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DUAL SPECIFICITY PROTEIN PHOSPHATASES: Therapeutic Targets for Cancer and Alzheimer's Disease

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■ **Abstract** The complete sequencing of the human genome is generating many novel targets for drug discovery. Understanding the pathophysiological roles of these putative targets and assessing their suitability for therapeutic intervention has become the major hurdle for drug discovery efforts. The dual-specificity phosphatases (DSPases), which dephosphorylate serine, threonine, and tyrosine residues in the same protein substrate, have important roles in multiple signaling pathways and appear to be deregulated in cancer and Alzheimer's disease. We examine the potential of DSPases as new molecular therapeutic targets for the treatment of human disease.

INTRODUCTION

Cellular signaling networks are controlled by reversible covalent phosphorylation, which depends on a precise balance between protein kinase and phosphatase activities (1). These signaling networks govern processes such as cell growth, cell division, and cell death; perturbation of these pathways, whether by environmental stresses or genetic defects, underlies the pathophysiology of many diseased states. The sequencing of the human genome predicts approximately 428 protein kinases, the majority of which catalyze serine and threonine phosphorylation (Figure 1) (2). Although protein kinases were originally considered the prime regulators of signal transduction-mediated events, it is now recognized that protein dephosphorylation is an equally important component, playing a central role in cell cycle transitions and other signal transduction mechanisms (3). Furthermore, protein phosphatase activity critically regulates fundamental cellular processes that are perturbed in diseased states. The human genome is estimated to encode 99 protein phosphatases,

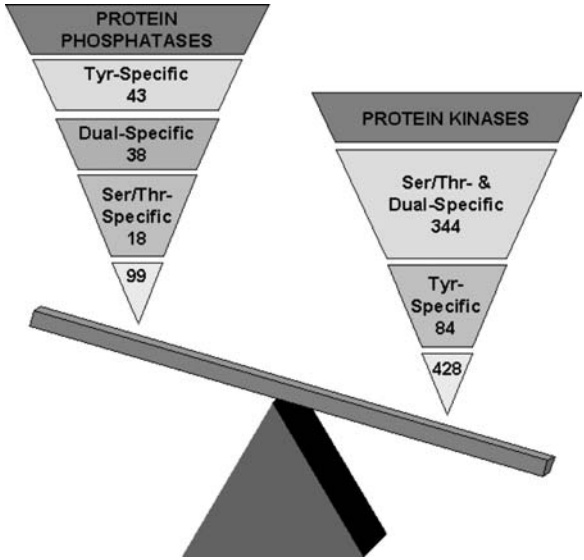

Figure 1 The balance of protein kinases and phosphatases in the human genome. This figure is based on DNA sequence and protein structural analyses described by others (2, 3, 6a). The total predicted number of human protein tyrosine (Tyr), serine (Ser), threonine (Thr), and dual-specificity kinases and phosphatases are indicated. Catalytically inactive phosphatases and kinases and the phosphatases with lipid or nucleic acid substrates are not included. See text for details.

approximately one quarter the number of protein kinases, suggesting functional redundancy and/or substrate promiscuity (Figure 1) (2, 3). Protein phosphatases are classified according to their substrate specificity, either serine/threonine-specific protein phosphatases (PS/TPases) or tyrosine-specific protein phosphatases (PTPases) (4), although there have been recent efforts to exploit structural information (3), which may result in some reassignments. Dual-specificity phosphatases (DSPases) represent a subclass of the protein tyrosine phosphatase superfamily by virtue of their highly conserved PTPase active site motif and because they employ the PTPase catalytic mechanism, which proceeds via the formation of a transient enzyme-phosphosubstrate intermediate [4; reviewed in Zhang (5)]. DSPases, however, are unique in their ability to dephosphorylate protein substrates containing both phosphotyrosine and phosphoserine or phosphothreonine, either immediately adjacent or separated by one amino acid; such substrates are exemplified by the cyclin-dependent kinases (Cdks) and the mitogen-activated protein kinases (MAPKs), which play essential roles in the signaling pathways that regulate cell division and cell growth (Figures 2 and 3). Recent structural analyses suggest the human genome encodes 38 DSPases, including 11 MAPK phosphatases (MKPs), 17 atypical DSPases, 4 PRL phosphatases, 3 Cdc14 phosphatases, and 3 Cdc25 phosphatases (3) (Figure 1). The DSPases share the conserved PTPase active site

and catalytic mechanism but they have a shallower active site cleft than PTPases, presumably to accommodate the sterically less accessible phosphoserine and phosphothreonine residues (4, 6). The most widely studied DSPases are the Cdc25 phosphatases and the MKPs, two protein families that play central roles in the biology of the cell.

CDC25 DSPASES

The first DSPases to be discovered were the Cdc25 phosphatases, which were functionally defined as promoters of the cell division cycle in yeast (7). More specifically, Cdc25 phosphatases dephosphorylate and activate the Cdks (Figures 2 and 4), which are key participants in the cellular division program induced in response to extracellular signals including growth factors. Cdks coupled to their cyclin partner are maintained in an inactive state by dual phosphorylation at adjacent threonine and tyrosine (-T-Y-) residues near their amino terminus; these inactivating phosphorylations are mediated by Wee1 and Myt1 protein kinases (8, 9). Cdc25s activate Cdks by dephosphorylating both phosphothreonine and phosphotyrosine residues (Figure 2); regulation of Cdk kinase activity remains an

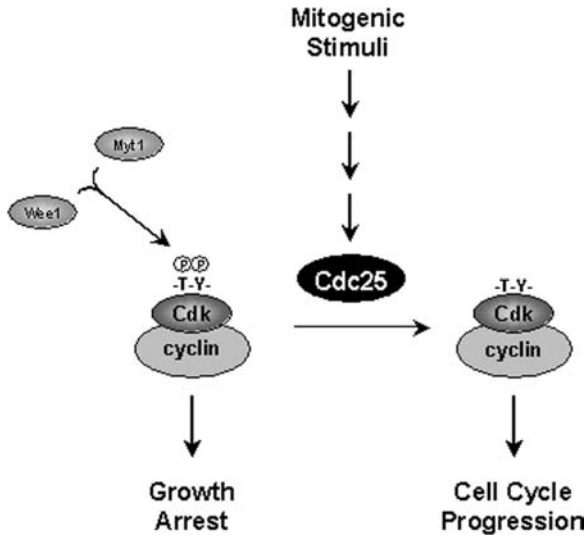

Figure 2 Cdc25 phosphatases dephosphorylate and activate the cyclin-dependent kinases. Mitogenic signal transduction cascades induce cell division. Progression through cell cycle transitions is achieved by dephosphorylation and activation of the cyclin-dependent kinases by Cdc25 phosphatases. In contrast to the MKPs, the Cdc25 phosphatases activate Cdks by dephosphorylating both residues in the Cdk -T-Y- motif (see text for details).

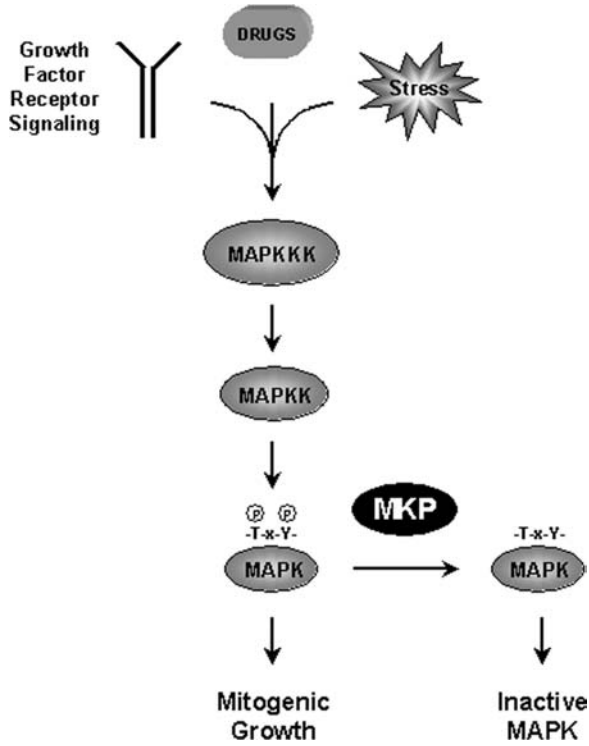

Figure 3 Mitogen-activated protein kinase phosphatases dephosphorylate and inactivate the mitogen-activated protein kinases. Growth factor receptor signal transduction cascades, cellular stresses, and chemotherapeutics can activate mitogenic signaling pathways, culminating in the activation of upstream mitogen-activated protein kinase kinase kinases (MAPKKKs), which phosphorylate and activate mitogen-activated protein kinase kinases (MAPKKs), which phosphorylate and activate mitogen-activated protein kinases (MAPKs) in the -T-x-Y- motif. Downregulation of mitogenic signaling through MAPKs is achieved by dephosphorylation of both residues in the -T-x-Y- motif, a process regulated by the dual-specificity MAPK phosphatases (DS-MKPs).

area of considerable investigation, and Cdks have emerged as a novel therapeutic target for the treatment of cancer (10).

The human Cdc25 DSPases comprise a family of three genes originally identified by their ability to complement a temperature-sensitive Cdc25 yeast strain, thus restoring a normal growth phenotype. The protein products of the three Cdc25 genes, Cdc25A, Cdc25B, and Cdc25C, possess a high degree of homology in their carboxy-terminal domain, the location of the catalytic active site, whereas their amino terminal domains are much less conserved, perform regulatory roles, and possibly contribute to the diverse nature of their biological activities (see

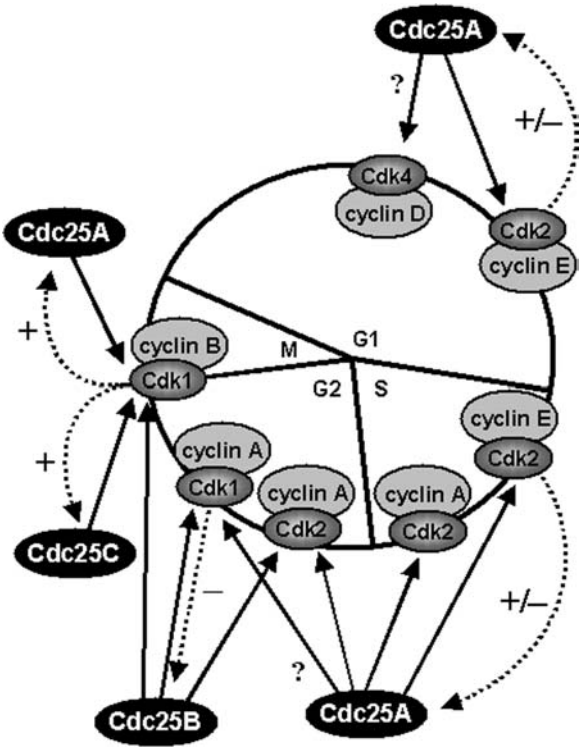

Figure 4 Cdc25 phosphatases promote mammalian cell cycle progression. Cdc25 phosphatases drive the cell division cycle by dephosphorylating and activating the Cdks. The three human Cdc25 isoforms, Cdc25A, Cdc25B, and Cdc25C, have overlapping roles in the cell cycle. Cdc25A exclusively promotes the G1/S transition and S phase progression and contributes to the Cdc25 activity necessary for G2 phase progression, the G2/M transition, and mitosis. Cdc25B contributes to G2 progression and is believed to be the trigger for initiating the G2/M transition. Cdc25C activity is restricted to mitosis. The Cdc25 phosphatases are targeted by the G1/S, intra-S, and G2/M cell cycle checkpoints to inhibit their activity in response to genotoxic stress. Cdc25 activity is influenced by Cdk activity in regulatory feedback loops: solid arrows indicate activation by Cdc25 and dotted arrows represent known positive (+) or negative (−) feedback loops. Cdk2 has both positive and negative effects on Cdc25A (+/−). It is unclear whether Cdk4/cyclin D is a bona fide substrate of Cdc25A in cells.

below). Cdc25C, the first human Cdc25 isoform identified, functions primarily in mitosis and catalyzes mitotic progression by activating Cdk1/cyclin B; microinjection of anti-Cdc25C antibodies into HeLa cells prevented mitotic entry (11–13). Cdc25B also activates Cdk1/cyclin B, and microinjection of anti-Cdc25B antibodies inhibits mitotic entry, leading many to speculate that Cdc25B is functionally

redundant to Cdc25C (14, 15). Nonetheless, Cdc25B and Cdc25C activities are temporally distinct, with Cdc25B activity peaking prior to that of Cdc25C. More recently, Cdc25B has been described as the trigger for the G2/M transition; Cdc25B appears to initiate the mitotic transition by activating a particular pool of Cdk1/cyclin B (15–18). Cdc25B also contributes to the Cdk phosphatase activity necessary to activate Cdk2/cyclin A in S phase and Cdk1/cyclin A in G2 (17, 19, 20). Cdc25A promotes the G1/S cell cycle transition and S phase progression by activating Cdk2/cyclin E (21, 22). Microinjection of anti-Cdc25A antibodies prevented S phase entry in cells following serum induction, and overexpression of Cdc25A accelerated S phase entry with premature Cdk2 activation (21–23). Cdc25A activity is rate limiting for the G2/M transition and mitotic progression by contributing to Cdk1/cyclin B activation (24, 25).

Although the emerging model for temporal and combinatorial contributions of Cdc25A, Cdc25B, and Cdc25C activities to achieve precise control over cell cycle progression is appealing, $Cdc25B^{-/-}$ mice and $Cdc25C^{-/-}$ mice are viable and cells isolated from these mice undergo normal mitotic cell division, implying that Cdc25A has the potential to drive the entire mitotic cell division cycle (26, 27). The preeminence of Cdc25A is further illustrated by the prompt inactivation of Cdk activity and cell cycle arrest seen with rapid Cdc25A degradation (24, 28–30). Cdc25A has, thus, been dubbed the "master Cdk phosphatase" (31), as it appears to be responsible for Cdk activation to promote the G1/S cell cycle transition, for maintaining Cdk activity throughout S phase and G2 progression, and for contributing to the Cdk phosphatase activity necessary for the G2/M transition and mitotic progression (Figure 4) (21, 22, 24, 25). It remains unclear why cells have multiple Cdc25s to regulate mitotic cell division, although it is possible that their combined activities ensure optimal Cdk activation to promote the irreversible process of mitotic division. In such a model, the multiple Cdc25s would impose a switch-like regulatory mechanism, consisting of a biological threshold of Cdk activation, to achieve strict unidirectional control of cell division (32).

Cdc25 Regulation

As key controllers of cell division, Cdc25 DSPases are subject to precise regulation, including enzyme-substrate feedback loops involving specific Cdk/cyclin complexes and their activating Cdc25. For example, Cdc25A activity is upregulated by Cdk2/cyclin E following its activation, and Cdc25A protein stability is increased by Cdk1/cyclin B phosphorylation (21, 24); Cdk2 activity also appears to negatively regulate Cdc25A protein stability (33). Cdc25B protein stability is negatively regulated by Cdk1/cyclin A (16) and Cdc25C catalytic activity is upregulated by Cdk1/cyclin B (Figure 4) (34). In addition, the Cdc25 DSPases are regulated by alternative gene splicing, which results in the expression of 12 splice variants. The precise role of alternative splicing in Cdc25 biology remains unclear, although the splice variants could have altered tissue or cell cycle phase activity profiles, or they may have different specific catalytic activities as a result of loss of consensus regulatory phosphorylation sites (35, 36).

Throughout the cell cycle, Cdc25C protein expression does not appreciably fluctuate; however, Cdc25C remains inactive during interphase by 14-3-3-mediated sequestration in the cytoplasm (37). Cdc25A and Cdc25B, on the other hand, are labile proteins, most likely owing to their role as the major catalysts of the cell cycle transitions (25, 38). Cdc25B protein levels accumulate throughout late S and early G2, peaking at the G2/M transition (17, 39). Although a detailed understanding of Cdc25B protein turnover is lacking, its proteolysis requires prior phosphorylation by Cdk1/cyclin A (16). Like Cdc25C, Cdc25B activity is also regulated by its subcellular localization, which is facilitated by interactions with 14-3-3 (40). Cdc25A protein levels and activity remain elevated past S phase and increase as cells enter mitosis. Cdc25A activity is primarily regulated by protein turnover, although 14-3-3 can prevent the phosphatase from interacting with its mitotic substrate, Cdk1/cyclin B. Furthermore, Cdc25A activity has been reported to be upregulated by phosphorylation in response to mitogenesis (41, 42). Cdc25A protein turnover is catalyzed by the ubiquitin-proteasome pathway; Cdc25A ubiquitination is catalyzed by the APC/C^{Cdh1} ubiquitin ligase during mitotic exit and early G1 and by the $SCF^{\beta-TrCP}$ ubiquitin ligase during interphase [reviewed in Busino et al. (43)]. The subcellular localization of Cdc25A remains a matter of some debate, as Cdc25A has been reported to localize in the nucleus, the cytoplasm, and the plasma membrane and to interact with proteins that reside in each of these cellular compartments (21, 41, 42, 44–46).

Cell Cycle Checkpoints

As major promoters of cell cycle progression and the main drivers of passage through the cell cycle transitions, the Cdc25s are targets of cell cycle checkpoint proteins, which are activated in response to genotoxic stress and terminate cell cycle progression in an effort to preserve genomic integrity. The Cdc25-dependent cell cycle checkpoints appear to be independent of p53 and serve as a rapid and primary response to genotoxic stresses (29). Whereas Cdc25B and Cdc25C are targets of the G2/M cell cycle checkpoint, Cdc25A is targeted by the G1/S, intra-S, and G2/M cell cycle checkpoints (24, 28–31, 47). Cdc25s are inactivated at cell cycle checkpoints by one or a combination of Chk1-, Chk2-, and p38 MAPK-mediated phosphorylations (Figures 5–7); checkpoint-dependent Cdc25 regulation has been the subject of several recent reviews (31, 43, 48, 49). In response to genotoxic stress, checkpoint kinases phosphorylate Cdc25C, resulting in 14-3-3 binding and cytoplasmic sequestration (Figure 5); in addition, checkpoint-mediated Cdc25C inactivation has been reported to occur via APC/C-mediated ubiquitination and proteolytic degradation, specifically in response to arsenite treatment (50). Although Cdc25B is a labile protein under physiologic conditions (see above), cell cycle checkpoint-mediated inactivation is thought to be due to 14-3-3 binding (Figure 6) (31, 49, 51). In contrast, the cell cycle checkpoints targeting Cdc25A appear to be independent of 14-3-3 binding and involve ubiquitin-mediated proteolytic degradation [reviewed in Donzelli & Draetta (31); Busino et al. (43)]. In response

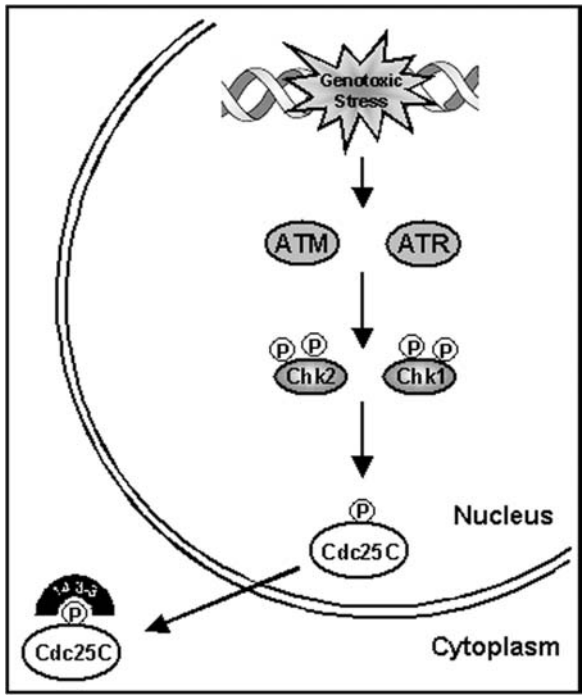

Figure 5 Cdc25C inactivation by the G2/M cell cycle checkpoint. In response to genotoxic stress, checkpoint kinases Chk1 and Chk2 phosphorylate Cdc25C, promoting its cytoplasmic sequestration by 14-3-3 binding.

to genotoxic stresses, Cdc25A is phosphorylated by Chk1, Chk2, and p38, which promote its polyubiquitination catalyzed by the $SCF^{\beta-TrCP}$ ubiquitin ligase (Figure 7) (31, 43, 52, 53). However, neither Chk1, Chk2, nor p38 can phosphorylate the Cdc25A serine residues necessary for recruitment to the $SCF^{\beta-TrCP}$ ubiquitin ligase, indicating that other kinases are necessary for promoting Cdc25A turnover (52, 53). Mutations in one or several of the components of these checkpoint pathways are common in cancers, resulting in a defective response to genotoxic stress and promoting genetic instability (31, 54)

In addition to their role in cell cycle control (Figures 2 and 4), Cdc25s regulate mitogenic and steroid receptor signal transduction pathways and the apoptotic response to cellular stresses (see below) (Figure 8) [reviewed in Lyon et al. (55)].

MKP DSPASES

MKPs dephosphorylate and inactivate MAPKs on threonine and tyrosine residues (Figure 3). MAPKs are widely studied protein kinases that play pivotal roles in

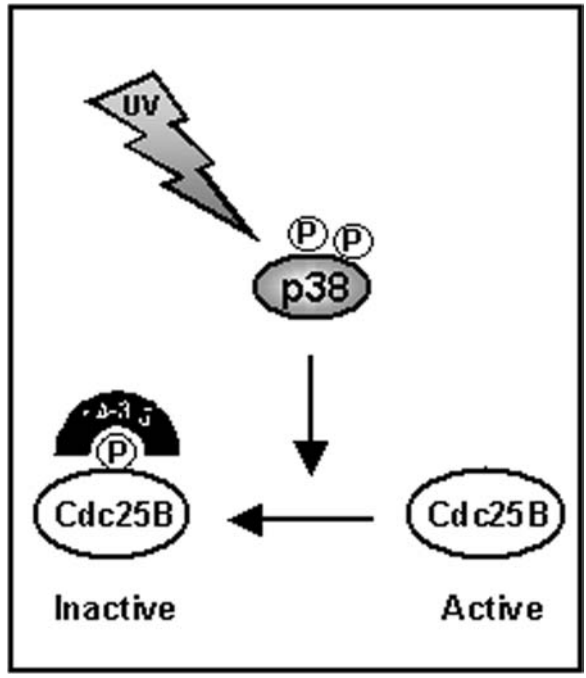

Figure 6 Cdc25B inactivation by the G2/M cell cycle checkpoint. In response to genotoxic stress (predominantly UV irradiation), p38 MAPK phosphorylates Cdc25B, promoting its association with 14-3-3, which inhibits Cdc25B activity.

mitogenic signal transduction, survival, stress response, and programmed cell death. There are currently three members of the MAPK family: extracellular signal-regulated kinase (Erk), c-Jun terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38/high osmolarity glycerol response kinase (HOG) MAPK. Although activation of Erk is most often associated with growth and survival, JNK and p38 are thought to primarily mediate stress responses and programmed cell death (apoptosis) [reviewed in Chang & Karin (56)]. Extensive studies addressing the activation of MAPK pathways by upstream kinases and cell-surface receptor-mediated events have placed MAPK signal transduction cascades at the heart of a sophisticated signaling network with multiple levels of complexity. In contrast, the events that regulate termination of MAPK signaling are less well understood, although it is clear that MKPs play a major role, and a large body of evidence now demonstrates that the regulation of MAPKs at the level of the protein phosphatases is as sophisticated as that mediated by the protein kinases [reviewed in Tonks & Neel (57); 58]. MKPs have been grouped into three major categories: dual-specificity MKPs (DS-MKPs), tyrosine-specific MKPs, and serine/threonine-specific MKPs (58). In this review, we have limited our discussion to the DS-MKP family because of their similarities to the Cdc25 DSPases.

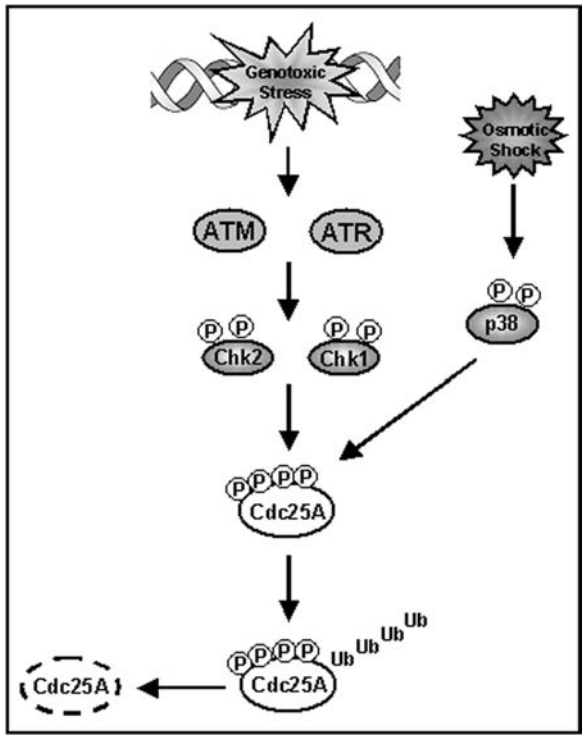

Figure 7 Cdc25A inactivation by cell cycle checkpoints. Cdc25A is rapidly and irreversibly inactivated by the G1/S, intra-S phase, and G2/M cell cycle checkpoints. In response to genotoxic stress or interruptions to DNA synthesis, stress-responsive p38 MAPK and checkpoint kinases Chk1 and Chk2 phosphorylate Cdc25A (at multiple sites), promoting its association with ubiquitin ligases. Following polyubiquitination (Ub), Cdc25A is degraded by the 26S proteasome; dashed outlined Cdc25A indicates degraded protein.

To date, 12 bona fide human DS-MKPs have been cloned and characterized (Table 1). Table 1 also contains two putative DS-MKPs, namely hVYH1, whose substrate has not been identified, and JSP-1, which fails to dephosphorylate MAPK in cells but nonetheless specifically activates the JNK pathway by an as of yet undetermined mechanism (59). The first MKP discovered was 3CH134/MKP-1 (60), which was later found to have PTPase activity (61) and DSPase activity (62). The human homolog of 3CH134/MKP-1, CL100 or DUSP1, was independently cloned (63). Other DS-MKPs were subsequently discovered in a variety of organisms [a comprehensive listing of DS-MKPs from various species was compiled by Farooq & Zhou (64)].

The DS-MKPs have unique but overlapping MAPK substrate specificities, as recently reviewed by Farooq & Zhou (64). For example, the Erk isoforms are

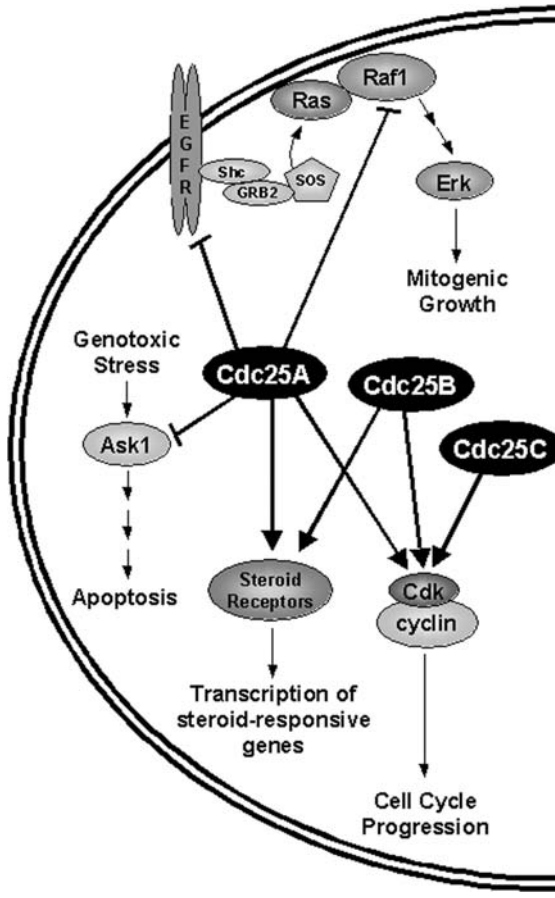

Figure 8 Cdc25 phosphatases regulate multiple signaling pathways. In addition to driving cell cycle transitions, Cdc25 phosphatases promote hormone-responsive gene expression by affecting steroid receptor activity, downregulate apoptotic responses to genotoxic stresses by blocking Ask1 homo-dimerization (which is necessary for Ask1 activation), and downregulate mitogenic signaling by dephosphorylating the epidermal growth factor receptor (EGFR) and Raf-1, which can also have a cytoprotective effect.

selectively dephosphorylated by MKP-3, whereas M3/6 selectively dephosphorylates JNK. MKP-1 recognizes JNK, ERK, and p38, and MKP-2 recognizes Erk and JNK. PAC-1, a DSPase from human T cells that is similar to MKP-3, is specific for Erk. MKP-5 appears to be somewhat selective for p38. The prototype DSPase VHR dephosphorylates Erk and JNK. There is also evidence for cross-talk between the MAPK pathways. For example, MKP-7 interacts with Erk, JNK, and p38, but shows substrate specificity for JNK and is phosphorylated in an Erk-dependent manner (65).

Table 1 Human DS-MKPs identified by DUSP nomenclature based on analysis described in Reference 64

DUSP	Synonyms	GenBank Accession Number
DUSP1	HVH1, CL100, MKP-1, PTPN10	NM_004417
DUSP2	PAC1, PAC-1	NM_004418
DUSP3	VHR	NM_004090
DUSP4	MKP-2, TYP, HVH2	NM_001394
DUSP5	HVH3	NM_004419
DUSP6	MKP-3, PYST1	NM_001946
DUSP7	MKP-X, PYST2	NM_001947
DUSP8	HB5, HVH8, HVH-5	NM_004420
DUSP9	MKP-4	NM_001395
DUSP10	MKP-5	NM_007207
DUSP14	MKP6, MKP-L	NM_007026
DUSP16	MKP-7	NM_030640
DUSP12	YVH1	NM_007240
DUSP22	JKAP, JSP1	NM_020185

DS-MKP Regulation

The DS-MKPs are regulated on multiple levels. The majority of DS-MKPs are inducible genes, and basal levels of DS-MKPs are low in nonstressed or unstimulated cells [reviewed in Keyse (58)]. Some DS-MKPs are immediate early genes. For example, MKP-1, MKP-2, MKP-X (PYST2), and PAC-1 are rapidly induced in response to serum stimulation (66–68). In contrast, MKP-3 (PYST1), MKP-4, MKP-5, MKP-X, and M3/6 are not encoded by immediate early genes (58). MKP-3 and VHR are constitutively expressed (67), and while MKP-3 is moderately inducible after several hours of stimulation (67, 69), VHR is not known to be inducible. Different DS-MKPs respond to different stimuli: MKP-1 is inducible by mitogens, oxidative stress, heat shock (63, 69), and hypoxia (70–72). In contrast, MKP-X is only moderately induced by serum but not by cellular stress (67).

Inducible expression of DS-MKPs is thought to be a mechanism for attenuation of mitogenic signaling. Induction of MKP-1 in NIH3T3 cells (62) and CCL39 hamster lung fibroblasts temporally correlates with Erk inactivation and is dependent on Erk activity (66). An additional mechanism by which Erk induces MKP-1 is through stabilization of MKP-1 protein levels. This is achieved by direct phosphorylation of MKP-1 by Erk, leading to reduced MKP-1 ubiquitination and proteasomal degradation (73). Furthermore, some DS-MKPs are activated by activated forms of their respective substrates. MKP-3 experiences a 25-fold increase in catalytic activity when complexed to its phosphorylated substrate, Erk2 (74).

This activation is specific, as neither p38 nor JNK activated MKP-3, but they did activate a nonspecific DS-MKP (MKP-4) (74). Taken together, the data indicate that inactivation of the Erk cascade is regulated through induction and stabilization of DS-MKPs in an inhibitory feedback loop.

ABERRANT DSPASE REGULATION IN DISEASED STATES

The pathogenic mechanisms underlying disease progression frequently involve perturbations in molecular signaling pathways. Cdc25A and Cdc25B are overexpressed in multiple human tumors, and high levels correlate with a poor prognosis (55, 75, 76). Cdc25A and Cdc25B have also been observed to be highly expressed in the brains of patients with Alzheimer's disease and may contribute to the pathology of neurodegeneration (77, 78). Although the mechanism by which Cdc25A and Cdc25B are overexpressed in human cancers is poorly understood, their expression may be elevated by increased gene expression, increased protein stability as a result of deficiencies in protein turnover, or both (31, 43, 55, 75, 76). Cdc25A and Cdc25B have oncogenic activity and can transform normal cells in cooperation with an activated Ras oncogene or inactivation of the Retinoblastoma (Rb) tumor suppressor protein (79). Targeted overexpression of Cdc25B in transgenic mice resulted in the formation of mammary gland tumors and an increased susceptibility to carcinogen-induced tumor formation (80, 81). Cdc25C, on the other hand, has not been found to be overexpressed in human tumors and does not transform cells (79); induction of premature mitosis by ectopic overexpression of Cdc25C was inefficient when compared to Cdc25B, providing a possible rationale for the lack of Cdc25C-associated oncogenic activity (18). Deregulated Cdc25 expression may contribute to the malignant phenotype by a combination of several mechanisms (Figure 8). As major targets of cell cycle checkpoints, overexpression of Cdc25A and Cdc25B may enable cell division in the presence of compromised genetic material by overwhelming the cell cycle checkpoint machinery, promoting genetic instability (24, 29, 51). Cdc25A and Cdc25B function as coactivators for steroid hormone receptors, independent of catalytic activity, and Cdc25 overexpression may promote expression of steroid hormone-responsive genes in the absence of ordinarily required stimuli or lower the threshold for such gene expression (82). Cdc25A functions as a liaison between mitogenic signaling pathways and the cell cycle, and overexpression of Cdc25A may promote unwarranted cell cycle activation in the absence of mitogenic stimuli, leading to a deregulated hyperproliferative state (41, 42). Furthermore, Cdc25A possesses antiapoptotic potential. Cdc25A downregulates the proapoptotic kinase apoptosis signal-regulating kinase 1 (Ask1) through a noncatalytic protein-protein interaction mechanism; overexpression of Cdc25A may block Ask1 activation in response to apoptotic stimuli (83). Cdc25A also downregulates Erk MAPK signaling by inactivating Raf1 and the epidermal growth factor receptor (44, 45). Prolonged Erk activation has been reported to promote cell cycle arrest and cytotoxicity in several cell types (84, 85); Cdc25A overexpression may thus provide a selective growth advantage by downregulating the deleterious effects of prolonged Erk MAPK activation in cells

transformed by upstream components of the Erk MAPK signaling cascade. Therefore, overexpression of Cdc25A and Cdc25B may contribute to the transformed phenotype by endowing cells with a proliferative advantage or by generating resistance to genotoxic stress-induced cell cycle arrest and apoptosis.

The role of the Cdc25s in neurodegeneration remains unclear. Cdc25A and Cdc25B are expressed and active in the brains of Alzheimer's disease patients (77, 78), and there is increasing evidence that expression and activation of the cell cycle machinery is associated with neurodegeneration in postmitotic neurons (86–89). Cell cycle activation appears to be a critical element of the apoptotic response to DNA damage in postmitotic neurons, and Cdk activation is a precursor to the neurodegeneration characteristic of Alzheimer's disease (78, 90, 91); moreover, inhibition of Cdk activity provides a neuroprotective effect, substantiating a role for the cell cycle machinery in the pathophysiology of neurodegeneration (92). The Cdc25 DSPases, therefore, constitute attractive potential targets for cancer and neurodegenerative disease drug discovery.

DS-MKPs in Neoplastic Disease

The chromosomal locations for all the human DS-MKP genes have been mapped, and many DS-MKPs reside in regions that are deleted in human tumors. For example, frequent loss of heterozygosity at 12q21 and 12q22-q23.1 has been observed in primary pancreatic cancers, and DUSP6/MKP-3 gene expression is lost in the majority of pancreatic cancer cell lines; MKP-3 maps to chromosome 12q22 (93). Consequently, a tumor suppressor function has been proposed for MKP-3; consistent with this hypothesis, exogenous expression of MKP-3 induced apoptosis in pancreatic cancer cells (93). Furthermore, MKP-X, MKP-5, and MKP-2 were mapped to chromosomes 3p21, 1q41, and 8p11-p12, respectively, where frequent deletions have been reported in multiple tumors (94–99).

Although a tumor suppressor function might be intuitively expected for phosphatases that deactivate Erk (i.e., MKP-3 and MKP-X), which is conventionally believed to promote growth and survival, phosphatases involved in JNK signaling are also found in regions of the genome suspected to harbor tumor suppressors. For example, hVH5, the human homolog of mouse M3/6, maps to 11p15 (100), a locus deleted in non-small-cell lung cancer (101). MKP-7 maps to chromosome 12p12–13 (102), where deletions have been found in several human tumors (103). Functional evidence that MKP-7 may be a tumor suppressor comes from a study by Hoornaert et al., who showed that BCR-Abl transformed cells reverted to a normal phenotype following MKP-7 overexpression (102). MKP-1 maps to chromosome 5q35 (104), and 5q gains have been found in malignant glioma cell lines (105) and in breast fibroadenomas (106), although there are also reports of 5q deletions in testicular (107, 108) and ovarian germ cell cancers (109). Although it was initially hypothesized that MKP-1 was a tumor suppressor (110), no evidence has been found to support this hypothesis. On the contrary, initial reports indicate mice with a targeted disruption of the *MKP-1* gene developed normally and had no increased frequency of malignancies compared to wild-type animals, even when the mice were over 1-year-old (111, 112).

A number of investigators have observed high basal levels of MKP-1 in human tumors, including prostate (113), gastric (114), breast (115), and pancreatic cancer (116). In ovarian cancer samples, MKP-1 expression was correlated with decreased progression-free survival (117). High levels of MKP-1 expression were also found in the early stages of prostate, colon, and bladder carcinogenesis (118). Evidence that MKP-1 may actually support the transformed phenotype comes from a recent study by Liao et al., who showed that PANC-1 human pancreatic cancer cells stably transfected with a full-length MKP-1 antisense construct had longer doubling times, decreased ability to form colonies in soft agar, and were unable to form tumors in nude mice (116). The precise mechanism by which loss of MKP-1 expression affected tumorigenicity, however, remains unknown. MKP-1 can protect cells against UV irradiation-induced apoptosis (119) and can inhibit JNK activity and AP-1-dependent gene expression in response to UV irradiation and the DNA damaging agent methyl methane sulfonate (120). Ectopic expression of MKP-1 also protects cells against cisplatin-induced apoptosis, whereas a catalytically inactive mutant of MKP-1 enhanced cisplatin toxicity (121). Thus, MKP-1 may have a cytoprotective role. It is interesting to note, however, that Liao et al. (116) found that MKP-1 antisense expression did not affect apoptosis by actinomycin D, which activates the JNK pathway. The MKP-1 antisense oligonucleotides also did not increase JNK or p38 phosphorylation, but did increase basal Erk phosphorylation and prolonged Erk phosphorylation in response to epidermal growth factor stimulation. This suggests that the primary mechanism by which MKP-1 supports the transformed phenotype may be mediated by an Erk, but not JNK, dependent process. Consistent with this hypothesis, several groups have shown that MKP-1 and activated Erk can coexist in malignant tissue (114, 115) and in cancer cells (116). This has led to a model where cells balance mitogenic overstimulation by expressing MKPs, the end result being a higher basal level of Erk signaling in tumors than in normal tissues. Additional evidence suggesting a role for MKP-1 in cancer comes from DNA microarray experiments, where high levels of MKP-1 in recurrent acute myelogenous leukemia (AML) were found concomitant with an activation of the Ras-Raf-Erk pathway (122). Furthermore, a recent report by Kang et al. has identified MKP-1 as one of 53 genes that were upregulated (4.03-fold) in highly metastatic breast cancer sublines compared to the parental MDA-MB 231 cells or cells with low metastatic potential (123). It should be noted, however, that the functional significance of many of these observations remains unclear, and more work needs to be done to precisely determine the roles that MKP-1 plays in the context of neoplastic disease.

DSPASES AS THERAPEUTIC TARGETS

Protein kinases have been a major focus of recent molecular-targeted drug discovery efforts, producing drugs such as imatinib mesylate (Gleevec®) and gefitinib (Iressa®), and the success of these drugs has prompted a substantial effort to target

other kinases, such as the Cdks, mitogen-activated protein kinase kinase (MEK), Raf, and mTOR (124). Based on their roles in multiple signaling pathways and altered expression in diseased states, there has been increasing interest in identifying DSPase inhibitors that are more potent and selective than the general tyrosine phosphatase inhibitor sodium orthovanadate (55). Such targeted agents may provide value as therapeutics for cancer and Alzheimer's disease. The shallow nature of the DSPase active site, combined with the conserved nature of the PTPase active site cleft, has lead some investigators to believe that DSPase-selective inhibitors may be difficult to identify. Although the three Cdc25 isoforms possess the common, highly conserved PTPase active site motif, the architecture of their active site appears to be different. Thus, the Cdc25 phosphatases have a shallow catalytic domain, whereas the PTPases have a deep 9 Å cleft (125,126). Indeed, several groups have identified lead compounds with favorable selectivity profiles, suggesting that phosphatase-selective inhibition is plausible (55).

Structure-activity relationships of natural and synthetic inhibitors of DSPases have been partially reviewed (55, 127). Representative members in this group include the natural products dnacin B_1 (**1**), dysidiolide (**2**), menadione (**3**), and coscinosulfate (**4**), which inhibited the Cdc25 family with IC_{50} values in the 1–10 μM range (Figure 9) (128–132). The biological activities of these natural products inspired total syntheses as well as the preparations of synthetic analogs and chemical libraries (133–138). Structurally most conspicuous among the small-molecule inhibitors discovered through combinatorial library and random screening are highly lipophilic acids [e.g., **5** (139), **6** (140), **7** (141)] as well as annulated *para*-quinones [e.g., **8** (142), **9** (143), **10** (144), **11** (145)] (Figure 9). In addition, moderately potent heterocyclic [e.g., **12** (146), **13** (147)] and phenolic derivatives [**14** (148)] have also been identified (Figure 9).

To date, compounds with quinone moieties have demonstrated the highest potency as well as considerable specificity in DSPase screens. Specifically, **10** was found to inhibit Cdc25B and VHR with IC_{50} values of 206 nM and 4.0 μM, respectively. Compound **11** had IC_{50} values of 22, 125, and 57 nM for Cdc25A, B, and C, respectively, and showed partial mixed-inhibitory kinetics. It is also interesting to note that indolyldihydroxyquinone **9** was found to bind competitively with the substrate at the active site of Cdc25 and yield a K_i of 470 nM (143). Molecular modeling of enzyme-inhibitor complexes is possible (145, 149) because several crystal structures of Cdc25 isoforms are available, but rational design has so far met with limited success for the improvement of the binding characteristics of lead structures. A recent report presents the homology-modeling of a Cdc25B-inhibitor complex, which might provide a more suitable starting point for rational inhibitor design (150).

Whether Cdc25 isoform specificity can be achieved is a challenging issue. All three Cdc25 isoforms possess identical amino acids in their highly conserved PTPase active site motif (HCEFSSER), and they share a high degree of sequence homology outside of this catalytic loop, posing a high hurdle for selective targeting of the individual Cdc25 isoforms. Nonetheless, selective protein kinase inhibition

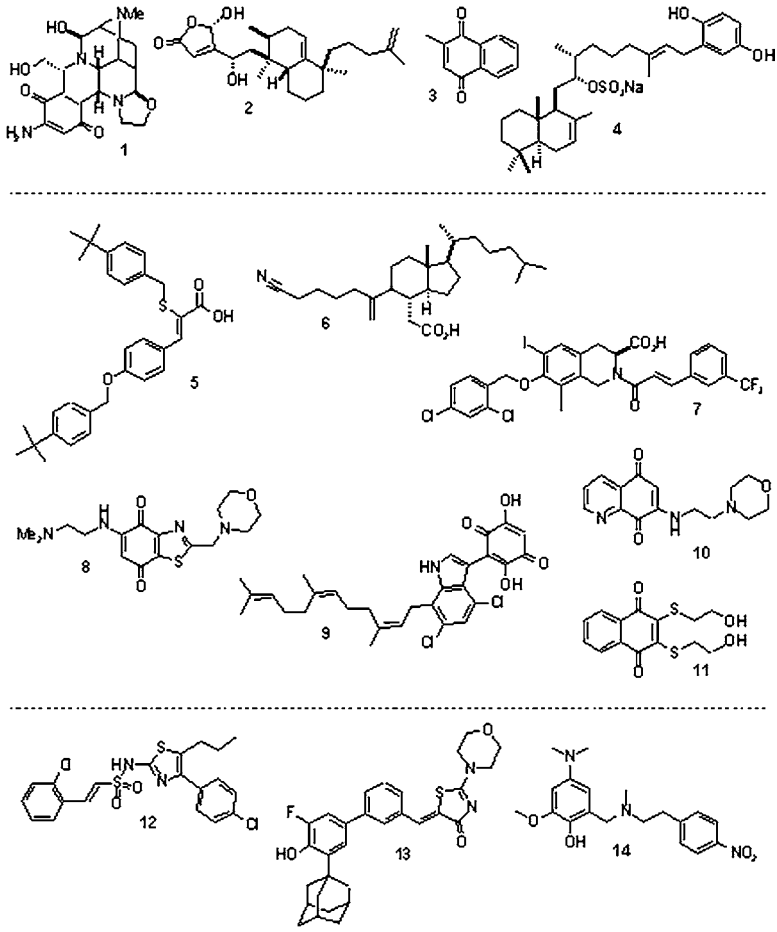

Figure 9 Natural product and synthetic inhibitors of the Cdc25 family of DSPases. Representative examples for small-molecule inhibitors of this enzyme class are natural products (**1–4**), lipophilic acids (**5–7**), quinones (**8–11**), heterocycles (**12**, **13**), and phenols (**13**, **14**).

has been achieved with ATP competitive inhibitors, which target similar active site structures and catalytic mechanisms (124), lending credence to the hypothesis that selective Cdc25 inhibition may yet be achieved. It is worth mentioning that Cdc25-specific inhibitors lacking isoform selectivity may have some theoretical therapeutic appeal, but additional small-molecule inhibitors will be required to fully test this hypothesis.

Although screening strategies for Cdc25 inhibitors have focused on identifying active site inhibitors, an alternate mechanism for targeted inhibition of Cdc25

phosphatases may exist, as exemplified by the naphthoquinone NSC 95397, which inhibits Cdc25A activity by a bimodal mechanism. Although NSC 95397 was identified as an inhibitor of Cdc25A phosphatase activity in a high-throughput in vitro screen (145), treatment of prostate cancer cells (PC-3 and LNCap) with NSC 95397 resulted in decreased Cdc25A protein levels by stimulating its degradation (46). Cdc25A degradation promoted by NSC 95397 was independent of genotoxic stress, as p53, Chk1, and Chk2 were not affected (46) and, therefore, presumably occurred through the ubiquitin-proteasome pathway that regulates physiological Cdc25A protein turnover. NSC 95397 therefore represents a novel class of Cdc25 inhibitors that can inhibit Cdc25A activity via the combined mechanism of catalytic inhibition and increased protein turnover (46). One advantage of such a novel class of Cdc25 inhibitors would be that, in addition to inhibiting Cdc25 catalytic activity, these compounds could also downregulate Cdc25 expression, thereby functioning as inhibitors of the noncatalytic activities of Cdc25.

Like the Cdc25 DSPases, MKP-1 may be an important regulator of the malignant phenotype, and it thus represents a rational target for anticancer drug discovery; however, selective small-molecule inhibitors of MKP-1 are lacking. This has been hampered, at least in part, by the lack of an available X-ray crystal structure and the lack of definitive assays for detection of cellular DS-MKP inhibition. Recently, a high-content fluorescence-based cellular assay for detection of MKP-3 inhibition was published (151). This assay was used to identify novel inhibitors of MKP-3 and might be applicable for MKP-1 in the future.

CONCLUSIONS

DSPases have critical roles in regulating cellular phosphorylation signaling networks and are deregulated in human cancer and Alzheimer's disease. The uniqueness of their biochemical mechanism and the central role of their substrates make DSPases an attractive target for further pharmacological studies. In recent years, several natural products and novel small organic molecules have been identified that can block phosphatase activity. Nonetheless, there continues to be a need for more potent and selective inhibitors of DSPases to permit a further dissection of their roles in biological systems and to clinically validate their potential as anticancer targets.

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