site required for secretion of the promyogenic cytokine IL-6 [191]. Secondly, in the absence of DUSP10/MKP-5 increased JNK and p38 mediated phosphorylation and activation of STAT3, increases the expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl2) thus preventing apoptosis during regenerative myogenesis and also leads to improved antioxidan defence capacity due to a sustained increase in catalase expression that protects mitochondrial function [192].
Finally, a recent study has used DUSP10−/− mice to address the possible function of this MKP in modulating the development of DSS-induced intestinal inflammation and colitis associated cancer (CAC) (Table 4). Surprisingly, given the previous findings that mice lacking DUSP10/MKP-5 were more sensitive to inflammatory tissue damage in skin and lung [189,190], DSS treated DUSP10−/− mice exhibited lower levels of intestinal inflammation, better intestinal crypt architecture and lower levels of pro-inflammatory cytokine/chemokine expression than wild type animals. This protection was secondary to improved intestinal epithelial cell (IEC) barrier function, which serves to separate luminal contents from the mucosal immune system, as evidenced by reduced IEC leakage of fluorescein isothiocyanate (FITC)-dextran [193]. Mechanistically, this was due to increased ERK-mediated expression of Kruppel like factor-5 (KLFL5) a transcription factor which up-regulates cyclinB expression and promotes IEC proliferation during intestinal regeneration and wound healing. In accordance with this increased numbers of proliferating Ki67-positive cells were observed in the intestinal crypts of DSS-treated DUSP10−/− colon compared with wild type tissue [193]. Again these results suggest possible tissue specific variation in DUSP10/MKP-5 activity towards MAPKs as ERK but not JNK or p38 activity was affected by DUSP10/MKP-5 loss. Finally, although protective against DSS-induced inflammation, the combined treatment of DUSP10−/− mice with the mutant azoxymethane (AOM) and DSS resulted in an increased incidence of adenomatous polyps of larger size, which stained positive for Ki67 and β-catenin. Overall, this strongly suggests that the IEC and the subsequent tumours were more proliferative in the absence of DUSP10/MKP-5 indicative of a tumour suppressor function for this MKP, an idea supported by the observation that higher levels of DUSP10/MKP-5 expression correlated with better survival amongst patients with colorectal cancer [193].

4.3. DUSP16/MKP-7

DUSP16/MKP-7 was the last of the 10 dual-specificity MKPs to be identified and was characterised as a JNK and p38-specific MKP with a possible function as a regulator of JNK activity in macrophages [194-196]. Although relatively little is known about this phosphatase, three recent studies using knockout mice have begun to shed some light on its possible physiological role(s). Firstly, using a gene trap null mutation Niedzielska et al. reported that loss of DUSP16/MKP-7 caused perinatal lethality [197]. Observing that DUSP16/MKP-7 was inducible in macrophages in response to TLR agonists, they used fetal liver cells from the null mice to reconstitute the lymphoid and myeloid lineages in lethally irradiated syngeneic CD45.1+ animals. They found that T and B cell populations were present in the normal numbers, that >95% of resident macrophages were derived from the DUSP16/MKP-7 null donor cells and the mice had normal numbers of granulocytes and plasmacytoid dendritic cells, indicating that DUSP16/MKP-7 is not essential for homeostasis of the immune system under steady state conditions. However, there was a deficit in numbers of splenic CD11c+ /CD11b + myeloid dendritic cells secondary to impaired granulocyte-macrophage colony-stimulating factor (GM-CSF)-driven proliferation of bone marrow progenitors [197]. Subjecting reconstituted mice to LPS challenge revealed no undue sensitivity to sepsis, but did reveal JNK-dependent overproduction of IL-12Beta (IL-12p40) by macrophages in response to LPS [197]. Overall these results reveal a dual function for DUSP16/MKP-7 in the innate immune system involving selective control of differentiation and cytokine production (Table 2), but further work is required to map out the physiological consequences of this regulation.

Zhang et al also reported that loss of DUSP16/MKP-7 was lethal and used recombination experiments to study the role of this MKP in adaptive immunity (Table 2) [198]. In agreement with Niedzielska et al. [178], they found that T cell development and numbers were normal, but that CD4+ T cells lacking DUSP16/MKP-7 were hypersensitive to activation, produced much more IL-2 and had higher rates of proliferation when compared to wild type cells. To study T cell differentiation and function they cultured naïve DUSP16−/− CD4+ T cells under Th1, Th2, or Th17 conditions in vitro and found that while functional Th1 and Th2 cells were produced normally, DUSP16−/− Th17 cell populations produced less IL-17A and IL-17F, and contained nearly 50% less IL-17A-producing cells compared with WT cells [198]. Interestingly, U0126 a specific MEK inhibitor, efficiently reversed the deficit in IL-17A producing Th17 cells in vitro, indicating that regulation of ERK and not JNK or p38 was responsible. Given the role that Th17 cells play in autoimmunity, the susceptibility of the reconstituted animals was also assessed using the MOG-induced model of EAE and consistent with the functional Th17 deficit, these animals were less susceptible to disease indicating an essential role for this MKP in autoimmune responses [198].

Finally, a recent study has explored the reason for the embryonic/ prenatal lethality in mice lacking DUSP16/MKP-7 and revealed an essential role for this MKP in brain development. Embryos lacking DUSP16/MKP-7 exhibited congenital obstructive hydrocephalus together with brain overgrowth. This was secondary to blockage of the midbrain aqueduct by the expansion of neural progenitor cells, eventually preventing the outflow of cerebrospinal fluid. Interestingly only an increase in cells staining positively for phospho-p38 was observed in the affected regions of the CNS indicating that this MAPK, rather than ERK or JNK could be the relevant target [199].

Taken together these studies reveal the first essential role for a member of the MKP family of enzymes in brain development and as the phenotype of DUSP16−/− mice recapitulates aspects of different human neurodevelopmental disorders suggests that either DUSP16/MKP-7 or the pathways it regulates may be implicated. They also reveal specific defects in innate and adaptive immunity in mice lacking DUSP16/MKP-7. In particular the specific role of DUSP16/MKP-7 in promoting autoimmunity make it a potential therapeutic target in a range of human disorders such as inflammatory bowel disease, rheumatoid arthritis and lupus.

5. Conclusions and future perspectives

The past decade has seen an acceleration in the use of GEM models to probe the physiological and pathophysiological roles of the MKPs and these have provided a wealth of information for certain members of the family, particularly in relation to the functional regulation of the immune system, but also in metabolic disease and cancer. As is the case for other classes of protein phosphatases, it is abundantly clear that MKPs are not merely passive “erasers” of protein phosphorylation, but instead form a complex network of activities in cells and tissues that act to regulate the spatiotemporal activity of the different MAPK isoforms targeted references in the MAPK pathways and play essential roles in regulating key physiological outcomes.

Several themes have emerged, one of which is the importance of compartmentalised regulation of MAPK signalling by activities in the nucleus and cytoplasm as exemplified by the regulation of nuclear JNK activity by DUSP1/MKP-1 in metabolic control [50] and nuclear ERK activity by DUSP5 in cancer [128]. It is also clear that there are both tissue and cell type specific differences in the MAPK isoforms targeted by particular MKPs, one example being the preference of DUSP1/MKP-1 for inactivation of p38 MAPK in macrophages and dendritic cells [30,35] whereas JNK is the relevant substrate in T cells [33]. Thus far, we do not have any detailed grasp of how these specificities may be altered in vivo. This must be addressed in future work as must the validity of putative non-MAPK substrates for certain MKPs invoked in disease models. The latter include the possibility that STAT3 is directly targeted by DUSP2 in innate immunity [87], the potential regulation of FOXP1 and as yet uncharacterised non MAPK substrates by DUSP6/MKP-3 in metabolic regulation [154] and endothelial inflammation [151] respectively and modulation of PKC activity by DUSP7/MKP-X in oocyte meiotic progression [173].
Finally, there are numerous examples where knockout phenotypes indicate that certain MKPs may be therapeutic targets in human disease. These include inhibition of DUSP1/MKP-1 in combattig obesity and depression (50,73) and DUSP2 as a potential anti-inflammatory drug target (86). However, caution must be exercised here in inhibition of DUSP1/MKP-1 could also result in more severe inflammatory responses and many existing anti-inflammatory agents, such as glucocorticoids, actually enhance the expression and activity of this MKP as part of their mode of action. Clearly specificity of action towards individual MKPs would also be crucial in any strategy to target these enzymes and the avoidance of undesirable side effects. In this regard and due to the involvement of a redox active cysteine residue in catalysis, the PTPase superfamily has long been regarded as “undraggible”. However, recent progress in the development of allosteric PTPase inhibitors [200,201] gives hope that future work will lead to the development of highly specific small molecules able to target MKP activity, which will then allow a meaningful exploration of their therapeutic potential.

Transparency document

The Transparency document associated with this article can be found in online version.

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