RESEARCH ARTICLE

Cloning and Functional Characterization of *Ptpcd2* as a Novel Cell Cycle Related Protein Tyrosine Phosphatase that Regulates Mitotic Exit

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Abstract

Faithful transmission of genetic information depends on accurate chromosome segregation as cells exit from mitosis, and errors in chromosomal segregation are catastrophic and may lead to aneuploidy which is the hallmark of cancer. In eukaryotes, an elaborate molecular control system ensures proper orchestration of events at mitotic exit. Phosphorylation of specific tyrosyl residues is a major control mechanism for cellular proliferation and the activities of protein tyrosine kinases and phosphatases must be integrated. Although mitotic kinases are well characterized, phosphatases involved in mitosis remain largely elusive. Here we identify a novel variant of mouse protein tyrosine phosphatase containing domain 1 (Ptpcd1), that we named *Ptpcd2*. Ptpcd1 is a Cdc14 related centrosomal phosphatase. Our newly identified *Ptpcd2* shared a significant homology to yeast Cdc14p (34.1%) and other Cdc14 family of phosphatases. By subcellular fractionation *Ptpcd2* was found to be enriched in the cytoplasm and nuclear pellets with catalytic phosphatase activity. By means of immunofluorescence, *Ptpcd2* was spatiotemporally regulated in a cell cycle dependent manner with cytoplasmic abundance during mitosis, followed by nuclear localization during interphase. Overexpression of *Ptpcd2* induced mitotic exit with decreased levels of some mitotic markers. Moreover, *Ptpcd2* failed to colocalize with the centrosomal marker γ-tubulin, suggesting it as a non-centrosomal protein. Taken together, *Ptpcd2* phosphatase appears a non-centrosomal variant of Ptpcd1 with probable mitotic functions. The identification of this new phosphatase suggests the existence of an interacting phosphatase network that controls mammalian mitosis and provides new drug targets for anticancer modalities.

Keywords: *Ptpcd1* - mitotic exit - cell cycle - Cdc14 - protein tyrosine phosphatase

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Introduction

Alternating dynamic activities of protein kinases and phosphatases ensure tight cell cycle regulation and DNA damage response control (Maya-Mendoza and Jackson, 2009; Shimada and Nakanishi, 2013).

Correct execution of mitosis in eukaryotes relies on timely activation and inactivation of cyclin dependent kinase1 (Cdk1) and its activating subunit, cyclin B (CycB) (Visconti et al., 2012). Anti-mitotic drugs are current drugs used to treat cancer by selectively perturbing progression through mitosis. Cancer cells treated with anti-mitotic drugs will delay mitotic progression due mitotic checkpoint (Janssen and Medema, 2011). This prolonged mitotic delay is often followed by mitotic cell death (Gascoigne and Taylor, 2008). However, a subset of cells can escape and exit mitosis. Blocking mitotic exit might circumvent drug resistance seen in those cells and enhance the efficacy of anti-mitotic drugs (Huang et al., 2009; Manchado et al., 2010; Hunt, 2013). Mitotic exit comprises all events that occur after completion of the spindle assembly checkpoint, including chromosome segregation, cytokinesis and reassembly of interphase cell structures. This is regulated through degradation of key mitotic factors and removal of phosphorylations from mitotic substrates (Wurzenberger and Gerlich, 2011; Gascoigne and Cheeseman, 2013). The Cdc14 family of dual specificity phosphatases (DSP) has been extensively studied in the context of yeast via its role in regulating late mitotic events, promoting Cdk substrate (s) dephosphorylation and mitotic exit (Bremmer et al., 2012; Hancioglu and Tyson, 2012; Sanchez-Diaz et al., 2012). Mammalian cells possess two orthologs, hCdc14A and hCdc14B (Vázquez-Novelle et al., 2005; Mocciaro and Schiebel, 2010). Interestingly, the essential role of Cdc14 in mitotic exit control is not conserved among species, where knockout studies of human Cdc14 orthologs in established cell lines have shown no obvious

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mitotic defects (Mocciaro and Schiebel, 2010). This strengthens the notion that alternative phosphatase (s) might function during mitosis (Berdougo et al., 2008; Mocciaro et al., 2010). We have previously identified the centrosomal protein tyrosine phosphatase containing domain 1 (Ptpcd-1) as a mitotic phosphatase involved in cytokinesis and regulation of centrosomal duplication (Zineldeen et al., 2009). Ptpcd1 is a Cdc14B homologue that is colocalized with Polo like kinase (Plk1), an oncogene overexpressed in many cancers (Zineldeen et al., 2009). In mammalian cells, the phosphatase (s) that counteracts Cdk1–CycB is yet to be identified, but is likely to impact mitotic exit regulation (Skoufias et al., 2007; Bremmer et al., 2012), here we functionally characterized a non-centrosomal isozyme of Ptpcd1, that we named; *Ptpcd2* which is a cell cycle regulated non-centrosomal phosphatase involved in mitotic exit.

Materials and Methods

Cloning of mPtpcd2

The data base of the NCBI (National Center for Biotechnology Information) was searched for sequences that bear homology to a protein sequence corresponding to the entire region of mouse Ptpcd1 **No. NM 207232** (Zineldeen et al., 2009).

A mouse eyeball cDNA clone: **E130020P12**, from the RIKEN full-length enriched library was identified and we named it *Ptpcd2* (with gene bank accession No. **AK053480**). The complete ORF of *Ptpcd2* was amplified by RT-PCR. Total RNA was extracted from MEF (mouse embryonic fibroblast) using Isogene (Nippon Gene, Japan) and reverse transcribed using RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific).

A 1.6 kb PCR product corresponding to the full length *Ptpcd2* was amplified by PCR using a primer set incorporating KpnI and NotI restriction sites as follows: *Ptpcd2*- KpnI forward primer: 5`TTGGTACCAT GTTCCATGGCGTGT`3 and *Ptpcd2*- NotI reverse primer: 5`AAGCGGCCGCCAACAAACTCATT `3. pcDNA3.1-Myc/His-*Ptpcd2* was then generated by directional cloning of KpnI/NotI *Ptpcd2* fragment into pcDNA3.1Myc/His vector that was subsequently sequenced.

Chemicals and antibodies

All chemicals unless otherwise described were purchased from Sigma (Sigma-Aldrich; USA). Antibodies used in this study were as follows: anti-c-myc (sc-789; Santa Cruz, sc-40; Santa Cruz), anti-γ-tubulin (T3559; Sigma), anti-β-tubulin (8226; Abcam) anti-Cyclin B cyclin B1 (GNS; sc-245; Santa Cruz) anti- Phospho-Cdk1 (Tyr15) (#9111; Cell signalling), anti-SIRT2 (rS8447; Sigma-Aldrich; USA) and anti-phosphohistone H3, serine 10 (06-570; Milipores).

Cell culture and transfection

HeLa or U2OS cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco) and streptomycin (0.1 mg/ml; Gibco) at 37°C in a 5%CO_2 incubator.

For Transient plasmids transfection, Lipofectamine Plus reagent (Life Technologies, Inc.) was used according to manufacturer's protocol. Cells were also co-transfected with a green fluorescent protein (GFP) expression vector pEGFP-C1 (Clontech, CA, USA) as a marker of transfection efficiency.

Protein phosphatase assay

Phosphatase assay was performed according to a method previously reported (Uchida et al., 1993). Briefly Cells were harvested in RIPA buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 1% sodium deoxycholate and complete protease inhibitor tablets (1/10 ml) (Roch). Lysates were then incubated with anti-c-myc monoclonal antibodies (2μg) or mouse normal IgG as a control at 4°C for 1hour and then precipitated with 20 μl of protein G beads. Beads were washed once with a buffer containing (50 mM Tris-HC1 (pH 7.6), 150 mM NaCl, and 0.1% Triton X-100), twice with 1 ml of buffer containing (50 mM Hepes (pH 7.6), 150 mM NaCl, 0.1% Triton X-l00), and twice with assay buffer containing (40 mM MES (2-N-morpholino ethanesulfonic acid, pH 5.0) and 1.6 mM dithiothreitol). The assay mixture contained 25 mM p-nitrophenyl phosphate (pNPP) as a substrate. Immune complex pellet was incubated at 30°C for 30 min. The reaction was terminated by the addition of 1N NaOH, and the absorbance was determined at 410 nm through a spectrophotometer (Biosystems).

Flow cytometry assessment of cell cycle

Cells were cultured in 6-well plates and transfected with plasmid DNA 48h later, assessment of cellular DNA content by flow cytometry was performed as described elsewhere (Massey et al., 2010). Cells were analyzed with a BD flow cytometer (FACScan, Becton-Dickinson). Cell cycle distribution was determined by Cyflogic software.

Immunoblotting

Whole cell extract and immunoblotting were performed as previously reported (Méndez and Stillman, 2000; Shimada et al., 2008). Subcellular fractionation and preparation of cytosolic, nuclear fractions and insoluble nuclear pellet were according to (Méndez and Stillman, 2000; Niida et al., 2007). Protein concentrations were determined by protein assay kit (Biodiagnostic., Egypt). Equal amounts of Proteins were resolved by 10% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were probed with the corresponding antibodies as in figure legends; β-actin was used as a loading control. Protein expression levels were determined using ECL detection kit (Amersham Biosciences) and chemiluminescence was detected by gel documentation (Biometra, Goettingen, Germany).

Immunofluorescence microscopy

Secondary antibodies for immunofluorescence were; goat anti-mouse IgG, Cy3 conjugated (Jackson immunoresearch), goat anti-rabbit IgG, Alexa Flour 594 conjugated and goat anti-rabbit IgG, Alexa Fluor

488 conjugated (Molecular probes). Cells were either grown on glass bottomed culture dishes (MatTech) or on acid washed glass coverslips. Immunostaining was performed as in (Naruyama et al., 2008; Zineldeen et al., 2009), DNA was visualized with DAPI (4',6-diamidino-2-phenylindole) (2mg/ml). Immunofluorescence microscopy (Olympus BX51) was performed with the combinations of antibodies specified in figure legends.

Bioinformatic analysis

Multiple sequence alignments were performed using Clustal W and Clustal X programs (Larkin et al., 2007). Motif analysis was performed according to (Dinkel et al., 2012) and for coiled domains prediction was according to (Lupas et al., 1991).

Statistical analysis

Data were expressed as mean±SD (standard deviation). Comparisons were achieved using, unpaired t -test according to the Graph Pad PRISM software version 5 (GraphPad Software, Inc., San Diego, CA).

Results

Identification and cloning of mouse Ptpcd2

Loss of function studies of human Cdc14A and B revealed that neither of them is essential for cell viability nor cell proliferation (Berdougo et al., 2008; Mocciaro and Schiebel, 2010; Mocciaro et al., 2010). Thus, we speculated that other mammalian homolog (s) of Cdc14 phosphatases may exist and participate in regulation of late mitotic events such as mitotic exit. We previously identified Ptpcd1 as a centrosomal phosphatase that regulates its duplication and cytokinesis (Zineldeen et al., 2009).

By homology search of protein sequence of Ptpcd1, a clone with the gene bank accession number (**AK053480**) was identified. The protein encoded by the open reading frame comprises 525 amino acids, with a predicted molecular mass of 58.7 kDa, and exhibits 69.8% overall sequence identity with mouse Ptpcd1 (**NP_997115.1**), 55.7% with human Ptpcd1 (**NP_689635.3**), 24.9% with hCdc14 A (**NP_003663.2**), 23.5% with hCdc14B (**NP_001070649.1**), 34.1% with Cdc14p (**NP_116684.3**) and 22.8% with mouse calcineurin (**NP_032939.1**). Thus, we named this gene mouse *Ptpcd2* (Figure 1A, C). Phylogenetic analysis placed *Ptpcd2* and Ptpcd1in the Cdc14 subfamily of the calcineurin phosphatase (Figure 1D). Sequence analysis of *Ptpcd2* revealed that it possessed PTP and DSP catalytic domains. Its carboxyl terminal lacks the unique coiled coil domain of Ptpcd1(Zineldeen et al., 2009), however it contains PDZ binding domain (Beuming et al., 2005). RXXL motif (APC/C binding motif), STAT5 Src Homology 2 (SH2) domains, nuclear export signals, and bipartite nuclear localizing signal were also found (Figure 1A, B).

Ptpcd2 has protein phosphatase catalytic activity

To functionally characterize our newly identified *Ptpcd2*, we first examined for the catalytic activity of ectopically expressed *Ptpcd2* protein, HeLa cells

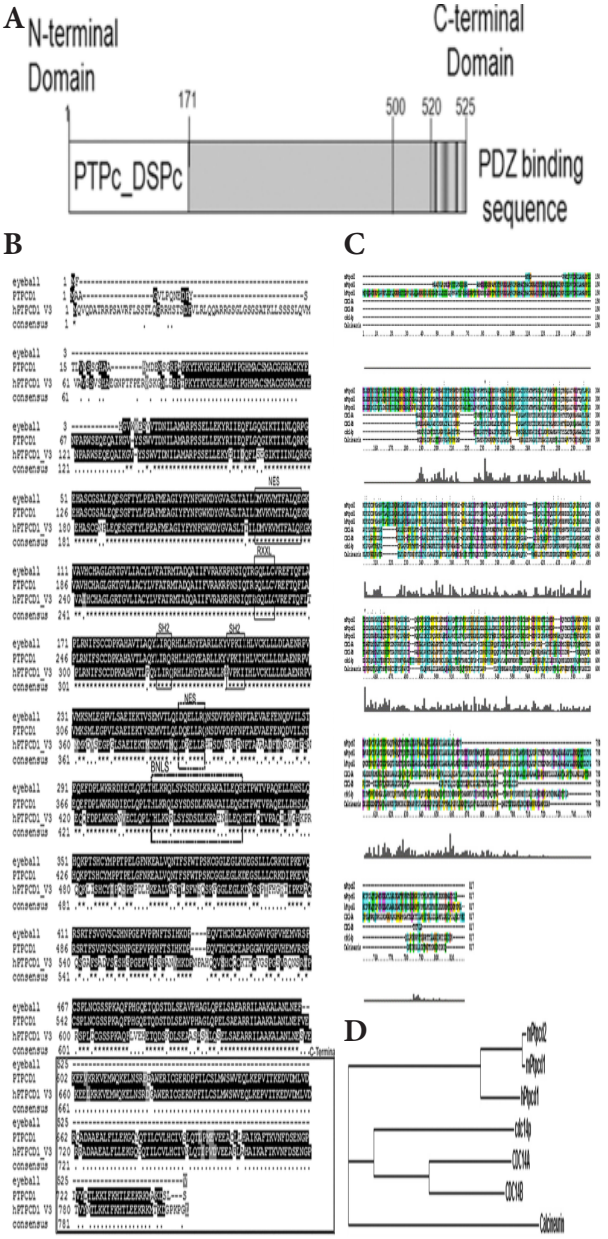

Figure 1. Sequence Analysis of *Ptpcd2*. (A) Amino acid sequence analysis of *Ptpcd2* showing PTP and DSP catalytic domains and PDZ binding domain. (B) Alignment of the amino acid sequences of the mouse *Ptpcd2*, mouse Ptpcd1 and human Ptpcd1. Black and gray backgrounds indicate residues that are identical or conservative substitutions, respectively. Hyphens within the sequences represent gaps introduced to optimize alignment, and residue numbers are shown on the left. (Generated by Clustal X 2.1; computer program). The conserved nuclear export signals (NES), bipartitite nuclear localizing signal (BNLS), RXXL motif that binds to APC/C (anaphase promoting complex/ cyclosome) and SH2 (Src Homology 2) domains are boxed. (C) Multiple alignment of the amino acid sequences of mouse *Ptpcd2*, mouse Ptpcd1, human Ptpcd1, human Cdc14A, human Cdc14B, S. cereviseae (Cdc14p) and mouse calcineurin. Identical and conservative residues are colored. Hyphens within the sequences represent gaps introduced to optimize alignment, and residue numbers are shown on the left. (D), a phylogenic tree of the aligned proteins (generated by Calsustal x 2.1, a computer program). The accession numbers of the compared proteins were as follows: mouse Ptpcd1 (NP_997115.1), human Ptpcd1 (NP_689635.3), hCdc14 A (NP_003663.2), hCdc14B (NP_001070649.1), Cdc14p (NP_116684.3) and mouse calcineurin (NP_032939.1)

were transfected with *Ptpcd2*-pcDNA3.1/Myc-His or empty vector, followed by immunoprecipitation with increasing amounts of HeLa cell lysates. PTP activity of the precipitated *Ptpcd2* was then assayed using pNPP as a phosphatase substrate.

PTP activity was recovered in the immune complex in a dose-dependent manner (Figure 2 A). Furthermore, the PTP activity in the immune complex was completely abolished by 1 mM sodium vanadate, an inhibitor of PTP activity (Figure 2A). Overexpression of *Ptpcd2* in U2OS cells was performed with transfection efficiency of about 60% when the efficiency was monitored by co-transfection with EGFP vector (Figure 2B). *Ptpcd2* encoded a 525 amino acid protein with a predicated molecular weight of 58.7 kDa. A band corresponding to 60 kDa was detected by western blotting (Figure 2C). Since sequence analysis of *Ptpcd2* identified multiple nuclear localizing and export signals (Figure 1B), therefore we went further for subcellular fractionation of asynchronous HeLa cells expressing *Ptpcd2* followed by western blotting. *Ptpcd2* was almost detected in all cellular compartments (Figure 2 D), with enrichment at cytosolic fraction and insoluble nuclear pellet.

Overexpression of Ptpcd2 drives mitotic exit

To investigate the effect of *Ptpcd2* overexpression on protein levels of various cell cycle key players, western blotting of U2OS cells expressing *Ptpcd2* or empty vector (control) was performed. We found minor decrease in cyclin B1 levels relative to control. As for inhibitory Cdk1 phosphorylation at tyrosine 15, we noted mild increase in Cdk1–cyclin B1 activity in cells expressing *Ptpcd2* when compared to control cells, probably due to rapid exit into the next G1 phase of the cell cycle. Sirtuin 2

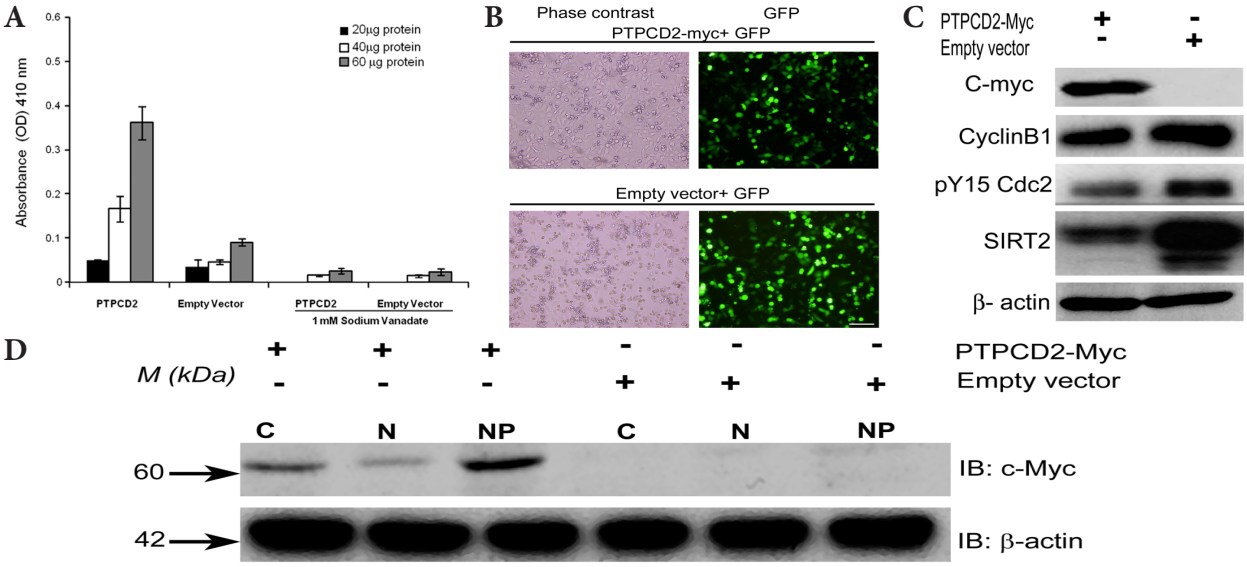

Figure 2. Functional Characterization of *Ptpcd2* Relative to Some Cell Cycle Key Players. (A) Varying protein amounts of HeLa cells were immunoprecipitated and assayed for PTP activity in absence and presence of 1M vanadate using pNPP as a phosphatase substrate. The value of the absorbance at 410 nm observed in the presence of control mouse immunoglobulin instead of antibody was subtracted from each value, and the net increase was expressed as a percentage of the control value. Each value is the mean of duplicate determinations. Result represents means±SD representative of three separate experiments. (B) Photomicrographs of U2OS cells transfected with *Ptpcd2*/c-myc or empty vector. EGFP-C1 was co-transfected to verify transfection efficiency. Scale bar; 100µm. (C) Whole cell extracts of cells from (B) 48h post-transfection were run onto 10% SDS-PAGE followed with immunoblotting with the indicated antibodies. β-actin served as a loading control. (D) Subcellular fractions of HeLa cells transfected with *Ptpcd2*/c-myc or empty vector that were run onto 10% SDS PAGE followed by immunoblotting. C: cytosolic fraction; N: soluble nuclear fraction; NP: nuclear pellet. Immunoblotting with anti-c-myc polyclonal antibody; β-actin was used as loading control

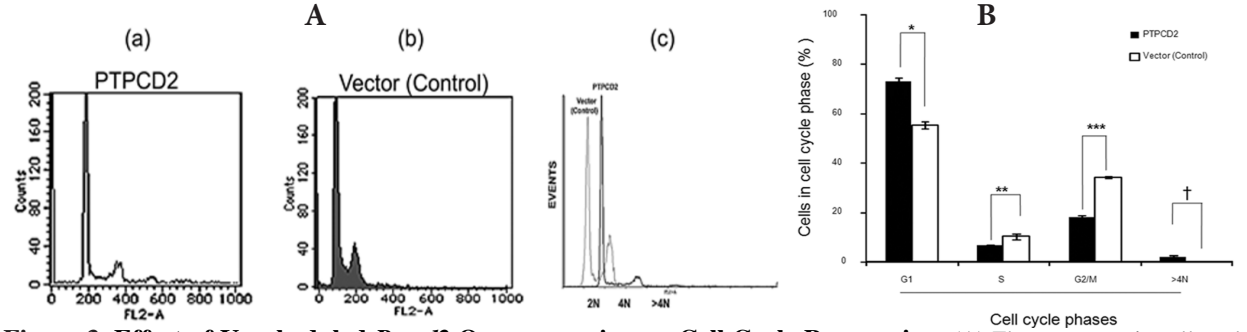

Figure 3. Effect of Unscheduled *Ptpcd2* Overexpression on Cell Cycle Progression. (A) Flow cytometric cell cycle profile of PI stained HeLa cells 48h after transfection with *Ptpcd2*/myc (a), empty vector (b) and overlay of both (c) (10000 events are shown). (B) Distribution of cells in different cell cycle phases of the above mentioned cells. Data represents means of three independent experiments error bars are±SD. P values are (*)=0.001, (**)=0.0353, (***)>0.0001 and (†)=0.0026 by student unpaired t-test. P< 0.005 was considered statistically significant

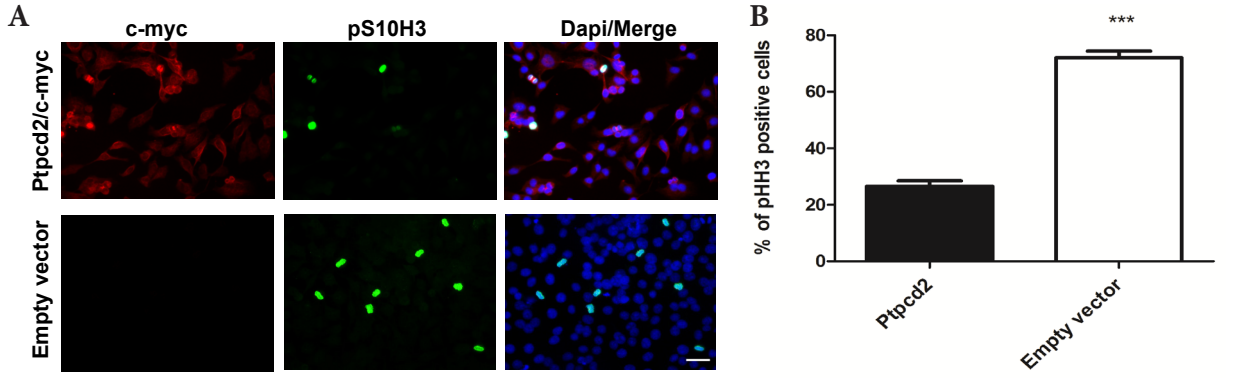

Figure 4. Overexpression of *Ptpcd2* Induced Mitotic Exit. (A) HeLa cells were co-transfected for 48h with Ptpcd-2/myc or mock control and immunostained for Ptpcd-2 myc (red), S10-pHH3 (Mitosis marker) (green). Nuclei visualized with DAPI (blue). Scale bars; 10μm. (B) Graph represents mean±SD percentage of mitotic cells with positive mitotic S10-pHH3. HeLa cells were transfected and immunostained as in (A) from 3 independent experiments and the data were presented as a percentage of total cells (n>100). P<0.0001; by student unpaired t-test. (***); indicates statistical significance

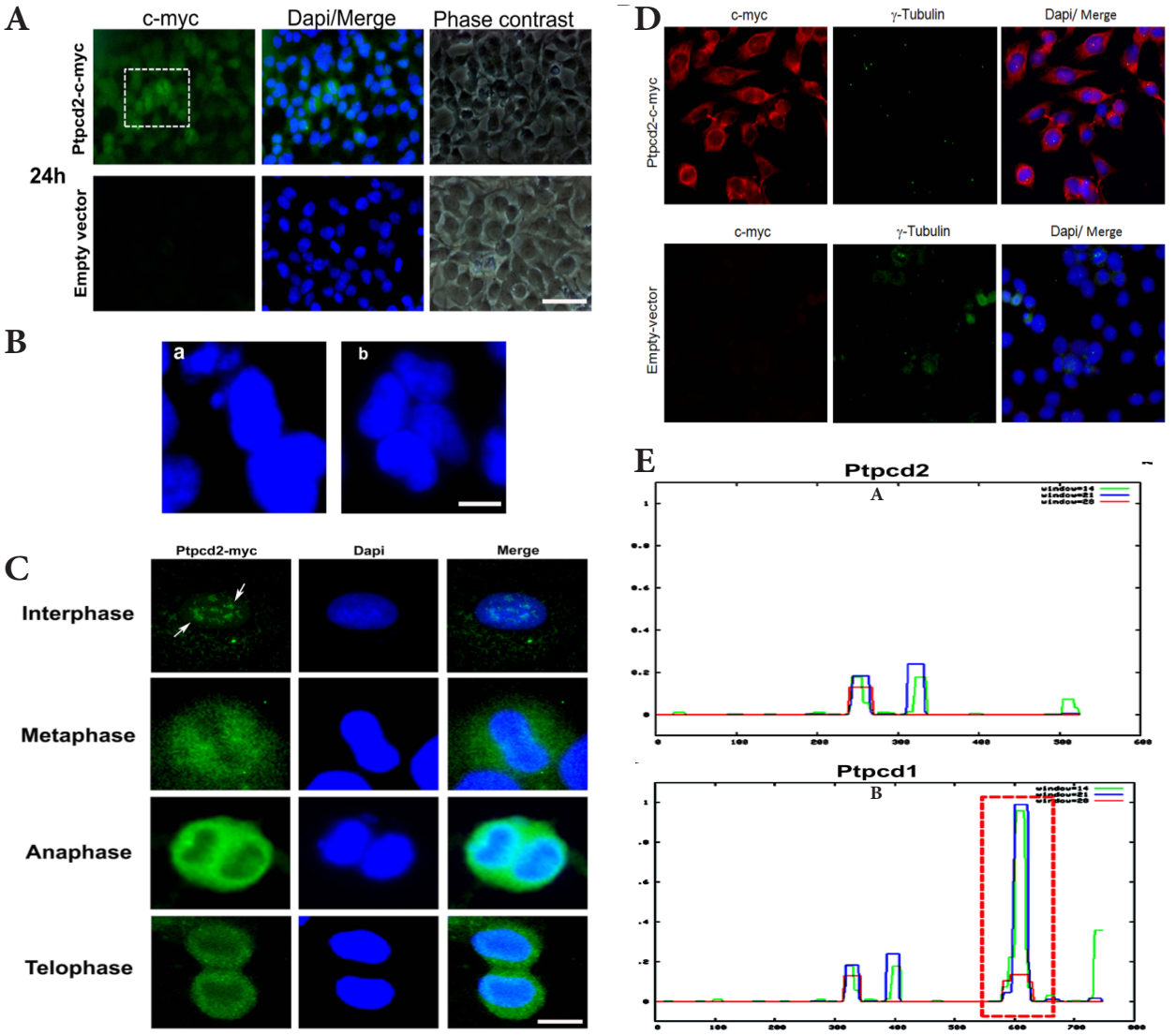

Figure 5. *Ptpcd2* Spatiotemporal Regulation Throughout the Cell Cycle. (A) Immunofluorescence microscopy pictures from HeLa cells grown on glass bottomed dishes 24h post-transfection with *Ptpcd2*/myc or Mock then processed for staining with anti-c-myc (green). Nuclei visualized with Dapi; white dotted box encircles metaphase cells. Scale bar; 100μm. (B) Dapi stained nuclei from (A) showing micronuclei (a) and multinucleation (b); Scale bar 5μm. (C) HeLa cells grown on glass coverslips transfected as in (A) and stained with c-myc (green), DNA was stained with DAPI (blue) Scale bar; 5μm. Pictures from interphase and different phases of mitosis are indicated. (D) Immunofluorescence micrographs of HeLa cells transfected as in (A) and immunostained with anti- c-myc monoclonal antibody (red) and anti- γ tubulin polyclonal antibody (green). Nuclei were visualized with Dapi (blue). Scal bar; 10μm. (E) *Ptpcd2* sequence analysis for coiled coils domains, *Ptpcd2* (a) and Ptpcd1 (b) sequences were analyzed by the coiled coil database; http://embnet.vital-it.ch/software/COILS_form.html (see methods section) and exported as histogram (green curves showed the highest coiled detected), red dotted box encircles the coiled structures

(SIRT2), a human class III histone deacetylase which is phosphorylated by Cdk1 during mitosis and was reported to be dephosphorylated by Cdc14 A and B during mitosis (North and Verdin, 2007). Interestingly, overexpression of *Ptpcd2* resulted in a decreased protein level of the mitotic SIRT2. (Figure 2B). To examine the effect of *Ptpcd2* overexpression on cell cycle progression, we performed flow cytometric analysis of HeLa cells expressing either *Ptpcd2* or control vector 48h after transfection. The majority of *Ptpcd2* expressing cells accumulated at G1 phase with few >4N cells (Figure 3A-a), probably due to endoreplication compared to mock transfectants (Figure 3 A(b) and A(c)).

The percentage of G1 and >4N *Ptpcd2* expressing cells was significantly higher (73%±1.53 and 2.6±1.2) when compared to mock cells (55.3±1.33 and 0.1±0.1), (P= 0.001 and 0.0026, respectively). On the other hand, mock transfected cells showed higher percentage of S phase and G2/M cells (10.3±1.2 and 34.2±0.4) when compared to *Ptpcd2* expressing cells (6.47±0.29 and 17.9±0.97), (P=0.0353 and >0.0001, respectively) (Figure 3B). Furthermore, immunofluorescence staining with the mitotic marker serine-10 phosophohistone-H3 (S10-pHH3) (Figure 4A) revealed that the percentage of mitotic cells expressing *Ptpcd2* had decreased S10-pHH3 immunoreactivity when compared to mock transfectants (P<0.0001) (Figure 4 B). Collectively, these data suggest a role of *Ptpcd2* in mitotic exit.

Ptpcd2 spatiotemporal regulation throughout the cell cycle

We then determined the subcellular localization of *Ptpcd2* by immunofluorescence analysis of HeLa cells expressing *Ptpcd2* that revealed a cell cycle dependent spatiotemporal localization (Figure 5C). *Ptpcd2* intensity was maximal during mitosis particularly in metaphase cells (Figure 5A, C) with some cells exhibiting micronuclei and multinucleation (Figure 5B a, b respectively) that may be due to mitotic slippage. *Ptpcd2* signal was decreased in cytoplasm of interphase cells (Figure 5C) and contained in the nuclei in the form of dots like signals (Figure 5C, white arrows), with onset of mitosis, *Ptpcd2* signal was mainly detected in the cytoplasm of metaphase, anaphase and telophase cells (Figure 5C). The observed immunostaining pattern of *Ptpcd2* characterizes it as a cell cycle regulated kinase with possible mitotic functions.

Ptpcd2 is a non centrosomal variant of Ptpcd1

Ptpcd1 was previously identified as a centrosomal phosphatase that controls proper centrosomal duplication during S phase and cytokinesis (Zineldeen et al., 2009). To investigate whether *Ptpcd2* has a similar centrosomal function, HeLa cells expressing *Ptpcd2*/Myc were double immunostained with c-myc antibody and an antibody that recognizes γ-tubulin, a known centrosomal marker (Zineldeen et al., 2009). Interestingly, *Ptpcd2* immunoreactivity was mainly cytoplasmic with small scattered nuclear dots (Figure 5D upper panel). Co-localization of *Ptpcd2* was not detected with γ-tubulin, suggesting that *Ptpcd2* is a non-centrosomal protein. We confirmed our results by bioinformatic analysis of the C-terminal part of *Ptpcd2* using the algorithm by Lupas et al. (1991) that showed lack of the coiled structures which are required for anchoring proteins to the centrosome (Figure 5E, a). In contrast, centrosomal Ptpcd1 exhibited multiple coiled structures at its C-terminal (Figure 5E, b). Taken together, our current data identify *Ptpcd2* as a non-centrosomal protein.

Discussion

Targeting mitotic exit is of great hope for anti-cancer modalities (Chan et al., 2012; Russell et al., 2012; Hunt, 2013). Hence, mammalian mitotic exit remained largely elusive, the present study characterizes *Ptpcd2* as a new member of the family of DSPs (Patterson et al., 2009) with possible function during mitotic exit. It shares homology to yeast and human Cdc14 and calcineurin; a phosphatase that has been reported to control meiotic M phase exit in xenopus (Mochida and Hunt, 2007) and completion of mitosis in drosophila (Takeo et al., 2010). Although mitotic functions of mammalian Cdc14B has been described (Cho et al., 2005; Wu et al., 2008), Cdc14B deficient cells were viable and lacked apparent mitotic defects (Mocciaro and Schiebel, 2010; Bremmer et al., 2012), suggesting that alternative phosphatase (s) is being able to complement mitotic functions of Cdc14B.

In the current study *Ptpcd2* is sequestered in the nucleus during interphase and is exported to the cytoplasm with mitotic onset and almost detected in all cellular compartments, with enrichment at cytosolic fraction and insoluble nuclear pellet. The nuclear pellet contains, in addition to nuclei, mitochondria and sheets of plasma membrane (Graham, 2002).

These data concluded that *Ptpcd2* is enriched in various cellular compartments that might be subjected to regulation by nucleocytoplasmic shuttling as predicted from its sequence analysis. Similarly, Cdc14B has been shown to be sequestered in the nucleolus then released in to the cytoplasm to induce mitotic exit (Wei and Zhang, 2011). Our identified *Ptpcd2*, thus strengthens the notion that highly structured intra-nuclear phosphatase (s) network might exist in mammalian cells. In accordance, previous report in living cells had revealed tight association of human Cdc14B with long filaments starting from nucleolar periphery to the nuclear envelope, and making connections with the nuclear pore complexes (Nalepa and Harper, 2004). In the present study we describe *Ptpcd2* as a novel non-centrosomal isozyme of Ptpcd1 (Zineldeen et al., 2009) that does not associate with centrosomes, and is sequestered in the nucleus during interphase and is exported to the cytoplasm with mitotic onset conveying a cell cycle dependent nucleocytoplasmic shuttle. Unscheduled overexpression of *Ptpcd2* induces exit into the next G1 phase with some cells showing multinucleation that may be due to mitotic slippage, where cells exit mitosis in a tetraploid state despite an active mitotic checkpoint (Janssen and Medema, 2011). Similarly, mitotic slippage in budding yeast was in part mediated by Cdc14p (Rossio et al., 2010). Forced *Ptpcd2* overexpression in HeLa cells is associated with micronuclei formation (Figure 5 B, a) indicating that some

cells exit mitosis in a tetraploid stat and are subjected to mitotic catastrophe that is characterized micronuclei (Galán-Malo et al., 2012). Targeting *Ptpcd2* thus could be of great help in combination with microtubule poisons and other anti-mitotic drugs to delay mitosis and induce apoptosis and tumor death. In line with this notion, suppression of ser/thr phosphatase 4 induced tetraploid cell death (Theobald et al., 2013). Moreover, inhibition of mitotic exit has led to tumor regression in conditional Cdc20 null mouse model (Manchado et al., 2010).

In the current work, *Ptpcd2* overexpression associates with altered cell cycle key players with decrease of Cdk1 phosphorylation of at tyrosine residue 15 (cdc2, Y15), and lowered protein levels of SIRT2, which could be due to *Ptpcd2* mediated proteasomal degradation of SIRT2 via its RXXL motif.

SIRT2 has been shown to be overexpressed in a subset of tumors like hepatocellular carcinoma and squamous cell carcinoma (Chen et al., 2013; Lai et al., 2013). In line with our findings, SIRT2 function has been described to be modulated by Cdc14B during mitotic exit, where Cdc14B overexpression induced dephosphorylation and decreased protein levels of SIRT2 (Dryden et al., 2003). SIRT2 has been shown to be phosphorylated by Cdk1 during mitosis and its overexpression causes delay of cells to exit mitosis (North and Verdin, 2007). The fact that *Ptpcd2* overexpression, caused loss of SIRT2 protein suggests that there is a functional link between *Ptpcd2* phosphatase activity, SIRT2 phosphorylation, and SIRT2 abundance, consistent with a role for these proteins in mitosis. Although Cdk1 activity was not completely abolished in cells overexpressing *Ptpcd2*, this could be attributed to a low Cdk1-CycB activity at G1 phase, after exiting from mitosis (Novak et al., 2007). Furthermore, we noted that the percentage of mitotic cells expressing *Ptpcd2* has decreased S10-pHH3 immunoreactivity, which could be due to mitotic exit of greater proportion of cells or due to unknown functions of *Ptpcd2*. Specifically, bioinformatic analysis of *Ptpcd2* sequence identifies two STAT5 Src Homology 2 (SH2) domains as well as PDZ protein-protein interaction binding domain; both are implicated in signal transduction cascades and tumor suppression by some phosphatases (van den Berk et al., 2005; Bard-Chapeau et al., 2011).

This could define *Ptpcd2* as an unidentified tumor suppressor phosphatase, which might be mutated in a subset of cancer cells. Interestingly, Cdc14 B has been proved to be of oncogenic potential which is an unusual feature for mammalian phosphatases (Chiesa et al., 2011; Wei and Zhang, 2011). Furtherly, Cdc14 down regulation has been proved to suppress glioblastoma growth (Galeano et al., 2013). An emerging role of phosphatases in cancer development has been recently highlighted (Zhang and Claret, 2012), that drives us to unravel new phosphatases and their role in cell cycle control and tumorgenesis. Conclusively, *Ptpcd2* is a novel cell cycle related non-centrosomal isozyme of Ptpcd1 with functional homology to Cdc14.

It is implicated in mitotic exit and mitotic slippage. *Ptpcd2* nuclear localization during interphase, may specifically affect transcriptional regulation, although this hypothesis deserve further investigations. Insights into substrate specificity of *Ptpcd2* are needed to explore its role in cell cycle, where targeting mitotic exit in malignant cells could open new avenues for anti-cancer therapeutics.

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