Chapter 4

Emerging Roles of Atypical Dual Specificity Phosphatases in Cancer

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1. Introduction

The dual specificity phosphatases (DUSPs) are a subfamily within the protein tyrosine phosphatase (PTP) family, with the unique property of being able to hydrolyze phospho-serine or phospho-threonine residues and phospho-tyrosine residues [1]. All DUSPs share the characteristic Class I PTP consensus sequence, D…HC(X)$_5$RS/T, with C representing the essential catalytic cysteine [1]. Unlike other PTPs, DUSPs lack the phospho-tyrosine recognition domain, resulting in a shallower catalytic cleft, most likely enabling DUSPs to dephosphorylate all three residues (S/T/Y) [1]. In addition to protein substrates, the DUSP subfamily contains members that dephosphorylate additional substrates including lipids, nucleic acids, and sugars [2].

DUSPs are regulators of multiple signaling pathways driving fundamental cell processes such as growth, proliferation, apoptosis, and migration, and as such they are often deregulated in a variety of diseases [2]. DUSPs can be further classified on substrate specificity and sequence homology, but all DUSPs share a highly conserved prototypical DUSP domain initially characterized in the Vaccinia virus's VH1 gene [3]. The best-characterized DUSPs include the MAP kinase phosphatases (MKP's), which directly antagonize the activating dual phosphorylation of mitogen activated protein kinases, and PTEN that functions to dephosphorylate phosphatidyl-inositol 3,4,5 triphosphate (PIP3), the product of PI3 kinase (PI3K) [2]. As these signaling pathways are intimately implicated in cancer initiation and progression, it is not surprising that their cognate phosphatases also functionally contribute to disease progression [2,4].

In addition to DUSPs described above, the DUSP subfamily contains a distinct subgroup described as the atypical DUSPs [2,5]. In humans there are at least 16 DUSPs classified as atypical (Table 1) based on the lack of sequence similarity to better-characterized DUSPs and/or due to their substrate specificity [2,5]. Physiological substrates for several atypical

Atypical DUSPs	
Name	**Aliases**
Laforin	EPM2A (epilepsy, progressive myoclonus type 2A)
STYX	Serine/threonine/tyrosine interacting protein
DUSP3	VHR (VH1-related)
DUSP11	PIR1 (phosphatase that interacts with RNA-ribonucleoprotein complexes)
DUSP12	hYVH1 (human YVH1)
DUSP13A	MDSP (muscle-restricted DUSP), SKRP4 (stress activated protein kinase pathway-regulating phosphatase-4), BEDP (branching-enzyme interacting DUSP)
DUSP13B	TMDP (testis-and skeletal muscle-specific DUSP)
DUSP14	MKP-L (MKP-1 like protein tyrosine phosphatases), MKP6
DUSP15	VHY (VH1-like member Y)
DUSP18	DUSP20, LMWDSP20 (low-molecular-weight DUSP20)
DUSP19	DUSP17, SKRP1
DUSP21	LMWDSP21
DUSP22	LMWDSP2, JSP1 (JNK-stimulating phosphatases 1), JKAP (JNK pathway-associated phosphatase), MKPX, VHX
DUSP23	DUSP25, LDP3 (low molecular mass DUSP3), VHZ
DUSP26	DUSP24, LDP4, MKP8, NEAP (neuroendrocrine associated phosphatase), SKRP3
DUSP27	DUPD1 (DUSP and pro-isomerase domain containing 1

Table 1. The atypical DUSPs and their various aliases.

DUSPs include proteins (MAPKs), nucleic aids (RNA), and phosphorylated carbohydrates (amylopectin and glycogen), but for many atypical DUSPs physiological substrates are unknown [2,5]. However, even for cases where phosphatase-substrate relationships are known, they are somewhat complicated by the fact that several DUSPs appear to function independently of their phosphatase activity and instead function as scaffolds in signal transduction pathways [6-8].

Several atypical DUSPs have been implicated in apoptosis and proliferation [2,5], but how the DUSPs contribute to these processes is largely also unknown. Emerging roles for atypical DUSPs in malignancy are beginning to be inferred from high throughput sequencing/ genomic approaches, which have demonstrated that, like MKP's and lipid phosphatases, the atypical DUSP genes are differentially expressed in a variety of cancers and may contribute to cancer initiation and/or progression [2,4,5]. The following sections present a current synthesis of what is known about how the atypical DUSPs function, and will focus specifically on how these proteins may contribute to cancer initiation and progression. Current gaps in knowledge on the function of these proteins in both normal and cancer cell biology is highlighted to hopefully inspire new research on these poorly understood proteins.

2. Atypical DUSPs currently implicated in cancer

2.1. Laforin

Due to alternative splicing of mRNA encoded by the *epm2a* gene, at least two isoforms of Laforin have been described that differ in their subcellular localization and phosphatase activity [9]. The major isoform encodes for a 331 amino acid protein containing a catalytically active DUSP domain and a N-terminal carbohydrate-binding module (CBM). Loss of function mutations of the major isoform are causative for Lafora's disease, a fatal form of progressive myoclonus epilepsy that is thought to occur as a result of deregulated glycogen metabolism [10-13] with the accumulation of insoluble complex carbohydrates [14]. Laforin regulates glycogen metabolism, in part, by its ability to directly dephosphorylate phosphorylated carbohydrates including glycogen [14]. Loss of epm2a or mutations in epm2a that prevent glycogen binding or alter its phosphatase activity, contribute to Lafora's disease [14,15]. Laforin also regulates glycogen metabolism in a phosphatase independent manner by functioning as an adapter protein to promote the ubiquitination of proteins involved in glycogen metabolism by recruiting Malin, an E3 ubiquitin ligase [16]. Proteins regulating glycogen metabolism are often complexed with glycogen, as Laforin can bind glycogen and independently recruit Malin this provides an additional regulatory mechanism [16]. As a result, mutations in epm2a that disrupt the ability to bind Malin also contribute to Lafora's disease [16]. Over the last several years, studies have revealed additional potential role(s) for Laforin in cancer development and/or progression where it most likely functions as a tumor suppressor, but may also contribute to cancer by promoting cell survival [17,18]. Here, we focus on potential roles of the major isoform in cancer, as it is the most studied.

A role for Laforin as a tumor suppressor was demonstrated by the observation that immunocompromised mice lacking Laforin produce spontaneous lymphomas [19]. Additionally, Laforin mRNA and protein levels were reduced in murine and human primary lymphomas [19]. In addition to the roles of Laforin in glycogen metabolism and Lafora's disease, Laforin regulates glycogen synthase kinase 3 (GSK3β) activity, a key signaling protein in the β-catenin/WNT signaling pathway [20] by removal of the inhibitory phosphate at Ser9 [21]. Cells lacking Laforin display decreased GSK3β activity resulting in increased cyclin D1 stability, and increased WNT signaling [21,22]. As both cyclin D1 expression and WNT signaling have been implicated in a variety of cancers [20], the ability of Laforin to regulate GSK3β to inhibit cyclin D1 and WNT signaling is a possible mechanism by which Laforin may function as a tumor suppressor. In addition to a GSK3β-dependent role in promoting cell cycle progression [21,22], Laforin additionally possesses pro-survival attributes and may be a potential therapeutic target in lymphomas where low Laforin expression promotes apoptosis induced by energy deprivation, while lymphomas with high Laforin expression are resistant [18]. Laforin's ability to promote cell survival could be related to glycogen metabolism, or alternatively due to its indirect regulation of the WNT signaling pathway via activation of GSK3β [18,19].

Laforin additionally has a role in stress induced proteostasis [23-26]. Knock down of Laforin in human embryonic kidney (HEK293) cells and the neuroblastoma cell line, SH-SY5Y,

resulted in increased apoptosis induced by endoplasmic reticulum (ER) stress [24]. Laforin also promotes autophagy by inhibiting the mammalian target of rapamycin (mTOR) pathway by a currently unknown mechanism to further protect cells from ER stress [25]. In addition to targeting genes involved in glycogen metabolism, recruitment of Malin further promotes ubiquitination of misfolded and aggregated proteins, thereby facilitating their proteosomal degradation [27]. Laforin also interacts with heat shock factor 1 (HSF1), an essential transcription factor in the heat shock response [26] and is necessary for up-regulation of HSF1-dependent gene expression and for protection from thermal stress in COS7 cells [26]. Accordingly, increased Laforin expression may allow cancer cells to survive in conditions where proteostasis has been perturbed.

2.2. DUSP3/VHR

Despite the ability of DUSP3 (alternatively named VHR for VH-1 related) to dephosphorylate the ERK1/2 and JNK MAPKs, it is generally not considered a MKP as it lacks the MAPK binding domain characteristic of MKPs [2,28,29]. Nevertheless, as a functional MAPK phosphatase, it is not surprising that DUSP3 is implicated in cancer, where it has been alternatively described as having both oncogenic [30-32] and tumor suppressive [33,34] properties.

dusp3 is up-regulated in cervical cancer cell lines and primary cervical cancers [30], and in HeLa cells, knock-down of *dusp3* inhibited proliferation by increasing the phosphorylation levels of ERK1/2 and JNK [31,35]. *dusp3* is also over-expressed in primary prostate tumors where it may function as a pro-survival phosphatase [32]. In prostate cancer cells (LNCaP), DUSP3 functions as an inhibitor of apoptosis, where *dusp3* knock-down resulted in increased JNK phosphorylation and increased apoptosis when treated with thapsigargin, an inducer of ER stress, or 12-*0*-tetradecanoylophorbol-13-acetate (TPA) [31].

DUSP3 may also inhibit tumor growth by regulating proliferation, particularly in the context of breast cancer cells expressing the oncogene, *brca1-iris. brca1-iris* is a splice variant of the breast cancer type 1 susceptibility (*brca1*) locus that promotes cell proliferation, cell migration, and invasion [34,36]. Over-expression of *brca1-iris* in breast cancer cell lines MCF7 and SKBR3 promoted proliferation through up-regulation of cyclin D1, and over-expression of *dusp3* was sufficient to suppress cyclin D1 expression [34]. *dusp3* expression is also down-regulated in primary non-small cell lung cancer (NSCLC) tumors [34]. A tumor suppressive function for DUSP3 in NSCLC is supported experimentally, as in both the NSCLC H1299 cell line and mouse xenographs, over-expression of *dusp3* suppressed growth of cells and tumors respectively [32]. Further supporting a tumor suppressor function for DUSP3, its expression suppressed phospholipase C (PLC) - protein kinase C (PKC) signaling [36]. The ErbB tyrosine kinase receptor, known to act upstream of PLC-gamma was subsequently identified as a direct DUSP3 substrate [37]. Increased ErbB tyrosine kinase activity drives several cancer-relevant properties including proliferation, cell motility, and invasion [36]. *dusp3* over-expression removed the activating phosphate from Y992 of ErbB, preventing ErbB kinase activity [33]. As the ErbB family of proto-oncogenes is strongly

associated with cancers in a kinase dependent manner [37], the ability of DUSP3 to inhibit its activity may have important implications for cancer therapies.

If DUSP3 functions as a tumor suppressor or oncogene, modulators of DUSP3 function(s) could be attractive anti-cancer drug targets. The ZAP70 cytoplasmic tyrosine kinase, a key component in signaling machinery downstream of the T-cell antigen receptor, directly phosphorylates DUSP3 on tyrosine 138 (Y138) and increases it's ability to temper Erk1/2 and JNK signaling and reduce expression of a NFAT-AP1 dependent luciferase reporter, as VHR^{Y138F} functions as a dominant negative [38]. This observation is consistent with Y138 increasing the catalytic activity VHR, but this hypothesis is technically difficult to confirm due to VHR's ability to auto-dephosphorylate [38]. ZAP70 has been implicated in cancer as it is a prognostic marker for chronic lymphocytic leukemia (CLL) and B-cell acute lymphoblastic leukemia (B-ALL) where increased ZAP70 expression correlates with poor clinical outcome [39,40]. Besides being a prognostic marker, ZAP70 contributes to cancer by promoting survival and migration in both CLL and B-ALL [41,42] but whether ZAP70's modulation of DUSP3 has importance in B-ALL and CLL remains unknown. ZAP70 is not the only tyrosine kinase to target DUSP3 at tyrosine 138. TYK2, the non receptor tyrosine kinase of the Janus kinase family that regulates the expression of type 1 interferons and interleukin 12 [43] also targets this site [44]. Phosphorylation of DUSP3 by TYK2 was required for DUSP3 to dephosphorylate and inhibit signal transducer and activator of transcription 5 (STAT5) [44]. The ability of DUSP3 to inhibit STAT5 transcriptional activity may have consequences in cancer cell biology, since STAT5 activity is known to promote cancer by transcriptionally regulating genes involved in proliferation and survival [45], therefore loss of DUSP3 activity could possibly result in increased STAT5 activity thereby promoting cancer development. Furthermore, TYK2 has a suggested role in breast cancer metastasis, as analysis of 140 tissue samples from 70 breast cancer patients revealed that TYK2 protein levels are reduced in tumors that have metastasized to the regional lymph nodes [46]. Additionally, knock-down of *tyk2* in the MCF10A breast epithelial cell line decreased cell migration and invasion [46]. Whether loss of TYK2 promotes metastasis, migration, and invasion due to reduced DUSP3 activity remains to be determined

DUSP3's activity is additionally activated by the pseudokinase, Vaccinia-related kinase 3 (VRK3) [47], whose expression is down-regulated in colorectal cancer [48]. VRK3 inhibits ERK1/2 signaling by the formation of a VRK3-DUSP3-ERK1/2 complex where VRK3 is able to enhance the activity of DUSP3 towards ERK1/2 in a kinase independent manner [47]. It would be interesting to examine whether tumors with decreased *vrk3* expression also have decreased DUSP3 activity. Since VRK3 promotes DUSP3's activity towards ERK1/2, one would expect tumors with decreased VRK3 to have increased ERK1/2 signaling, and these investigations could further indicate a potential tumor suppressor for DUSP3.

2.3. DUSP11

Due to interactions with ribonucleoprotein (RNP) complexes and RNA splicing factors and its ability to dephosphorylate RNA trinucleotides, DUSP11 is thought to have a role in RNA

splicing [49]. DUSP11 also associates with SAM68 (SRC-associated protein in mitotic cells), an ERK1/2 phosphorylated splicing factor that promotes the alternative splicing of cluster of differentiation 44 *(CD44)* mRNA encoding a glycoprotein involved in cell-cell interactions, cell adhesion, and migration [50,51]. Alternatively spliced isoforms of CD44 include up to 10 variant exon sequences (V1 to V10) that are though to contribute to human organismal complexity from a relatively restricted number of genes [50]. The v5 exon splice variant of CD44 is suggested to promote metastatic cancers [50,51] and this alternative splicing event is thought to be under the control of Ras singaling pathway through recruitement of SAM68. As forced overexpression of Sam68 leads to increased inclusion of the V5-exon sequence in CD44 and Dusp11 associated with SAM68, and variants of SAM68 that cannot be phosphrylated by ERK abrogate the inclusion of the V5-exon variant, it suggests that SAM68 function is under control of Ras signaling pathways in response to extracellular cues, but whether DUSP11 can counteract the ERK1/2 dependent phosphorylation of SAM68 similar to Sam68 varaints lacking all candidate ERK1/2 phosphorylation sites remains to be determined experimentally. As alternative splicing affects the activities of many oncogenes and tumor suppressors, DUSP11's role in splicing may extend beyond CD44, which could have important implications for cancer [52].

dusp11 is also a transcriptional target of the p53 tumor suppressor [53]. p53 is a potent tumor suppressor that induces cell cycle arrest, senescence, and apoptosis in response to DNA damage and oncogene activation, and is one of the most commonly mutated genes in cancer [54]. The ability of p53 to up-regulate *dusp11* may provide a link between p53 function and to the splicing machinery [53].

DUSP11 regulates proliferation as over-expression of *dusp11* in U20S and mouse embryonic fibroblasts resulted in inhibition of proliferation in a manner dependent on DUSP11's phosphatase activity [53]. Additionally, knock-down of *dusp11* increased proliferation in both untreated and UV or Doxorubicin DNA damaged U20S cells where DUSP11 most likely inhibits proliferation in response to genotoxic stress in a p53 dependent manner [53]. Loss of growth arrest in response to DNA damage in cells lacking *dusp11* could result in genomic instability, which can promote cancer development and/or progression [55].

2.4. DUSP12

dusp12 is up-regulated or amplified in a variety of cancers including neuroblastoma, retinoblastoma, intracranial ependymoma, and chronic myelogenous leukemia [56-59]. Additionally, *dusp12* is one of only two candidate genes for the target of a 1q21-1q23 amplification found in invasive liposarcomas [60] leading to the hypothesis that *dusp12* is an oncogene. Within the atypical DUSPs, DUSP12 is unique in that it contains an evolutionarily conserved cysteine-rich domain (CRD) at the C-terminus that binds zinc [61], but whose biological function remains obscure.

DUSP12 was initially identified as a pro-survival phosphatase from a high throughput siRNA screen in HeLa cells [62] as knock-down of *dusp12* induced spontaneous apoptosis [62]. Later, it was demonstrated that transient over-expression of *dusp12* in HeLa rendered

cells resistant to apoptotic stimuli including heat shock, oxidative stress, and FAS death receptor activation, but not against the DNA damage inducing agent, cisplatin [63]. In HeLa cells, the ability of DUSP12 to protect cells from apoptosis was dependent on phosphatase activity [63].

DUSP12 interacts with the heat shock protein 70 (HSP70) and the requirement of the enzymatic activity to protect cells from apoptosis activity raises the possibility that HSP70 may be a direct substrate for DUSP12. However, if DUSP12 does regulate HSP70, it does not appear to regulate HSP70's chaperone function as demonstrated by *in vitro* folding assays [63]. Conversely, HSP70 was able to promote DUSP12's phosphatase activity against the phospho-tyrosine analog, 6,8-di-fluoro-4-methylumbeliferyl phosphate (DiFMUP), in a manner that is most likely not a chaperone:substrate interaction as DUSP12 binds the ATPase domain of HSP70 [63]. The C-terminal CRD was also required for DUSP12 to protect from apoptosis [63]. As oxidation of catalytic cysteines is a common mechanism to post-translationally regulate DUSP's [64], it was later demonstrated that the zinc binding ability of the C-terminus protected DUSP12's phosphatase activity during oxidizing conditions *in vitro* [65].

DUSP12 also has a role in cell cycle regulation. Transient over-expression of *dusp12* in HEK293 cells resulted in an increase in the percentage of cells in the G2/M phase and polyploidy and knock-down resulted in cell cycle arrest and senescence [66]. Since, *dusp12* is amplified in several cancers [56,57,60], it is possible that DUSP12 may promote cancer by increasing genomic instability. Unlike the pro-survival properties described above, DUSP12's effect on the cell cycle was independent of its phosphatase activity and required the CRD domain [66]. The CRD function is also likely regulated post-translationally: replacement of serine 335 with alanine (S335A) elicited cell cycle profiles similar to wild type DUSP12, whereas the phosphomimetic S335E variant led to a significant increase in the percentage of G2/M cells (29.8 % for S335A vs. 36.6 % for S335E) [66]. This alteration also affects subcellular localization from the cytoplasm (WT and S335E) to the nuclear compartment (S335A), but does not affect DUSP12's phosphatase activity *in vitro* [66]. To the best of our knowledge this is the only study demonstrating phosphorylation dependent regulation of DUSP12 and the kinases and/or phosphatases responsible remain unknown. Additionally, The S335 kinases may be restricted to mammals, as the S335 site is not well conserved in other organisms [66].

To determine whether DUSP12 has oncogenic properties, we examined the oncogenic potential of DUSP12 in a cell culture model [67]. Unlike the cell cycle effects in HEK293 cells transiently over-expressing *dusp12* described above, we observed no difference in proliferation in HEK293 cells stably over-expressing *gfp-dusp12* (hereafter referred to as *dusp12* over-expression) [67]. Despite the lack of effect on proliferation, we did find that *dusp12* over-expression protected cells from apoptosis induced by both staurosporine and thapsigargin [67]. We additionally observed, that *dusp12* over-expression increased cell motility in scratch wound and transmigration assays [67]. *dusp12* over-expression also up-regulated the expression of two validated oncogenes; integrin alpha 1 (*itga1*) and the

hepatocyte growth factor receptor, *c-met* [67]. ITGA1, a component of the cellular receptor for collagen, promotes proliferation, invasion, and angiogenesis of cancer cells [68-70], but whether it is responsible for the DUSP12 dependent motility remains to be determined. The c-MET proto-oncogene is a well established regulator of growth, survival, and migratory signaling [71], but it seems unlikely that c-MET is responsible for the pro-survival and migratory function of DUSP12 overexpression, since we failed to observed increased c-MET activation in cells over-expressing *dusp12* [67]. This was not wholly unexpected as c-MET frequently requires its ligand, HGF, to be supplied in an autocrine or paracrine manner in order to function [72]. The ability of elevated DUSP12 to promote c-MET and ITGA1 expression has potential implications in cancer where, in the right tumor microenvironment, DUSP12-dependent up-regulation could co-opt the oncogenic potential of these validated oncogenes [73].

Although it is clear that DUSP12 regulates several important cancer-relevant processes, how DUSP12 accomplishes this is largely unknown. Insights into DUSP12's cellular function(s) can be obtained by investigating the budding yeast DUSP12 ortholog, Yvh1p. *YVH1* transcription is up-regulated by nitrogen starvation and low temperatures and *yvh1Δ* yeast strains exhibit a severe growth phenotype, display defects in sporulation, glycogen accumulation, and ribosome biogenesis [74-78]. Expression of the Yvh1p CRD domain in isolation was able to suppress all the mutant phenotypes of *yvh1Δ* strains, suggesting that neither phosphatase activity nor the N-terminal phosphatase domain is required for its cellular function in yeast [76]. The DUSP12 CRD has been alternatively described as a LIM-domain, a zinc-finger and a RING-variant domain due to its ability to coordinate zinc, but how it contributes to biological function is not clear [61]. However, either full-length human DUSP12 or a variant rendered catalytically inactive (C115S) were able to suppress the *yvh1Δ* phenotypes, suggesting that these proteins are functional orthologs [61]. It is important to note that to date, unlike human DUSP12, phosphatase-dependent function(s) of Yvh1p have yet to be characterized, suggesting that, DUSP12 has acquired additional cellular functions than those described in yeast.

Recent independent genetic screens in yeast have revealed that Yvh1p is a critical factor in ribosome biogenesis [77,78]. Ribosome biogenesis is an extremely complex process that is regulated both spatially and temporally [79]. Ribosomal RNA is transcribed and processed in the nucleolus and the pre-40S and pre-60S subunits, are assembled in the nucleus [79]. The pre-40S and pre-60S subunits are exported into the cytoplasm where additional maturation occurs including the binding of multiple translation initiation factors to the small ribosomal subunit to form a 48S complex [79]. Upon recognition of the Met initiation anticodon, the translation initiation factors are expelled to facilitate the joining of the large 60S ribosomal subunit to form a translationally competent 80S ribosome [79]. Defects within specific steps or association of proteins with defined complexes within this multistep process can be inferred from polysome analyses as each complex in endowed with a unique sedimentation coefficient within sucrose gradients [80]. Polysome analysis in yeast demonstrated that Yvh1p associates with pre-60S ribosomes and loss of *YVH1* results in an increase in half-mers (consisting of a 48S small subunit that fails to join to a large 60S

ribosomal subunit) [81]. The production of translationally incompetent half-mers likely results from defects in cytoplasmic 60S maturation [80,81]. In addition to specific temporal associations of initiation factors with the 40S subunit, the nuclear pre-60S subunits associate with Mrt4p, which is subsequently displaced by the P0/P1/P2 ribosomal stalk proteins upon export of the complex into the cytoplasm [78, 82]. The addition of the ribosomal stalk to the cytoplasmic 60S ribosomal subunits is one of the last essential steps prior to large subunit joining [82]. In *yvh1* null yeast strains Mrt4p remained associated with the cytoplasmic 60S subunits preventing the assemblage of the P0/P1/P2 proteins that form the ribosomal stalk [78]. Further supporting a genetic interaction between *YVH1* and *MRT4*, specific mutations of *mtr4* suppressed all *yvh1Δ* phenotypes, suggesting that the pleiotropic phenotypes associated with *yvh1Δ* strains may be an indirect consequence of aberrant ribosome maturation [83].

A similar role for DUSP12 regulating ribosome biogenesis in humans is borne from observations that knock-down of human *dusp12* in HeLa cells promoted the mislocalization of the ribosome factors MRTO4 and eIF6, a translation initiation factor that also binds to the 60S subunit preventing its association with the 40S subunit [84]. In addition, siRNA knock-down of the ribosome factor P0 in HeLa cells resulted in exclusion of DUSP12 from the nucleus [84], its initially characterized sub-cellular localization [61]. As was described in yeast, the reported pleiotropic roles for human DUSP12 may also be an indirect consequence of defects in the production of translationally competent ribosomes, as several proteins affecting ribosome biogenesis are known oncogenes [85] and many other oncogenes are selectively regulated at the translational level [86].

2.5. DUSP18

dusp18 mRNA is expressed in a variety of primary tumors and cancer cell lines [87]. The crystal structure of DUSP18 demonstrates that the phosphatase domain adopts a structure similar to the phosphatase domain of DUSP3/VHR with some minor modifications, including alterations in charge distribution within the active site pocket, suggesting the possibility that it may have distinct substrate specificity profile [88]. Additionally, DUSP18 contains a C-terminal motif (CT) that specifically interacts with the catalytic domain, which may be responsible for Dusp18's elevated thermostability, with a temperature optimum of 55°C [89]. DUSP18 localizes to the nuclear and cytoplasmic compartments as well as the intermembrane space of the mitochondria [90,91] although it appears that the mitrochondrial localization is native as reports of DUSP18 localizing to the cytoplasm and nucleus was due to over-expression of *dusp18* with an N-terminal tag that disrupted the mitochondrial localization [91]. Like components of the intrinsic pathway of apoptosis, mitochondrial DUSP18 can be released into the cytoplasm in response to apoptotic signals [91]. DUSP18 selectively dephosphorylates the JNK stress activated MAPK *in vitro* and *in vivo* [87,90,91], but since JNK is not thought to be mitochondrially resident, it seems unlikely that DUSP18 interacts with JNK under normal conditions [91]. However, due to the ability of intrinsic apoptosis to release DUSP18 from mitochondria, it is possible that DUSP18 may be able to interact with and dephosphorylate JNK under apoptotic conditions. The JNK

MAPK is a well-established regulator of tumorigenesis, and regulates processes such as cell proliferation [92], apoptosis [93], and inflammation [94].

2.6. DUSP22

DUSP22 expression is down-regulated in breast cancer and lymphomas [95, 96], and is used as a prognostic marker for B cell chronic lymphocytic leukemia patients [97]. In anaplastic lymphoma kinase (ALK)-negative anaplastic large cell lymphomas, the commonly found t(6;7)(p25.3;q32.3) translocation disrupts the *dusp22* gene [96]. Down-regulation of *dusp22* in cancer suggests a possible tumor suppressive role for DUSP22, but exactly how DUSP22 may function in cancer and normal cell biology awaits further characterization.

There are conflicting reports concerning the ability of DUSP22 to dephosphorylate MAPKs [97-100], but most studies indicate DUSP22 as a regulator of JNK [99-101]. Over-expression of both JNK and DUSP22 in COS7 suppressed JNK phosphorylation [99]. However, other reports have identified a phosphatase-dependent role for DUSP22 in promoting JNK activity [100,101]. Glutathione S-transferase (GST) pull downs and immunoprecipitations revealed that DUSP22 can bind the JNK activating kinase, MKK7, but not JNK itself, and the association with MKK7 activates MKK7's phosphorylation of JNK [99]. Exactly how DUSP22 activates MKK7 and JNK activity is unclear but the requirement is biologically significant as mouse embryonic stem cells lacking DUSP22 were unable to activate JNK in response to cytokines [100].

Another reported substrate of DUSP22 is the estrogen receptor (ERα) an important prognostic marker for breast cancer regulating proliferation and apoptosis [102,103]. DUSP22 most likely functions within a negative feedback loop to regulate ERα, as activation of ERα induces *dusp22* mRNA expression and DUSP22 dephosphorylates and inhibits ERα's transcriptional activity in breast cancer cells [102]. Additionally, *dusp22* expression is down-regulated in breast cancers, specifically those containing the 8p11-12 amplicon [95]. This amplicon contains the potential oncogene, *ppapdc1b,* which is thought to be responsible for DUSP22 down-regulation as siRNA knock-down of *ppapdc1b* increases *dusp22* expression [95]. The oncogenic ability of *ppapdc1b,* in part, may be due to its ability to down-regulate *dusp22* expression to allow for increased estrogen receptor activity, as it was reported that all 11 tumor samples analyzed that contain the 8p11-12 amplicon, had ER-positive statuses [95].

DUSP22 may also regulate metastasis as it dephosphorylates tyrosines 576/577 and 397 of focal adhesion kinase (FAK) [104]. FAK is a key regulator of integrin-mediated attachment and FAK inhibition results in detachment and apoptosis in some cell lines [105]. *dusp22* over-expression inhibited cell migration and reduced FAK phosphorylation while *dusp22* knock-down promoted cell migration and FAK phosphorylation in H1299 cells [104]. DUSP22 is myristoylated [106], which may allow for its co-localization with FAK at actin filament enriched regions of lamelapodia [104]. The subcellular localization of DUSP22 likely contributes to its biological function as myristoylation-deficient variants of DUSP22 do not display altered enzymatic activity *in vitro,* but unlike wild-type DUSP22, induced cell detachment and apoptosis when over-expressed [106].

2.7. DUSP23

DUSP23 dephosphorylates ERK1/2 *in vitro,* but DUSP23 is an activator of JNK and p38 in COS7 cells [6]. In addition, the regulation of JNK and p38 is phosphatase independent, and the MAPKKs for JNK and p38, MKK4 and MKK6, also have increased phosphorylation when *dusp23* is over-expressed and the cells are treated with sorbitol, suggesting that DUSP23 may act as a scaffold to promote MKK binding to JNK and p38 [6].

The *dusp23* gene is highly methylated and decreased *dusp23* mRNA expression is observed in neuroblastoma [107]. Interestingly, *dusp23* mRNA levels were lower in tumors from deceased patients than patients exhibiting no clinical symptoms, suggesting that DUSP23 levels could be a prognostic marker for neuroblastomas [107]. The generality of *dusp23* functioning as a tumor suppressor is called into question by observations that it is amplified in many other cancers, including breast, colon, lung, squamous carcinoma, pancreatic, brain, esophageal, stomach, bladder, kidney, skin, ovary, prostate, and testicular cancers [108], and selective over-expression of *dusp23* in MCF7 cells increased proliferation while knock-down of *dusp23* decreased proliferation [108].

2.8. DUSP26

A role for *dusp26* in cancer is borne by observations that it is located on an 8p12 amplicon found in anaplastic thyroid carcinoma tissue [109] and differential expression of *dusp26* has additionally been observed in glioblastoma tissues, neuroblastoma, brain, and ovarian cancer cell lines, where *dusp26* is down-regulated [110,111]. Additionally, knock-down of *dusp26* in immortalized ovarian epithelia HOSE17.1 cells increased both colony formation and proliferation [111]. Over-expression of *dusp26* in immortalized breast epithelial MCF10A, cells suppressed colony formation and acinar growth in 3D culture [111]. Alternatively, in anaplastic thyroid carcinoma primary tumors and cell lines, over-expression of *dusp26* promoted colony formation, while knock-down of *dusp26* expression reduced proliferation [109]. Defining a more precise role for DUSP26 is further complicated by the fact that different groups have come to opposing conclusions regarding *dusp26* expression in neuroblastoma cell lines [111,112].

The cellular function of DUSP26 is also unclear as the substrates for DUSP26 are debated. DUSP26 can dephosphorylate the tumor suppressor p53 at Ser20 and Ser37, inhibiting p53-mediated apoptosis induced by genotoxic stress [112], suggesting a pro-survival role. In addition to p53, several other *in vitro* DUSP26 substrates have been described, including p38 [109]. In HEK293T cells, over-expression of *dusp26* resulted in reduction of p38 activity and p38-mediated apoptosis [113]. The small molecule inhibitor NSC-87877 functions as an *in vitro* DUSP26 inhibitor with an IC_{50} of 16.7 uM that additionally prevented the DUSP26-dependent dephosphorylation of p38 in HEK293 cells [114]. However, in COS7 cells, over-expression of *dusp26* increased both JNK and p38 activities [6], and in the rat neuronal cell line, PC12, and epithelial cells DUSP26 had no affect on MAPKs [17,112] questioning the generality of DUSP26 functioning as a p38 phosphatase.

DUSP26 is also implicated in regulating the kinesin superfamily 3 (KIF3) microtubule-directed protein motor complex by dephosphorylating the kinesin-associated protein 3 (KAP3) [111]. The KIF3 motor complex has been implicated in cancer due to its ability to traffic cancer relevant proteins including, adenamaous polyposis coli (APC), β-catenin, cadherins, and the polarity complex, PAR3 [115,116]. Consistent with DUSP26 functioning as a positive regulator of KIF3, over-expression of *dusp26* in the mouse fibroblast cell line, NIH3T3, increased cell-to-cell adhesion and intracellular transport of N-cadherin and β-catenin to the cell surface [111].

3. Less well-characterized atypical DUSPs.

The previous sections discussed the atypical DUSPs that have been described in the literature as having a relationship, however tenuous or controversial, to tumor suppressive and/or oncogenic properties and are often supported by genetic/genomic analyses of primary tumors samples. Although the previous sections should reaffirm that the function(s) of many of the atypical DUSPs are likely both cell type and context dependent, we nevertheless undertook a comparison of the expression profiles of all the atypical DUSPs in tumors of the prostate to normal prostate tissue using the cBio Cancer Genomics Portal (http://www.cbioportal.org/) and microarray data deposited by the Memorial Sloan-Kettering Cancer (MSKCC) Center's Prostate Oncogenome Project [117]. Comparison of the transcriptome of 85 tumors to normal prostate tissue revealed that many atypical DUSPs have aberrant expression in prostate cancer (Table 2). In at least two cases this difference

Gene	Down	Up	Total
EPM2A	13%	0%	13%
STYX	2%	7%	9%
DUSP3	40%	0%	40%
DUSP11	4%	5%	8%
DUSP12	1%	15%	16%
DUSP13	0%	5%	5%
DUSP14	6%	11%	16%
DUSP15	4%	0%	4%
DUSP18	40%	4%	44%
DUSP19	1%	1%	2%
DUSP21	0%	7%	7%
DUSP22	15%	2%	18%
DUSP23	11%	8%	19%
DUSP26	1%	2%	4%
DUSP27	2%	5%	7%
Androgen Receptor (AR)	2%	7%	9%

Table 2. Using the cBio Cancer Genomics protal (http://www.cbioportal.org/), and microarray data deposited by MSKCC Prostate Oncogenome Project [117]. we compared the expression of atypical DUSPs in tumors to normal prostate tissue using a Z score threshold of +/- 2. The androgen receptor (AR) is included for comparison as a gene already implicated in cancer progression [117]

was reflected clinically as patients harboring tumors with aberrant *dusp22* or *dusp23* expression had faster disease relapse than those harboring tumors with normal *dusp22* or *dusp23* expression (Figure 1). Although differential expression of any particular gene in prostate cancer may not functionally contribute to the initiation or progression of the disease, this analysis serves as an example of one way to potentially identify cancer-relevant markers. The following sections describe what is currently known about the remaining atypical DUSPs that, to date, have not been specifically associated with cancer.

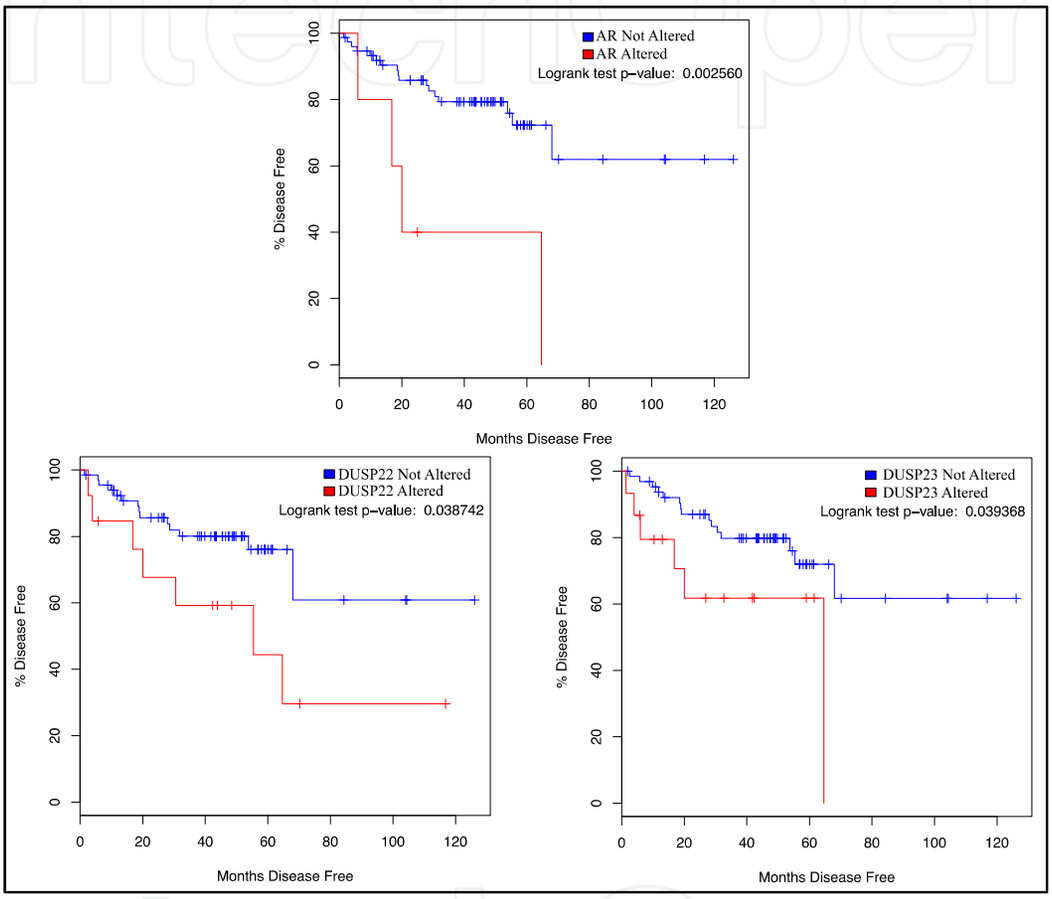

Figure 1. Kaplan-Meyer analysis of disease free survival for patients with altered expression of the androgen receptor or atypical DUSPs. This graph was generated by the cBio Cancer Genomics Portal (http://www.cbioportal.org/) using data deposited by the MSKCC Prostate Oncogenome Project [117]. Microarray expression data from tumors compared to normal prostate was used in this analysis with a Z-score threshold of +/- 2.

3.1. STYX

The prototypical pseudophosphatase, STYX, contains a substitution of the catalytic cysteine for glycine, rendering it catalytically inactive [118]. As this mutation abrogates catalysis, but not substrate binding, pseudophosphatases are though to function as substrate traps preventing dephosphorylation of the target protein(s) [119]. In mice, *styx* expression is restricted to the testis, and is essential for spermatogenesis, as STYX knock-out male mice are infertile [118].

3.2. DUSP13A/B

The *dusp13* gene encodes for two similar protein products via alternative reading frames [120,121]. Designated DUSP13A and DUSP13B, the proteins are expressed in the muscle and testis respectively [121,122]. Both proteins have phosphatase activity *in vitro,* with DUSP13B exhibiting higher activity than DUSP13A [122]. Interestingly, both DUSP13A and B regulate apoptosis, but by different mechanisms. Knock-down of DUSP13A in mouse embryonic fibroblasts reduced apoptosis signal regulating kinase 1 (ASK1) kinase activity and intrinsic apoptosis induced by *ask1* over-expression [7]. Furthermore, autophosphorylation assays of ASK1 with increasing amounts of DUSP13A demonstrated that DUSP13A increases ASK1 autophosphorylation in a phosphatase independent manner [7]. In contrast to DUSP13A, DUSP13B appears to be pro-survival [123]. Over-expression of DUSP13B in COS7 cells resulted in reduced phosphorylation of the stress activated MAPKs, JNK and p38 [123]. Over-expression of DUSP13B resulted in reduced activity of the down-stream JNK effector AP-1 in a phosphatase dependent manner [123]. AP-1 activity is associated with a large number of cellular processes including transformation, proliferation, differentiation, and is specifically implicated in apoptosis [124].

3.3. DUSP14

dusp14 is located on a chromosomal region that is amplified in gastric cancer, but it may not be the target for the genetic amplification, as *dusp14* expression is not increased [125]. *In vitro,* DUSP14 dephosphorylates all three MAPK isoforms, leading to its alternate designations of MKP-L (MKP1-Like) and MKP-6 [126]. However, DUSP14 is classified as an atypical DUSP as it lacks the characteristic MAPK binding domain found in the MKP DUSP subfamily [2]. In T-cells, expression of the catalytically inactive DUSP14 C111S variant enhanced ERK1/2 and JNK phosphorylation, suggesting that *in vivo,* p38 is not a DUSP14 substrate [126]. Additionally, in β pancreatic cells, knock-down of *dusp14* expression or the expression of a dominant negative DUSP14 variant resulted in increased ERK1/2 phosphorylation and cell proliferation [127]. Elucidating a role for DUSP14 may be aided by the discovery of the PTP inhibitor IV, an inhibitor of DUSP14 *in vitro* that increases hydrogen peroxide induced JNK activation in a concentration dependent manner [128].

3.4. DUSP15

The DUSP15 crystal structure reveals it lacks the MAPK substrate recognition domain and it has a unique additional alpha helix located at the back end of the active site suggesting that DUSP15 has unique substrate recognition mechanisms [129]. DUSP15 displays phosphatase activity against the artificial substrate pNPP *in vitro* [130], but physiological substrates for DUSP15 have yet to be reported. DUSP15 contains an N-terminal myristoylation signal, resulting in targeting of the protein to plasma membrane [90] and in mice DUSP15 has been identified as a candidate gene in a quantitative trait locus (QTL) thought to harbor genes that control for the predisposition to growth and fatness in mice [131].

3.5. DUSP19

Like DUSP13A, DUSP19 is thought to facilitate ASK1 activation leading to MKK7 activation and in turn activating JNK as DUSP19 directly binds MKK7, but not JNK *in vitro*, and co-immunoprecipitates with ASK1 and MKK7 [8,132]. The proposed model for how DUSP19 differentially regulates JNK activation is that at high levels of DUSP19 ASK1 is sequestered by DUSP19 thereby inhibiting MKK7 and JNK activation, while at low levels DUSP19 functions as a scaffold to promote the activation of MKK7 by ASK1 [8,132].

3.6. DUSP21

Similar to DUSP18, DUSP21 contains a highly conserved mitochondrial localization signal, however DUSP21 localizes to the peripheral membrane of the inner membrane of the mitochondria, which is the opposing side to which DUSP18 is found [91]. DUSP21 exhibits activity against synthetic MAPK peptides *in vitro,* but cell based assays fail to demonstrate that DUSP21 has activity against any cellular MAPKs [90].

3.7. DUSP27

Substrates for the newest atypical DUSP, DUSP27, are unknown but solution of the DUSP27 3D structure suggests that it may have substrates other than the MAPKs [133]. The catalytic site can accommodate dually-phosphorylated residues separated by two amino acids, which differs from the catalytic site of DUSPs that can dephosphorylate the characteristic MAPK activation loop (T-X-Y) [133].

4. Conclusion

Although many atypical DUSPs display differential expression in tumor samples, significant amounts of work will be required to determine whether and how these differences contribute to malignancy, especially with the common discrepancy between *in vitro* and *in vivo* results. Due to the central localization of the MAPK signaling cascade and the role of MKPs in malignancy, much of the initial work has been to evaluate if and how atypical DUSPs affect MAPK signaling. The MAPK pathway however represents a doubled edged sword, although it is strongly associated with disease, it is difficult to modulate pharmacologically due to complex crosstalk and feedback loops. We envision that specific inhibitors for atypical DUSPs, particularly those that do not target MAPK isoforms but other cancer-relevant substrates, could have important therapeutic value. Even in the event that these inhibitors fail to function as therapeutics, we think that selective inhibitors will be instrumental in advancing the elucidation of the cellular functions, substrates, and expression of the atypical DUSPs. Even in instances where the mechanism of oncogenesis remains unknown, we anticipate that continued large-scale expression profiling of the atypical DUSPs may be increasingly used to for clinical benefit to patients through the identification of potential novel biomarkers.

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