Dual-specificity phosphatases in NUCLEAR SIGNALING

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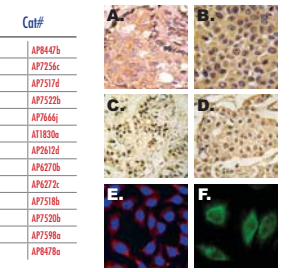
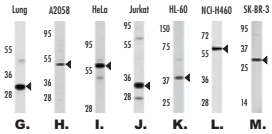
Selected Abgent Products

Figure	Target	Tissue/Cell line	Cat#
A.	DUSP4	Human breast carcinoma	AP8447b
B.	CDC25B	Human breast carcinoma	AP7256c
C.	CDK1	Human breast carcinoma	AP7517d
D.	CDK6	Human breast carcinoma	AP7522b
E.	PDGFRA	Human carcinoma (HeLa)	AP7666j
F.	DUSP6	Human carcinoma (HeLa)	AT1830a
G.	Cyclin D1	Mouse lung tissue lysate	AP2612d
H.	Cyclin E1	A2058 cell line lysate	AP6270b
I.	CDC25A	HeLa cell line lysate	AP6272c
J.	CDK2	Jurkat cell line lysate	AP7518b
K.	CDK4	HL-60 cell line lysate	AP7520b
L.	Cyclin B1	NCI-H460 cell line lysate	AP7598a
M.	DUSP3	SK-BR-3 cell line lysate	AP8478a





Dual-specificity phosphatases

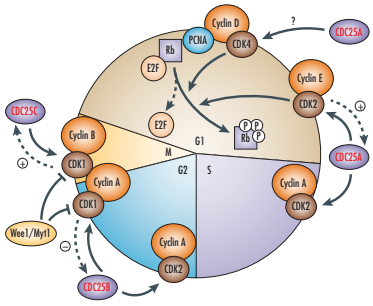


Fig. 1 CDC25 phosphatases promote mammalian cell-cycle progressi Dual-specificity phosphatases (DSPases) have a central role in the complex regulation of signaling pathways that are involved in cell stress responses, proliferation and death (1). The cell-division cycle 25 (CDC25) family of DSPases regulates cell-cycle progression by dephosphorylating and activating cyclin-dependent kinases (CDKs). In the event of DNA damage, CDC25 are key targets of the checkpoint machinery that ensures genetic stability. Inactive CDKs are phosphorylated at adjacent threonine and tyrosine residues near their amino termini. Dephosphorylation at both sites by CDC25 phosphatases catalyses their activation and allows the CDKs to propagate cell-cycle signal transduction (1-12). Indicated in red are the protein targets for ABGENT's antibody products

Examples for dual-specificity phosphatases and their biological functions

Gene	Name	Role in nuclear signaling	Cellular process / disease
CDC25A, B, C	cell-division cycle 25	DNA damage	Cell cycle control, checkpoint pathways
CDC14A, B, C	cell division cycle 14	p53 regulation	Cell cycle control, cytokinesis, cancer
PTEN	phosphatase and tensin homolog	DNA repair	Cell cycle control, chromosome stability
PTPN11	SHP2	Transcriptional regulation	Mitogenic activation, metabolic control
DUSP1	dual-specificity phosphatase 1	Transcriptional regulation	Cell cycle control, immune response
DUSP2	dual-specificity phosphatase 2	Nuclear accumulation of ERK	Immune response, heat shock
DUSP4	dual-specificity phosphatase 4	Nuclear accumulation of ERK	Control of cell cycle and MAP kinases
DUSP5	dual-specificity phosphatase 5	Nuclear translocation	Immune response
DUSP6	dual-specificity phosphatase 6	FGF signaling to the nucleus	Development, postnatal lethality
DUSP7	dual-specificity phosphatase 7	FGF signaling to the nucleus	Development
DUSP9	dual-specificity phosphatase 9	Transcriptional regulation	Development
DUSP10	dual-specificity phosphatase 10	Transcriptional regulation	Immune response
DUSP12	dual-specificity phosphatase 12	Heat stress response	Cell survival, diabetes
DUSP14	dual-specificity phosphatase 14	Transcriptional regulation	Immune response, CD28 signaling
DUSP22	dual-specificity phosphatase 22	STAT3 activation, ER α signaling	Immune response, proliferation
EPM2A	laforin	β-catenin accumulation in nucleus	Lafora progressive myoclonus epilepsy

Overexpression of CDC25 in cancer

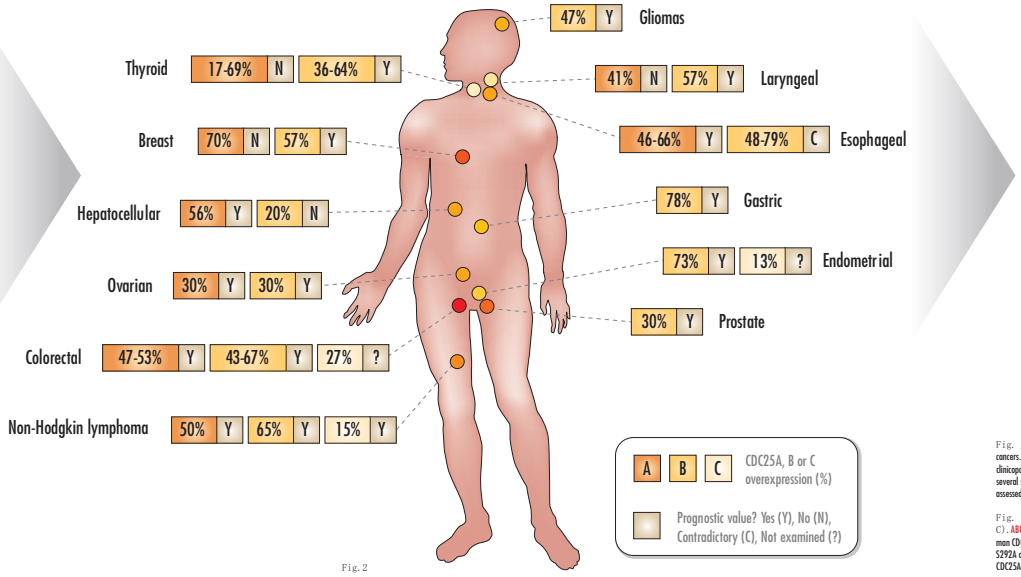


Fig. 2

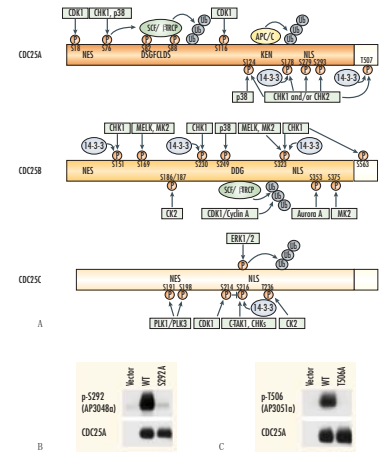


Fig. 2 Overexpression of CDC25 protein in human cancer CDC25 proteins are overexpressed in a wide variety of cancers. Percentages of tumors in which CDC25A, CDC25B or CDC25C are overexpressed are indicated. CDC25 overexpression is linked to clinicopathological features, including tumor grade or stage, metastases, depth of invasion, residual or recurrent disease. Cases for which several studies reported contradictory prognostic values are marked by a (C), and studies in which clinicopathological features were not assessed are indicated by an (?). The intensity of the red circles in the human body corresponds to the cancer mortality (2, 5).

Fig. 3 Domain organization of CDC25 homologs (A) and detection of CDC25 in transfected cells (C). ABGENT's antibody AP3048a was generated against synthetic phosphopeptide corresponding to amino acids surrounding S292 of human CDC25A (B). The antibody was used in Western blot to detect Phospho-CDC25A-S292 in cells transfected with wild type (wt) or mutant S292A of CDC25A. Antibody #AP3051a was generated against phosphopeptide corresponding to amino acids surrounding T506 of human CDC25A (C). The antibody was used to detect Phospho-CDC25A-T506 in cells transfected with wild type or mutant T506A of CDC25A.

Control of the cell cycle

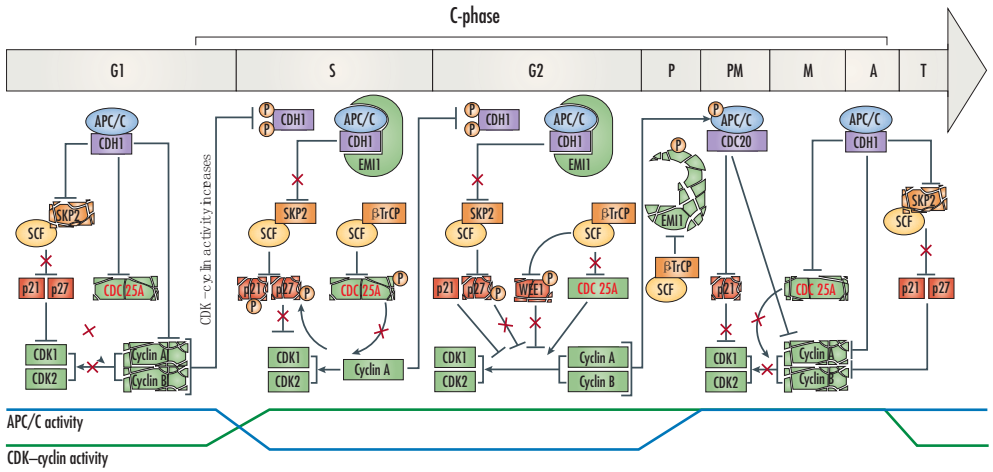


Fig. 4 Control of the cell cycle by the ubiquitin – proteasome The cell division cycle is regulated primarily by the activity of cyclindependent kinases (CDKs) and protein degradation by the ubiquitin–proteasome system (UPS). Each CDK complex contains one of many activating subunits, termed cyclins, the levels of which oscillate during the cell cycle. CKIs (CDK inhibitors), such as p27 and p21, inhibit CDK activity and promote cell cycle arrest and/or delay. SCF complexes and the APC/C (anaphase-promoting complex/cyclosome) provide the specific, rapid and timely proteolysis of cell cycle regulators, which ultimately controls CDK1 and CDK2 to timely modulate their activities during cell cycle progression. The best characterized cell cycle ubiquitin ligases are SCFSKP2, SCFFBXW7 (not shown), SCF βTrCP, APC/CDH1 and APC/CDC20. SCFSKP2 is a positive regulator of cell cycle progression (by promoting the degradation of p21 and p27), whereas SCF βTrCP is both a positive and negative regulator of the cell cycle (by targeting CDC25A (cell division cycle 25A), despin, WEE1 and EMI1 (also known as F-box protein 5)). APC/CDH1 and APC/CDC20 always attenuate CDK1 activity (by directing the degradation of cyclins A and B), except in early mitosis, when APC/CDC20 targets p21 for degradation. Finally, SCFFBXW7 attenuates CDK1 and CDK2 by inducing the degradation of cyclin E. SCF complexes and the APC/C control each other, with SKP2 being ubiquitylated by APC/CDH1 in G1 and SCF βTrCP targeting EMI1, which is an inhibitor of APC/CDH1, for proteolysis in early mitosis. Additionally, SCF complexes and the APC/C share common substrates that are targeted by their respective ubiquitin ligase(s) only at particular times during the cell cycle. For example, SCFSKP2 targets p21 for degradation at G1–S, whereas APC/CCDC20 targets p21 during prometaphase. This scenario is also true for the targeted degradation of CDC25A by APC/CDH1 in G1 phase, which is followed by SCF βTrCP-mediated degradation during S phase. Moreover, phosphorylation by CDKs modulates the activity of SCF complexes and the APC/C. CDK activity inhibits binding of CDH1 to the APC/C while promoting the activation of APC/CDC20, and phosphorylation of certain SCF substrates by CDKs allows recognition by the F -box protein subunit. β-TrCP (βTransducin repeat-containing protein; CDH1, also known as FZR1 (Fizzy/cell division cycle 20 related 1); FBXW7, F-box protein with WD domain 7; SKP2, S-phase kinase-associated protein 2 (4).

Product abbreviations

DUSP4 : dual specificity phosphatase 4; MAP kinase phosphatase 2; VH1 homologous phosphatase 2
CDC25A & B : cell division cycle 25 homolog A & B
CDK1 : cell division cycle 2, G1 to S and G2 to M; cyclin-dependent kinase 1; p34 protein kinase; CDC2
CDK6 : cyclin-dependent kinase 6; cell division protein kinase 6
PDGFRA : platelet-derived growth factor receptor, alpha polypeptide
DUSP6 : dual specificity phosphatase 6; MAP kinase phosphatase 3
Cyclin D1 B-cell CLL/lymphoma 1; G1/S-specific cyclin D1; CCND1
Cyclin E cyclin E1; cyclin E1; CCNE1
CDK2 : cyclin-dependent kinase 2; cdc2-related protein kinase; cell devision kinase 2; p33 protein kinase
CDK4 : cyclin-dependent kinase 4; cell division kinase 4; melanoma cutaneous malignant, 3
CCNB1 : G2/mitotic-specific cyclin B1; CCNB1
DUSP3 : dual specificity phosphatase 3; vaccinia virus phosphatase VH1-related; VHR

References

1. Patterson KI, Brummer T, O'Brien PM and Daly RJ. (2009) Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J. 418(3), pp.475-489.

2. Boutros R, Lobjois V and Ducommun B. (2007) CDC25 phosphatases in cancer cells: key players? Good targets?. Nat Rev Cancer. 7(7), pp.495-507.

3. Jeffrey KL, Camps M, Rommel C and Mackay CR. (2007) Targeting dual specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov. 6(5), pp.391-403.

4. Frescas D and Pagano M. (2008) Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. Nat Rev Cancer. 8(6), pp.438-449.

5. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. (2008) Cancer statistics, 2008. CA Cancer J Clin. 58(2), pp.71-96.

6. Brezak MC, Kasprzyk PG, Galcera MO, Lavergne O and Prévost GP. (2008) CDC25 inhibitors as anticancer agents are moving forward. Anticancer Agents Med Chem. 8(8):857-62.

7. Planchon SM, Waite KA and Eng C. (2008) The nuclear affairs of PTEN. J Cell Sci. 121(Pt 3), pp.249-253.

8. Lyon MA, Ducruet AP, Wipf P and Lazo JS. (2002)Dual-specificity phosphatases as targets for antineoplastic agents. Nat Rev Drug Discov. 1(12), pp.961-976.

9. Dickinson RJ and Keyse SM. (2006)Diverse physiological functions for dual-specificity MAP kinase phosphatases. J Cell Sci. 119(Pt 22), pp.4607-4615.

10. Lang R, Hammer M and Mages J. (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. J Immunol. 177(11), pp.7497-7504.

11. Hutchins JR and Clarke PR. (2004) Many fingers on the mitotic trigger: post-translational regulation of the Cdc25C phosphatase. Cell Cycle. 3(1), pp.41-45.

12. Wu TR, Hong YK, Wang XD, Ling MY, Dragoi AM, Chung AS, Campbell AG, Han ZY, Feng GS and Chin YE. (2002) SHP-2 is a dual-specificity phosphatase involved in Stat1 dephosphorylation at both tyrosine and serine residues in nuclei. J Biol Chem. 277(49), pp.47572-47580.