Biochemical and Biophysical Research Communications 452 (2014) 42–47

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Leucine methylation of protein phosphatase PP4C at C-terminal is critical for its cellular functions

JungJin Lee, Dong-Hyun Lee *

Department of Biological Sciences, College of Science, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 22 July 2014
Available online 15 August 2014

Keywords:
DNA damage response
Double-strand DNA breaks repair
Protein phosphatase 4
Leucine methylation
Homologous recombination
Non-homologous end joining

ABSTRACT

Background: Protein phosphatase 4 (PP4) has been known to have critical functions in DNA double strand break (DSB) repair and cell cycle by the regulation of phosphorylation of its target proteins, such as H2AX, RPA2, KAP-1, 53BP1. However, it is largely unknown how PP4 itself is regulated.
Methods: We examined the PP4C methylation on L307 at C-terminal by using methylated-leucine specific antibody. Then with PP4C L307A mutant, we explored that how nonmethylated form of PP4C affects its known cellular functions by immunoprecipitation, immunofluorescence, and DNA DSB repair assays.
Results: Here we show that PP4C is methylated on its C-terminal leucine residue *in vivo* and this methylation is important for cellular functions mediated by PP4. In the cells PP4C L307A mutant has significantly low activity of dephosphorylation against its known target proteins, and the loss of interaction between L307A PP4 mutant and regulatory subunits, R1, R2, or R3α/β causes the dissociation from its target proteins. Moreover, PP4C L307A mutant loses its role in both DSB repair pathways, HR (homologous recombination) and NHEJ (non-homologous end joining), which phenocopies PP4C depletion.
Conclusion: Our results demonstrate the key site of PP4C methylation and establish the physiological importance of this regulation.

1. Introduction

DNA double-stranded breaks (DSBs) or replication stress is hazardous due to its ability to promote both genomic instability and cellular transformation. To prevent such detrimental consequences, organisms have evolved a sophisticated response system, so called DNA Damage Response (DDR), which is generally initiated by the phosphatidylinositol-3 (PI3) kinase-like family of protein kinases. In mammalian cells, these kinases phosphorylate about 700 proteins in response to exogenous DNA damage [1,2]. The phosphorylated proteins include factors involved in DNA replication and repair, apoptosis and/or cell cycle progression. The functional consequences of phosphorylation have been studied for only a small subset of these factors, and in many cases these modifications impact upon the DDR. There is increasing evidence that protein phosphatases play an important role in the DSB induced signaling cascade [3]. Two recent studies investigating the dynamics of phosphorylation following induction of DSBs show that over one-third of the captured phospho-peptides were dephosphorylated within minutes of DNA damage [4,5]. These data suggest that phosphatases not only play a role in counteracting the DSB-induced phosphorylation later in the damage response but also play a primary role in initiating the repair process. Interestingly, this suggestion is consistent with our recent studies showing that PP4 dephosphorylates the essential replication protein A (RPA), KAP-1, and 53BP1 after DNA damage and that these dephosphorylation events are critical for efficient repair of DSBs [6–8].

PP4C has 65% homology with protein phosphatase 2AC (PP2AC) and functions in complex with regulatory subunits (PP4R1, PP4R2, PP4R3α, PP4R3β, and PP4R4) that are distinct from PP2A [9–13]. It was reported that PP2AC was methylated on its C-terminal leucine residue (Leu^{309}). This modification leads to increase of PP2A activity and has indispensable role in modulating phosphatase function [14–22]. Due to high sequence homology between PP2AC and PP4C especially at C-terminal, we hypothesized that PP4C also can be methylated on its last leucine residue (Leu^{307}), which might impact its cellular functions. Here we elucidated the importance of the PP4C methylation and showed that PP4C methylation is critical for interactions with regulatory subunits and its target proteins as well.

* Corresponding author. Fax: +82 625303409.
E-mail address: donghyunlee73@jnu.ac.kr (D.-H. Lee).

http://dx.doi.org/10.1016/j.bbrc.2014.08.045
0006-291X/

2. Materials and methods

2.1. Cell culture, antibodies, and reagents

HeLa S3, U2OS, HeLa-DR-GFP, and HeLa-EJ5-GFP cells were grown in DMEM supplemented with 10% (v/v) FBS. Antibodies used were against PP4R1 (Bethyl), PP4R2 (Bethyl), PP4R3α (Bethyl), PP4R3β (Bethyl), PP4C (Bethyl), Phospho-histone H3 (Ser10) (Cell Signaling), 53BP1 (Cell Signaling), pS1618-53BP1(Cell Signaling), KAP-1 (BD Transduction Laboratories), pS824-KAP-1 (Bethyl), Cyclin A (Santa Cruz), PP2AC methylated leucine309 (Covance), Flag-tag (Sigma) and α-tubulin (Sigma). Nocodazole, thymidine and camptothecin (CPT) were obtained from Sigma–Aldrich.

2.2. siRNAs and plasmids

Cells were transfected with siRNA duplexes (Invitrogen) using RNAi MAX (Invitrogen). The PP4C siRNAs were as follows: siRNA #1, sense 5′-CGCUAAGGCCAGAGAGAUCU UGGUA-3′, antisense 5′-UACCAAGAUCUCUCUGGC CUUAGCG-3; siRNA #2, sense 5′-GGA CAAUCGACCGAAAGCAAGAGGU-3′, antisense 5′-ACCUCUUGCUU UCGGU CGAUUGUCC-3′. The siRNA-resistant PP4C construct was made by mutating the siRNA target site to 5′-CGCGAATGCAAGCG AGATCTTGGTA-3′. PP4C mutants were constructed by QuikChange II XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. Primers used were the following:Y305E-F, 5′-CCGCTCACAG GAACTCGTCGGCCACGGGC-3′; Y305E-R, 5′-GCCCGTGGCCGACGAGTTCCTGTG AGCGG-3′; L307A-F, 5′-TGCGGCCGCTCACGCGAAGTAGTCGGCC-3′; L307A-R, 5′-GGCCGA CTACTTCGCGTGAGCGGCCGCA-3′; PP4C siRNA-resistant-F, 5′-TGT GCGC GAATGCAAGCGAGATCTTGGTAGAGGA-3′; PP4C siRNA-resistant-R, 5′-TCCTCTAC CAAGATCTCGCTTGCATTCGCGCACA-3′. To replace endogenous PP4C with WT or mutant, cells were transfected with siRNA-resistant PP4C plasmids using Lipofectamine 2000, and after 30 h cells were reverse transfected with PP4C siRNA by RNAi MAX for 48 h.

2.3. Co-immunoprecipitation

HeLa S3, expressing FH-PP4C WT or mutants, were lysed in buffer containing 50 mM Tris–HCl, pH 7.5, 250 mM NaCl, 5 mM EDTA, 0.5% (v/v) NP-40 and protease inhibitor cocktail (Roche). Anti-Flag-agarose (Sigma) were incubated with lysate at 4 °C for 16 h. Immunocomplexes were washed three times with buffer containing 50 mM Tris–HCl, pH 7.5, 250 mM NaCl, 5 mM EDTA and 0.5% (v/v) NP-40. The immunoprecipitated proteins were resolved by SDS–PAGE and analyzed by immunoblot.

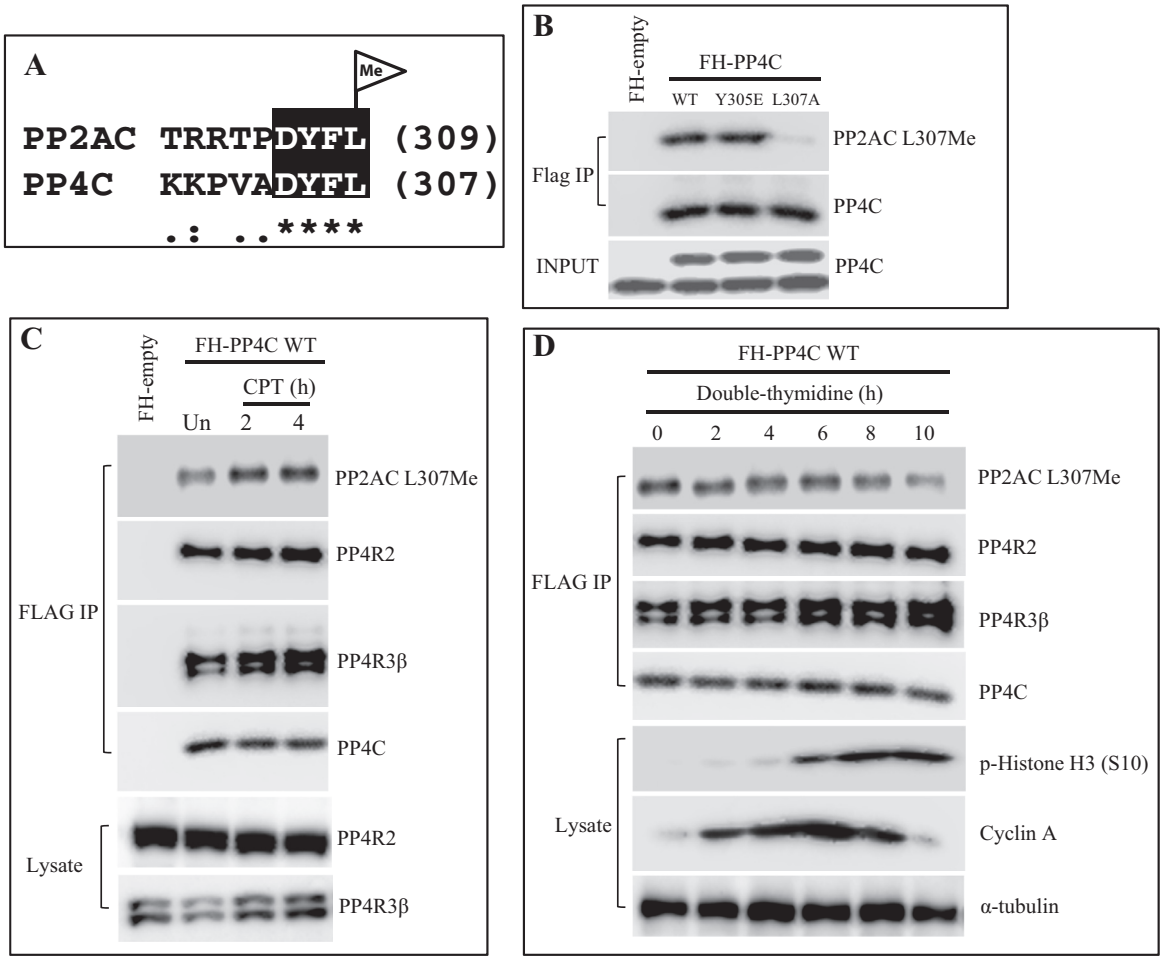

Fig. 1. PP4C is methylated on L307 *in vivo*. (A) Sequence comparison of amino acids adjacent to PP2AC L309 and PP4C L307 sequence in the C-terminal region. Sequence alignment was performed with ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2). (B) PP4C on L307 was methylated. PP4C WT or mutants were immunoprecipitated with flag antibody from HeLa S3 cells, and its methylation was detected by methylated-leucine^{309} PP2AC monoclonal antibody. (C and D) PP4C was constitutively methylated. Methylation on L307 of PP4C did not show any significant changes after DNA damage or through the cell cycle. HeLa S3, stably expressing FH-PP4C, was treated either with camptothecin (CPT) or double-thymidine. Then cell lysate was pulled down by flag antibody and immunoblotted with methylated-leucine^{309} PP2AC monoclonal antibody. Antibodies against phospho-Histone H3 (S10) or Cyclin A were used as cell cycle controls.

2.4. DNA DSB repair assay

DSB repair assays were as described [23]. Briefly, 1×10^5 of HeLa cells with a single, stably integrated copy of the transgenic reporter DR-GFP or EJ5-GFP plated overnight in 12-well plates were first transfected with PP4C siRNA and siRNA-resistant PP4C plasmid. After 24 h, cells were transfected with 0.8 μg of I-SceI expression plasmid (pCBASce) using Lipofectamine 2000. Two days later, GFP-positive cells were assayed by FACScan.

2.5. Immunofluorescence

Cells plated on glass slides were fixed for 10 min with fixative (3% (w/v) PFA, 2% (w/v) sucrose and 1X PBS) and permeabilized for 1 min with 0.2% (v/v) Triton X-100 in PBS. Cells were rinsed with PBS and incubated with primary antibody diluted in PBS with 2% (w/v) FBS for 1 h at room temperature (RT). Cells were washed three times, incubated with secondary antibody (diluted in PBS with 2% (w/v) BSA) for 30 min at RT in the dark, incubated with 4′,6-diamidino-2-phenylindole (DAPI) for 10 min and washed three times with PBS. Slides were mounted using DapiFuoromount-G (Southern Biotech) and visualized using a Zeiss Axioplan microscope. Secondary Alexa Fluor IgG antibodies used were as follows: 488 goat anti-rabbit, 594 goat anti-rabbit (Invitrogen).

3. Results

3.1. PP4C is methylated on L307 at C-terminal in vivo

PP2AC, having high sequence homology with PP4C, was methylated on leucine residue at C-terminal, which is critical for its interaction with regulatory B subunits and cellular functions as well [16,19,20,22]. Therefore, it was plausible that PP4C might have the same event happening on its leucine residue on C-terminal. Sequence comparison revealed that the residues adjacent to the last amino acid leucine 309 of PP2AC resemble those around PP4C leucine 307, suggesting that it might be the methylated site recognized by the anti-PP2AC-methylated-L309 antibody (Fig. 1A). We examined the status of leucine methylation on L307 of PP4C. With HeLa S3 cells expressing FLAG and HA (FH)-tagged PP4C, immunoprecipitation was performed. We analyzed the methylation of PP4C by immunoprecipitation/immunoblot assays using lysate from HeLa S3 cells expressing FH-PP4C WT, or mutants (Y305E or L307A). The methylation of both PP4C WT and Y305E is detected by methylated-leucine specific antibody, but PP4C

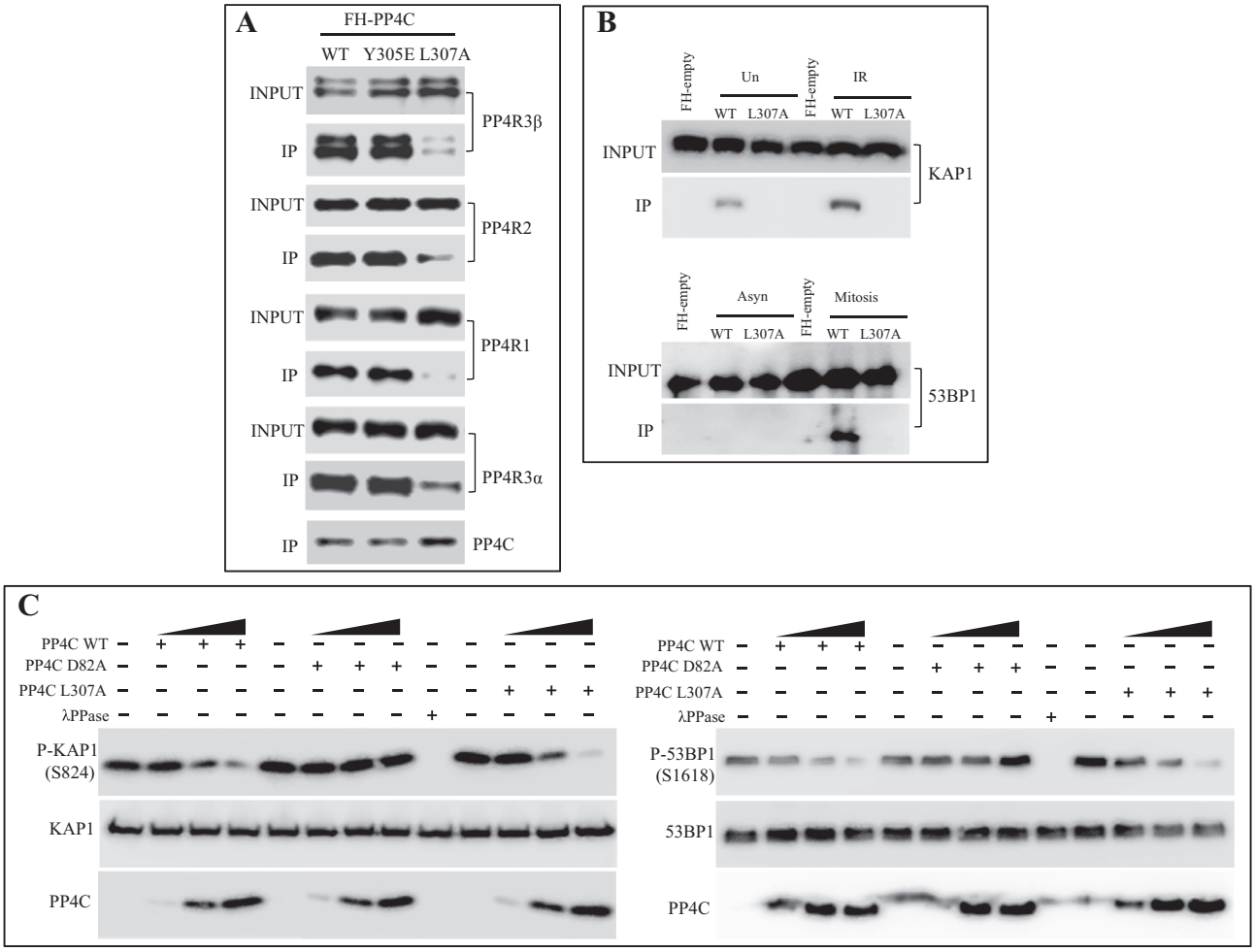

Fig. 2. PP4C methylation on leucine residue at C-terminal is required for its interaction with regulatory subunits and its targets. (A) Lysate from cells expressing FH-PP4C WT or mutants was immunoprecipitated by flag antibody and immunoblotted with PP4 regulatory subunits, PP4R1, PP4R2, PP4R3α, or PP4R3β. (B) FH-PP4C was pulled down from cells treated with IR for introduction of DSBs or nocodazole for mitotic arrest and immunoblotted with antibodies against its targets, KAP-1 or 53BP1. (C) Like PP4C WT, PP4C L307A has an ability to dephosphorylate its targets *in vitro*. PP4C WT and mutants (D82A or L307A) were purified using the baculoviral system and were serially diluted in the phosphatase reaction. λ phosphatase served as positive control for the reaction. Both PP4C WT and L307A dephosphorylate phospho-KAP1 on S824 and phospho-53BP1 on S1618 in a dose-dependent manner. Phosphatase reactions were probed with indicated antibodies.

L307A dramatically lost its ability of being methylated (Fig. 1B). Using cell lysates from the same cells which were treated with either CPT, a DNA damaging agent or double thymidine for G1 phase synchronization, we observed that the methylation of PP4C L307 is damage-independent and cell cycle-independent as well (Fig. 1C and D).

3.2. The loss of methylation on PP4C compromised the ability of interaction with regulatory subunits and subsequently with its known substrates

PP4C has four regulatory subunits (PP4R1, PP4R2, PP4R3α, PP4R3β, and PP4R4), each of which renders substrates specificity to PP4 complex. We suspected that methylation of PP4C is important for the interaction with regulatory subunits and its target proteins. To test this, we performed co-immunoprecipitation with HeLa S3 cells expressing FH-PP4C WT or two mutants (Y305E and L307A) and observed that the interaction of PP4C L307A, not Y305E or WT, with regulatory subunits is dramatically reduced in absence of DNA damage (Fig. 2A). Moreover, PP4C L307A was deprived of its ability to dephosphorylate target proteins, 53BP1 and KAP-1, due to loss of interaction with regulatory subunits (Fig. 2B).

3.3. PP4 L307A mutant can dephosphorylate its target proteins in vitro like wild type

In previous studies, we and other group showed that PP4C wild type, but not PP4C D82A, can dephosphorylate target proteins, which was demonstrated by *in vitro* dephosphorylation assay [6–8,24]. To test how efficiently PP4C L307A mutant dephosphorylates target proteins, such as phospho-KAP-1 and phospho-53BP1, we immunopurified endogenous phospho-53BP1 from mitotic cells or phospho-KAP-1 from CPT treated cells and performed dephosphorylation assays. We observed that PP4C L307A dephosphorylated both phospho-S1618 53BP1 and phospho-S824 KAP-1 in a dose-dependent manner (Fig. 2C). This tells us that PP4C L307A is catalytically active like PP4C WT and able to dephosphorylate its targets *in vitro*. Catalytically inactive PP4C D82A and λ phosphatase served as controls.

3.4. PP4C L307A mutant is deprived of the ability to repair DNA double-strand breaks in the cells

Previous studies showed that PP4 complex was important for both double-strand break repairs, HR and NHEJ, through the dephosphorylation of their target proteins following induction of DSB [6–8,24]. To test HR and NHEJ repair efficiencies by PP4 complex with PP4 L307A mutant, we expressed the rare-cutting I-Sce1 endonuclease in HeLa cells containing a single, stably integrated copy of the artificial recombination substrate DR-GFP with an I-Sce1 site (HR) or EJ5-GFP with two I-Sce1 sites (NHEJ). In case of NHEJ, an artificial start codon is integrated out of frame with original start codon in the middle of I-Sce1 sites, thus preventing GFP translations. These systems permit quantification of HR or NHEJ efficiency through assay of the fraction of cells expressing GFP [23]. We confirmed our and other earlier observation that the both HR and NHEJ are significantly reduced in PP4C-silenced cells. To address whether the leucine methylation of PP4C on C-terminal had any impact on the efficiency of DSB-induced HR and NHEJ, we expressed I-Sce1 in cells replaced with PP4C L307A. Cells expressing PP4C L307A had a significantly lower HR

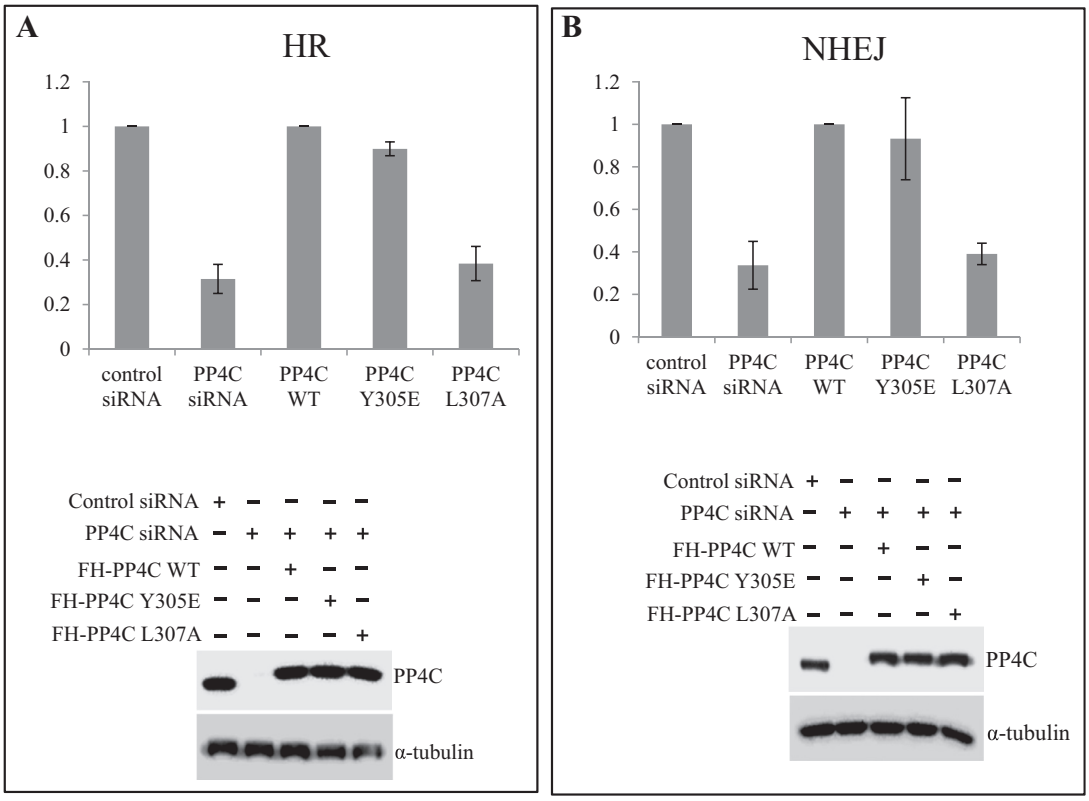

Fig. 3. Measurement of HR- and NHEJ-mediated repair of an I-Sce1-induced DSB. HeLa cells carrying a single copy of the recombination substrate were transfected with control siRNA or PP4C siRNA. Endogenous PP4C was silenced in HeLa cells with siRNA and replaced with siRNA-resistant PP4C constructs (WT, Y305E, or L307A). At 48 h after transfection, the I-Sce1 expression plasmid was transfected, and green fluorescent protein-positive cells were measured by flow cytometry. Immunoblots performed to confirm siRNA efficiency and expression of siRNA-resistant constructs are shown.

and NHEJ efficiency than cells expressing PP4C WT, which is comparable to PP4C depleted cells (Fig. 3A and B).

To further confirm the impact of PP4C L307A on DSB repair, we measured the foci formation of known PP4 target proteins after ionizing radiation (IR). Recently, we showed that the dephosphorylation of 53BP1 on T1609/S1618 mediated by PP4C/R3β prior to G1 phase is necessary for its recruitment to DSBs in G1 cells, which is a critical step for DNA damage response [7]. We also discovered that when PP4R3β was depleted, both dephosphorylation of 53BP1 on Thr 1609/Ser1618 and its recruitment to DSB sites (foci formation) failed in the cells. Therefore, the interaction of PP4C with PP4R3β, one of regulatory subunits of PP4, is critical for 53BP1 dephosphorylation in the cells [7]. To test the impact of PP4C L307A on 53BP1 recruitment in response to IR in G1 phase, we evaluated the localization of 53BP1 in the G1 phase of synchronized cells at various time points after IR. There is a striking reduction of 53BP1 foci formation when the cells were replaced with PP4C L307A in G1 phase, which is comparable to PP4C depletion (Fig. 4A). This is most likely because PP4C L307A is not able to interact with PP4R3β in the cells. As shown in a previous study [8], KAP-I is rapidly phosphorylated on Ser 824 by ATM kinase in response to IR, which peaks within 2 h and significantly drops by 4 h. PP4R3β interacts with KAP-1, but in absence of PP4R3β or PP4C there is significantly higher amount of pS824-KAP-1 in cells even after 8 h. We reasoned that PP4C L307A also impacts on dephosphorylation of KAP-1. To test this, using cells replaced with PP4C L307A we evaluated levels of phosphorylated KAP-1 at various time points after IR. We found that in cells expressing PP4C L307A there is a significantly higher amount of pS824-KAP-1 in cells even 8hr after IR, which is similar to that in PP4C depleted cells (Fig. 4B).

In conclusion, *in vivo* condition PP4C L307A, nonmethylated form of PP4C, phenocopies PP4C depletion due to loss of interaction with its regulatory subunits.

4. Discussion

The PP2A-like phosphatase family comprises PP2A, PP4 and PP6. The members have significant homology in their primary amino acid sequence and comparable sensitivity to chemical inhibitors, including okadaic acid. Each phosphatase-catalytic subunit forms a multimeric complex with regulatory and scaffolding subunits and these interactions are crucial for substrate specificity and functions [3,25]. There are well-established research data that PP2AC, a catalytic subunit of PP2 complex, is methylated on its C-terminal leucine residue (Leu^{309}), which is important for its functions [14–22]. Since PP4C has high sequence homology with PP2AC, especially in C-terminal including the last leucine residue, we hypothesized that PP4C was also methylated on leucine at

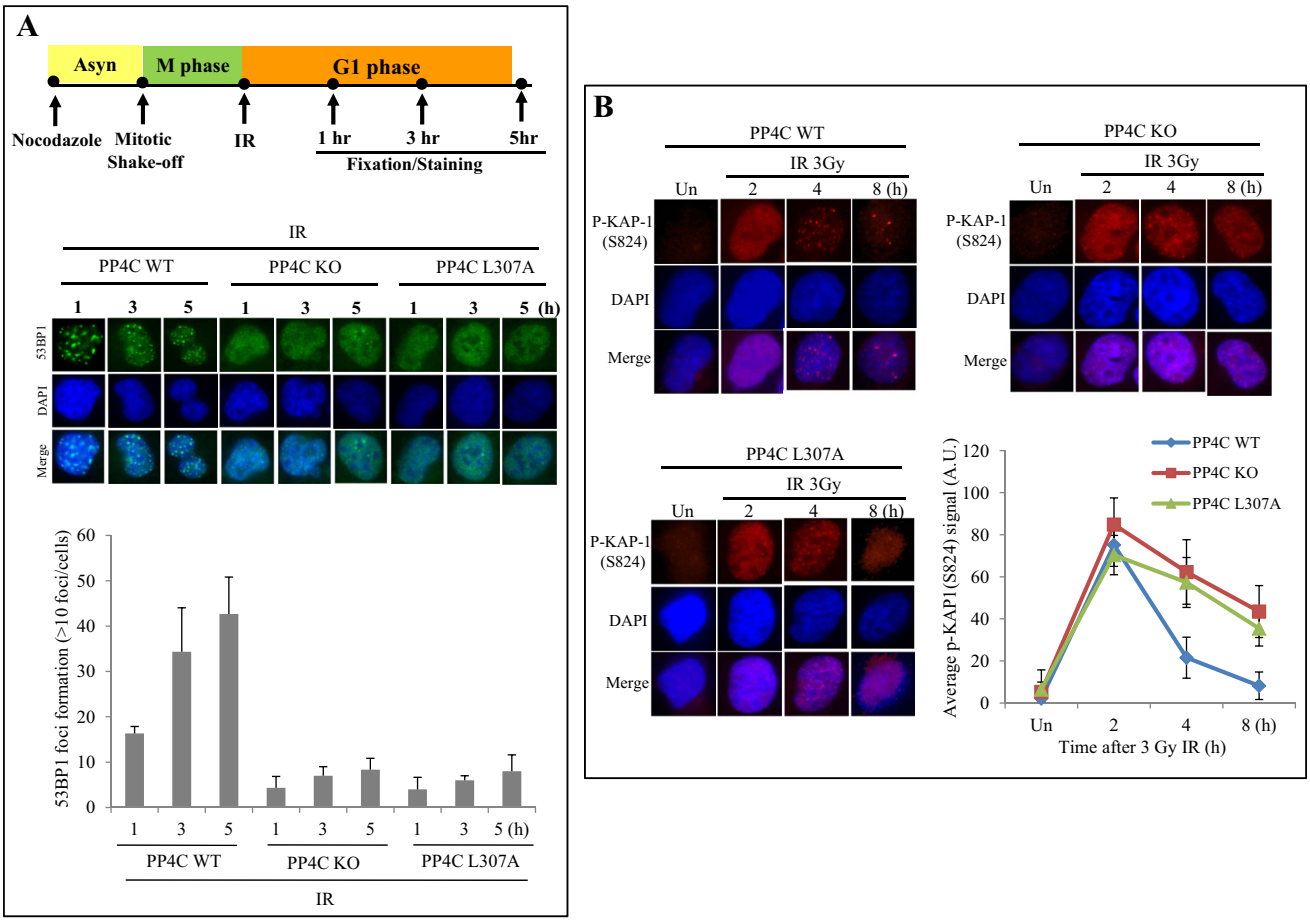

Fig. 4. Nonmethylated form of PP4C, PP4C L307A, impedes DSB repair. (A) In cells, expressing PP4C L307A, 53BP1 is not recruited to DSBs. (Upper left panel) Schematic to study 53BP1 focal recruitment. (Lower panel) U2OS cells expressing full-length FH-PP4C (WT or L309A) or depleted of PP4C by siRNA were prepared for immunofluorescence after mitotic shake-off and 10 Gy IR. 53BP1 foci were visualized using anti-53BP1 antibody. Quantification shows percent 53BP1-positive cells in G1. Cells displaying >10 foci were counted as positive. The data are expressed as mean ± SD; $n = 3$; >100 cells quantified (Upper right panel). (B) PP4C L307A mutant attenuates pS824-KAP-1 turnover after IR. U2OS cells were transfected with FH-PP4C (WT or L307A) or PP4C siRNAs. After 72 h, cells were irradiated, fixed at the indicated times and immunostained for pS824-KAP-1. Lower left panel: The average pS824-KAP-1 signal intensity per nucleus was quantified using ImageJ software. Data represent average and ± SD; $n = 3$; >100 cells quantified.

C-terminal affecting its cellular functions. With an antibody detecting methylated-leucine reside, we figured out that PP4C was also methylated at C-terminal leucine residue (Leu^{307}) constitutively. Nonmethylated form of PP4C (L307A) has significant loss of the interaction with its regulatory subunits and known target proteins as well. Recently, a couple of research groups reported that PP4 complex has significant roles in DNA double-strand break repair through the dephosphorylation of H2AX, KAP-1, RPA2, or 53BP1. We monitored the efficiency of DSB repairs by PP4C L307A mutant and discovered that PP4C L307A failed to dephosphorylate its target proteins, KAP-1 and 53BP1 after IR. Moreover, cells expressing PP4C L307A had significantly low efficiency of both HR and NHEJ.

In conclusion, PP4C is methylated at C-terminal on leucine, which is significantly important and nonmethylated form of PP4C phenocopies PP4C depletion in cells. At first, we demonstrated that the post-translational modification of PP4C and its a critical role. As mentioned, PP6 also has significant homology with PP2AC. Therefore, it is feasible PP6C may be methylated on its C-terminal leucine residue (Leu^{342}), implying a common phenomenon in PP2A-like phosphatase. Future studies will be followed.

Acknowledgments

This study was supported by grants from Basic Science Research Program through the National Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2013R1A1A1061207), and Chonnam National University, 2013.

References

[1] S. Matsuoka et al., ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage, Science 316 (2007) 1160–1166.
[2] J.J. Mu et al., A proteomic analysis of ataxia telangiectasia mutated(ATM)/ATM-Rad3-related (ATR) substrates identifies the ubiquitin–proteasome system as a regulator for DNA damage checkpoints, J. Biol. Chem. 282 (2007) 17330–17334.
[3] D.H. Lee, D. Chowdhury, What goes on must come off: phosphatases gate-crash the DNA damage response, Trends Biochem. Sci. 36 (2011) 569–577.
[4] M.V. Bennetzen et al., Site-specific phosphorylation dynamics of the nuclear proteome during the DNA damage response, Mol. Cell. Proteomics 9 (2010) 1314–1323.
[5] A. Bensimon et al., ATM-dependent and-independent dynamics of the nuclear phosphoproteome after DNA damage, Sci. Signal. 3 (2010). rs3.
[6] D.H. Lee et al., A PP4 phosphatase complex dephosphorylates RPA2 to facilitate DNA repair via homologous recombination, Nat. Struct. Mol. Biol. 17 (2010) 365–372.
[7] D.H. Lee et al., Dephosphorylation enables the recruitment of 53BP1 to double-strand DNA breaks, Mol. Cell 54 (2014) 512–525.
[8] D.H. Lee et al., Phosphoproteomic analysis reveals that PP4 dephosphorylates KAP-1 impacting the DNA damage response, EMBO J. 31 (2012) 2403–2415.
[9] G.I. Chen et al., PP4R4/KIAA1622 forms a novel stable cytosolic complex with phosphoprotein phosphatase 4, J. Biol. Chem. 283 (2008) 29273–29284.
[10] P.T. Cohen et al., Protein phosphatase 4—from obscurity to vital functions, FEBS Lett. 579 (2005) 3278–3286.
[11] M. Mourtada-Maarabouni, G.T. Williams, Protein phosphatase 4 regulates apoptosis, proliferation and mutation rate of human cells, Biochim. Biophys. Acta 1783 (2008) 1490–1502.
[12] M. Mourtada-Maarabouni, G.T. Williams, Protein phosphatase 4 regulates apoptosis leukemic and primary human T-cells, Leuk. Res. 33 (2009) 1539–1551.
[13] X. Zhang et al., Histone deacetylase 3 (HDAC3) activity is regulated by interaction with protein serine/threonine phosphatase4, Genes Dev. 19 (2005) 827–839.
[14] V. Stanevich et al., Mechanisms of the scaffold subunit in facilitating protein phosphatase 2A methylation, PLoS One 9 (2014) e86955.
[15] J.M. Sontag et al., Regulation of protein phosphatase 2A methylation by LCMT1 and PME-1 plays a critical role in differentiation of neuroblastoma cells, J. Neurochem. 115 (2010) 1455–1465.
[16] T. Ikehara et al., Methylation of the C-terminal leucine residue of the PP2A catalytic subunit is unnecessary for the catalytic activity and the binding of regulatory subunit (PR55/B), Biochem. Biophys. Res. Commun. 354 (2007) 1052–1057.
[17] E. Sontag et al., Downregulation of protein phosphatase 2A carboxyl methylation and methyltransferase may contribute to Alzheimer disease pathogenesis, J. Neuropathol. Exp. Neurol. 63 (2004) 1080–1091.
[18] N. Leulliot et al., Structure of protein phosphatase methyltransferase 1 (PPM1), a leucine carboxyl methyltransferase involved in the regulation of protein phosphatase 2A activity, J. Biol. Chem. 279 (2004) 8351–8358.
[19] X.X. Yu et al., Methylation of the protein phosphatase 2A catalytic subunit is essential for association of Balpha regulatory subunit but not SG2NA, striatin, or polyomavirus middle tumor antigen, Mol. Biol. Cell 12 (2001) 185–199.
[20] T. Tolstykh et al., Carboxyl methylation regulates phosphoprotein phosphatase 2A by controlling the association of regulatory B subunits, EMBO J. 19 (2000) 5682–5691.
[21] J. Wu et al., Carboxyl methylation of the phosphoprotein phosphatase 2A catalytic subunit promotes its functional association with regulatory subunits in vivo, EMBO J. 19 (2000) 5672–5681.
[22] J.C. Bryant et al., Methylated C-terminal leucine residue of PP2A catalytic subunit is important for binding of regulatory Balpha subunit, Biochem. J. 339 (1999) 241–246.
[23] D.M. Weinstock et al., Assaying double-strand break repair pathway choice in mammalian cells using a targeted endonuclease or the RAG recombinase, Methods Enzymol. 409 (2006) 524–540.
[24] J. Liu et al., Protein phosphatase PP4 is involved in NHEJ-mediated repair of DNA double-strand breaks, Cell Cycle 11 (2012) 2643–2649.
[25] R.E. Honkanen, T. Golden, Regulators of serine/threonine protein phosphatases at the dawn of a clinical era?, Curr Med. Chem. 9 (2002) 2055–2075.