Serine/threonine protein phosphatases in DNA damage response

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DNA damage response (DDR) is among the most important of the mechanisms that maintain genome stability which, when destabilized, predisposes organs to cancer. Reversible phosphorylation mediated by protein kinases and protein phosphatases regulates most, if not all, cellular activities, including DDR. Protein kinase inhibitors have become the main focus of targeted therapy and anticancer drug development. However, our limited knowledge of protein phosphatase function is compromising our capacity to develop therapeutic agents against phosphatases. In this review, we summarize the roles of serine/threonine protein phosphatases involved in DDR and propose that in situ dephosphorylation of phosphoproteins by protein phosphatases, instead of proteasome-mediated degradation of phosphoproteins, is mainly employed by cells.

reversible protein phosphorylation, protein phosphatase, DNA damage, cancer

The human genome is being constantly attacked by various DNA damaging agents. According to the source of the toxic agents, DNA damage can be categorized into 2 groups, endogenous (spontaneous) and exogenous (environmental) [1,2]. Endogenous DNA damage occurs at a high frequency within normal cells, mainly caused by by-products of metabolic and biochemical reactions, such as reactive oxygen species (ROS), reactive chemicals (e.g. aldehydes and S-adenosylmethionine). In most cases, these endogenous toxic agents result in the modification or hydrolysis of the base components of DNA, such as through oxidation, alkylation, deamination, depurination and depyrimidination. Mismatch is another kind of endogenous DNA damage occasionally introduced during DNA replication [3–5].

DNA damage caused by exogenous agents, such as ionizing radiation (IR) (ultraviolet (UV), X-rays, and γ-rays) and radiomimetic drugs is much more catastrophic than that caused by endogenous agents. UV radiation has deleterious effects in all living organisms, prokaryotic bacteria and eukaryotes, lower and higher plants, and animals including humans. UV radiation can be classified into 3 categories according to its wavelength or frequency: long wave UVA (315–400 nm), medium wave UVB (280–315 nm), and short wave UVC (100–280 nm) [6]. Most UV radiation (98.7%) is blocked by the Earth’s ozone layer when penetrating through the atmosphere, including most of UVB (95%) and all of UVC. Although UVA and UVB induce similar mutations in human skin cells [7], UVB is the primary cause of UV radiation damage, as it is directly absorbed by cellular DNA and an increasing amount reaches the Earth because of substantial damage to the protective ozone layer [6,8]. UVA causes less direct DNA damage because it is not absorbed by cellular DNA. Gamma rays and radiomimetic drugs are the most dangerous types of DNA damaging agents, which can cause lethal lesions, such as DNA double-strand breaks (DSBs), and they have been widely used to kill cancerous cells in cancer therapy. Radiomimetic drugs, such as camptothecin (CPT) and etoposide are both effective anticancer drugs, which specifically bind and inhibit the enzyme activity of topoisomerase I and II respectively, which unwind DNA during replication or transcription [9]. Adriamycin and bleomycin interact with DNA by intercalation and inhibit the macromolecular biosynthesis [10,11].
1 DNA damage response

One single cell may incur tens of thousands of DNA lesions daily. Fortunately, mammalian cells have developed evolutionarily conserved mechanisms to cope with genotoxic threats, which have been termed as the DNA damage response (DDR) (Figure 1). Upon DNA damage, cells detect the damaged DNA and relay the signal downstream to activate the cell cycle checkpoints to halt the cell cycle progression, allowing time for repairing the damage, or they tolerate the damage and continue replication, which may eventually cause mutations, or lead to apoptosis if the damage is too severe to be repaired [12–14]. Several conserved repair pathways responsible for eliminating specific types of lesions have been identified in mammalian cells. These include direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) repair, nonhomologous end joining (NHEJ) repair, fanconi anemia (FA) repair and translesion DNA synthesis (TLS) [2,15–18]. There is also considerable redundancy among these different pathways. A class of enzyme known as alkyltransferases can repair DNA base damage induced by alkylating agents by directly removing the alkyl group. Both BER and NER repair single-strand DNA damage. HR and NHEJ are mainly responsible for DSB repair [19–21].

DDR involves 3 main groups of evolutionarily conserved proteins that transduce the DNA damage signals and determine the cell fate (Figure 1). The first group comprises the DNA damage sensor proteins, such as ATM (ataxia telangiectasia mutated protein), ATR (ataxia telangiectasia and Rad3-related protein), and DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which belong to the phosphoinositide 3-kinase-related kinase (PIKK) family. It also includes their physical and functional partners, including MRE11-RAD50-NBS1, PARP-1 (poly(adenosine diphosphate ribose) polymerase 1), TIP60 (60 kD Tat-interactive protein), MDC1 (mediator of DNA damage checkpoint 1), ATRIP (ATR-interacting protein), Claspin, RAD17-RFC (replication factor C), Ku70/80, and Artemis. The second group comprises the transducer proteins, including checkpoint kinases CHK1 and CHK2 that relay the signals to the downstream effectors, which are the third group [22,23] and control the cell cycle progression, remodeling the chromatin or repairing the damage [13,24,25]. The DDR pathway is a cascade of reversible phosphorylation events of the sensors, transducers and effectors. Large-scale proteomic analysis of proteins phosphorylated in response to DNA damage (caused by ionizing radiation) on consensus sites recognized by ATM and ATR have identified more than 900 regulated phosphorylation sites encompassing over 700 proteins, most of which are involved in DDR [26].

For example, upon DSB formation, chromatin remodeling and protein and DNA modification result in dissociation of the sensor protein ATM, changing it from a dimer into a monomer and causing autophosphorylation on multiple residues required for its activation [27]. The activated ATM phosphorylates H2AX on serine (Ser) 139, located at the C-terminal, to form γH2AX. MDC1 directly binds to γH2AX through its C-terminal tandem BRCT domains (the C-terminal portion of the BRCA-1 gene) [28]. At the same time, MDC1 facilitates the transfer of the DNA damage signal through ATM-dependent phosphorylation of downstream targets on S/T-Q residues, generating a landing platform for RNF8 [29,30]. Furthermore, ATM activates CHK2 by phosphorylation of T68, and both kinases phosphorylate various substrates, such as p53 and CDC25, to activate the cell cycle checkpoints [31,32].

2 Protein phosphatases

Reversible protein phosphorylation, mediated by protein kinases and protein phosphatases, occurs on about one-third of all proteins in human cells, and controls almost every aspect of cellular activities, including DDR [33]. Disturbed protein phosphorylation has been identified in many human diseases, including cancer. Proteins, such as enzymes and receptors, are activated or inactivated by reversible phosphorylation, which results in a conformational change in the proteins structure.

Phosphorylation occurs predominantly on serine (Ser),
threonine (Thr) and tyrosine (Tyr) residues, with each accounting for approximately 86.4%, 11.8% and 1.8%, respectively, of the human phosphoproteome [34]. In addition, basic amino acid residues, such as histidine, arginine or lysine, in prokaryotic proteins can also be modified via phosphorylation [35]. The human genome encodes 518 protein kinases, 428 of which phosphorylate Ser or Thr residues, and the remaining 90 kinases belong to the Tyr kinase family [36–38]. By contrast, there are only about 147 protein phosphatase catalytic subunits identified in the human genome, with 107 serving as Thr phosphatases or dual-specificity phosphatases [38]. Surprisingly, the human genome only encodes 40 Ser/Thr phosphatases, whereas more than 98% of the phosphorylation occurs on Ser/Thr residues.

Phosphatases can be classified into 3 groups according to their sequence identity, structure, substrate specificity and catalytic mechanisms, namely Ser/Thr protein phosphatases, Tyr protein phosphatases, dual specificity protein phosphatases, and the FCP/SCP/HAD family. The FCP/SCP/HAD family uses an Asp-based catalysis mechanism with a DXDXT/V catalytic motif in which the first aspartate serves as a phosphoryl acceptor during the substrate dephosphorylation [39].

Ser/Thr protein phosphatases comprise the PPP (phosphoprotein phosphate) family and the PPM (protein phosphotyrosine phosphatase) family and are responsible for dephosphorylation of phosphoserines or phosphothreonines [40]. The catalytic subunit of the PPP family members itself does not have any enzymatic activity. It forms heterodimeric or heterotrimeric holoenzymatic complexes with regulatory/scaffolding subunits. These holoenzymes thus gain catalytic activity, specific subcellular localization, and specific substrate recognition. The PPP family is further divided into 3 subfamilies: PP1, PP2A and PP2B. PP4 and PP6 are closely related to PP2A, while PP7 was grouped into the PP2B subfamily because of its requirement for Ca2+ [41]. Most members of this family have roles in DDR [42]. The PPP members are catalytically Mg2+ or Mn2+ dependent, sharing structural similarity, however, there is no sequence homology with the PPP members at their active sites. The human genome encodes 18 PPM members [43]. The catalytic subunits of this family exhibit enzymatic activity without the requirement of additional regulatory subunits, however, their interacting partners may play a role in determining their subcellular localization and modulate their enzymatic activity. PPM1D or WIP1 (wild type p53-inducible phosphate 1) is a significant member of this family involved in DDR (Table 1).

To date, 107 protein Tyr phosphatases (PTPs) have been identified in the human genome, 106 of which have mouse orthologs, and only 81 of these are catalytically active. Compared with about 90 catalytically active Tyr kinases, it is reasonable to assume that the PTPs have comparable substrate specificities. PTPs have been found to play critical roles in human diseases, such as diabetes, Sézary syndrome, and Noonan syndrome, and at least 30 PTPs have been implicated in tumor growth [38]. PTPs are becoming a promising drug target in drug development [44].

We will summarize Ser/Thr protein phosphatases involved in DDR (Table 1) in the following sections.

### 2.1 PP1

Protein phosphatase 1 (PP1) is widely expressed in mammalian cells. It includes 3 highly-related isoforms, PP1α, PP1β, and PP1γ, encoded by separated genes [45]. These isoforms have more than 89% identity in amino acid sequence. PP1γ has two alternative splicing variants, PP1γ1 and PP1γ2 [46]. Localization patterns of PP1 isoforms are dynamic in a cell cycle-dependent manner. PP1α localizes to the nucleus during the interphase, while PP1β and PP1γ additionally accumulate in the nucleoli [47,48]. PP1 has been reported to interact with various subunits through its N terminus. Sequence analysis of all the interacting partners has revealed that most of these proteins contain a consensus PP1-binding motif: (R/K)×1(V/I)×2(F/W), where ×1 may be absent or any residue apart from the large hydrophobic residues, and ×2 denotes any amino acid except large hydrophobic residues, phosphoserine and probably aspartic

| Sub-family | Phosphatases | Catalytic subunits | Regulatory subunits | Involved in DDR | Identified substrates in DDR |
|------------|-------------|--------------------|---------------------|-----------------|-----------------------------|
| PPP        | PP1         | α, β, γ1, γ2       | >90                 | yes             | CDC25, CHK1, BRCA1, p53, KAP1, etc. |
|            | PP2A-like   | α, β               | B subunits          | yes             | γH2AX, p53, DNA-PKcs, Chk2, etc. |
|            | PP2A        |                     |                     |                 |                             |
|            | PP4         | PPP4C              | PP4R1, PP4R2, PP4R3α, PP4R3β, PP4R4 | yes | γH2AX, RPA2 |
|            | PP6         | PP6C               | PP6R1, PP6R2, PP6R3 | yes             | γH2AX |
|            | PP2B/PP3    | PPP3CA, PPP3CB, PPP3CC | unknown | – | – |
|            | PP5         | PP5                | –                   | yes             | – |
|            | PP7         | PP7                | –                   | unknown         | – |
|            | PPM/PP2C    | PPM1A, B, etc.     | –                   | PPM1D           | ATM, ATR, CHK1, CHK2, p53, γH2AX, etc. |
|            | (18 members)|                     |                     |                 |                             |
acid [49,50]. More than 180 conserved mammalian proteins have been identified as potential binding partners of PP1. PP1 exists in as many as 650 distinct complexes, which may contribute to its subcellular localizations, substrates specificity, and account for the roles of PP1 in the regulation of an enormous variety of cellular functions [50,51]. One prerequisite for activation of PP1 is the removal of the inhibitory phosphothreonine residue at the PP1 C-terminus, which is phosphorylated by cyclin-dependent kinases (CDKs) in a cell cycle-dependent manner [52].

The roles of PP1 in DDR have been reported in both DNA damage signaling and DNA repair. Upon DNA damage, PP1 is activated after ionizing radiation by removing the inhibitory phosphorylation sites in an ATM dependent manner [53]. PP1 also regulates the ATM activation by its the inhibitory phosphorylation sites in an ATM dependent manner [53,55,56]. The mechanisms underlying PP1 participation in DNA damage during interphase and mitosis have been elucidated by identification of several other PP1 substrates, such as CDC25, CHK1, BRCA1 and p53 [57–62]. CDC25, a crucial regulator of the G2/M transition, is a highly conserved dual-specific phosphatase and a key target of the checkpoint machinery that ensures genetic stability.

Dis2, the Schizosaccharomyces pombe homolog of PP1, is required for dephosphorylation and deactivation of CHK1 and recovery from DNA damage-induced G2 checkpoint arrest [59,60]. BRCA1 is also identified as a PP1 substrate during DNA damage recovery [61,63]. PP1α interacts with BRCA1 via its PP1-binding motif (898KVT6901) and mediates dephosphorylation of BRCA1 at several sites, which were previously phosphorylated by ATM, ATR, or CHK2 upon DNA damage. Mutation in the PP1-binding motif in BRCA1 impaired the DNA damage-induced Rad51 foci formation, and compromised its function in the HR [55,61,63]. Moreover, low levels of BRCA1 may be related to the variable levels of PP1α and β in primary sporadic human breast tumors, indicating an important role of PP1 during the development of breast cancer, and may result from an aberration in its phosphorylation status [62]. p53 is another substrate of PP1. As a multifunction factor, the roles and phosphorylation status of p53 have been well established during the past decades [64]. Upon DNA stress, such as γ-irradiation or UV irradiation, p53 is phosphorylated at Ser15 and Ser20 by ATM/CHK2 and ATR/CHK1 [65–68]. PP1 dephosphorylates UV-induced p53 phosphorylation at Ser15, and this can be disturbed by okadaic acid (OA) and GADD34 (growth arrest and DNA damage 34). GADD34, one of the PP1 binding partners, inhibits PP1 binding with p53, interferes with the dephosphorylation of p53 and sustains the amount of phospho-p53 after UV-treatment [69]. New data suggest that PP1 may be involved in the regulation of cross talk between different post-translational modifications in DDR, e.g., KAP1 (Krüppel-associated box associated protein 1) phosphorylation and sumoylation. KAP1 provides a prosurvival advantage by contributing to transcriptional repression of the DNA damage response genes p21, Bax, noxa and Puma. KAP1 phosphorylation antagonizes its sumoylation and keeps it inactive. PP1α is essential to establish the minimal level of Ser-824 phosphorylation required for KAP1’s coressor function in unstressed cells. By contrast, PP1β is recruited to KAP1 post Dox-treatment, and its stimulatory effect on sumoylation is expanded from KAP1 to RanGAP1. These findings provide novel mechanistic insights into how PP1 impacts DDR, influencing not only KAP1 Ser-824 dephosphorylation, but also its sumoylation and targeted gene expression [2].

2.2 PP2A

PP2A is a major Ser/Thr phosphatase with distinct roles in regulation of cell cycle progression, cell growth and development, cytoskeleton dynamics, and cell mobility [70]. Two isoforms of PP2A are encoded by 2 distinct and unlinked genes, PP2ACα and PP2ACβ. These 2 isoforms share 97% identity in primary amino acid sequences, there is no antibody available to date that can distinguish them [70]. They are expressed in all tissues and cell types and distributed in the cytosol, nucleus, and chromatin. Knockout of Ppp2car in mice leads to death at embryonic day 5.5. It has been assumed that they are functionally redundant. However, it would be interesting to see the phenotypes of Ppp2car knockout mice and if these two isoforms can rescue each other in vivo [71].

Active forms of PP2A exist as heterotrimeric complexes, each of which consists of a catalytic subunit C, a structural subunit A and a regulatory subunit B [70]. The structural subunit A interacts with the catalytic C subunit by its carboxyl terminus, and forms a horseshoe-shaped scaffold. There are two A isoforms encoded by two alternative genes, PR65α and PR65β, with 87% similarity in their primary amino acid sequences [72]. The heterodimeric AC remains inactive without the addition of the regulatory B subunits. To date, 15 genes have been identified in the human genome. These genes encode at least 26 different alternatively spliced variants representing the B subunits of the PP2A holoenzyme. These B subunits have been divided into 4 families based on sequence homology, namely, B (B55 or PR55), B’ (B56 or PR61), B” (PR48/59/72/130), and B”’ (PR93/110) families. It is believed that PP2A achieves regulatory flexibility and substrate specificity via the specific association of the core dimer with one of the regulatory B subunits [73].

Involvement of PP2A in DNA damage signaling, DNA repair and apoptosis has been established. Upon DNA damage, PP2A is activated, partially regulated by methyla-
upon mitotic DNA damage [79]. PP2A/B56 involved in the deactivation of PLK1 by dephosphorylation abrogates radiation-induced G2 arrest. PP2A might also be minimally truncated form of the B56 subunit in 3T3 cells also induced G2/M arrest [78]. Similarly, expression of an N-terminal dephosphorylation of Cdc2-Tyr15, and attenuates the IR-induced activation of ATR and CHK1, as well as more, inhibition of PP2A by inhibitors or siRNA abrogates PP4 is closely related to PP2A, exhibiting phosphatase activity in a heterodimeric or heterotrimeric holoenzyme [83, 84]. PP4 has been known to be a ubiquitous Ser/Thr phosphatase in several species for more than a decade. Mammalian PP4c share 65% amino acid identity with PP2cα and PP2cβ. Five regulatory subunits have been identified, PP4R1, PP4R2, PP4R3α, PP4R3β and PP4R4, forming 4 potential holoenzymatic complexes, PP4c-PP4R1, PP4c-PP4R4, PP4c-PP4R2-PP4R3α, and P4c-PP4R2-PP4R3β) [83,84]. PP4 has roles in several cellular processes, including organelle assembly, centrosome maturation, spliceosome assembly, regulation of histone acetylation and chromatin remodeling [85–87]. Its roles in DDR have been appreciated recently. Gingras and colleagues identified several novel, evolutionarily conserved PP4C-containing complexes involved in cisplatin sensitivity [88]. Deletion of any of the PP4 subunit orthologs in Saccharomyces cerevisiae elicited cisplatin hypersensitivity [89]. Furthermore, human PP4R3 complemented yeast psy2 deletion, and Drosophila melanogaster lacking functional PP4R3 (falaefal) exhibited cisplatin hypersensitivity, suggesting a highly conserved role for PP4 in DNA damage repair. Finally, PP4R3 was found to, at least in part, target PP4c to the DNA damage repair machinery via an interaction with Rad53 (tCHK2) [42,88,90]. Investigations in budding yeasts demonstrated that phosphatase Pph3-containing complex facilitated DNA damage checkpoint recovery by regulating the phosphorylation status of H2A. In addition, Pph3 forms a complex with PsyI (Pph3-PsyII) that specifically binds and dephosphorylates Rad53 during replication stress recovery, this is required to restart stalled replication forks [84,91].

Studies in mammalian cells have revealed that PP4C, PP4R2, and PP4R3β holoenzymatic complexes specifically dephosphorylate ATR-mediated γH2AX generated during DNA replication [83,92]. The PP4C-PP4R2 complex regulated the RPA2 phosphorylation level to regulate in turn the HR repair [93]. Overexpression of PP4 has been found in human breast tumors and lung tumors, and inhibition of its expression sensitizes cancer cells to cisplatin treatment [94]. Overexpression of PP4 and other protein phosphatases in tumors may confer resistance to chemotherapeutic drug-induced DNA damage because DNA damage-induced γH2AX may be dephosphorylated regardless of repair. This would allow cancer cells to continue proliferating in the presence of damaged DNA. These studies have established that PP4 is involved in DDR and could be a target for drug development and cancer therapy.

2.4 PP6

PP6 is the mammalian homolog of yeast Sit4, which is highly related in sequence to the PP2A-like Ser/Thr phosphatases. Phenotypes induced by inhibition of Sit4 could be rescued by ectopic expression of mammalian PP6 [95]. The phosphatase activity of Sit4 depends on its interacting partners, Sit4-associated proteins (SAP) [96]. Three regulatory
subunits containing SAP domains, which were renamed as PP6R1, PP6R2 and PP6R3, respectively, have been identified in mammals and shown to coprecipitate with PP6c, but not with PP4 or PP2A [97,98]. All three SAP subunits can individually associate with PP6c, but exhibit nonoverlapping substrate specificities. DNA-PK interacts with PP6C, and its regulatory subunits PP6R1, PP6R2 and PP6R3. Silencing of PP6c or PP6R1 resulted in sustained phosphorylation of γH2AX after IR, while neither of the phosphorylation states of DNA-PK or ATM was compromised [99]. This demonstrated that neither DNA-PK nor ATM is a substrate of PP6c. DNA-PK may instead recruit PP6 to sites of DNA damage to dephosphorylate γH2AX, remove IR-induced foci, and release from the G2/M checkpoint in vivo [99]. This appears contradictory to the findings of other reports [99–101], in which inhibition of PP6c or PP6R1 expression by siRNA impairs DNA-PK activation in response to IR, though all reports demonstrated that preventing PP6c expression sensitizes tumor cells to IR. Our laboratory further demonstrated that depletion of PP6c or PP6R2, but not PP6R1, compromised repair of CPT-induced DSBs or I-SceI-induced DSB, suggesting that DNA lesions induced by different agents may require different PP6 holoenzymes [102].

Our laboratory also examined expression status of PP6c and its regulatory subunits in breast tumor samples derived from a cohort of approximately 200 breast cancer patients [102]. It was unexpected to find that expression of PP6c, PP6R2, and PP6R3 was lowered in breast cancer samples in comparison with benign breast lesions, suggesting that PP6 may have a protective role during breast tumorigenesis.

2.5 PP5

PP5 is a unique phosphatase of the PPP family, not only in terms of its structure, but also its phosphatase activity. PP5 has a peptidyl-prolyl cis-trans-isomerase-like (PPase-like) domain and 3 consecutive tetratricopeptide repeat (TPR) domains responsible for protein interaction, neither of which have been identified in other protein phosphatases [103]. Its phosphatase domain resides in the C-terminus and shares about 48% sequence similarity to other phosphatases, such as PP1, PP2A and PP2B. PP5 exhibits low phosphatase activity under normal conditions, which may be a consequence of the inhibitory interaction between its C-terminus region (responsible for the phosphatase activity) and its N-terminus TPR region [103]. PP5 plays roles in proliferation, migration, differentiation, electrolyte balance, apoptosis, survival, and also DDR [103,104]. PP5 is required for the activation of both ATM-mediated and ATR-mediated DNA damage checkpoints through enhanced direct interaction in response to genotoxic stresses [105–107]. PP5 interacts with ATM depending on DNA damage, and inhibition of PP5 expression attenuated ATM activation. The mouse model lacking PP5 does not arrest the ATM-mediated cell cycle [108]. PP5 is also required for the activation of ATR and phosphorylation of Rad17 and CHK1 after UV or hydroxyurea treatment [107]. While no phosphorylation residues have been identified that can be reversed by PP5, the available data suggest that PP5 may regulate the phosphorylation status of 53BP1 in response to radiomimetic agents, such as neocarzinostatin (NCS) [109]. Overexpression of PP5 was found in human breast cancer, indicating that elevated PP5 protein levels may promote tumor progression [110].

2.6 PPM1D

PPM1D/WIP1 was originally identified as a wild-type p53 induced protein phosphatase in response to IR [111]. Subsequent studies have revealed that a wide range of damage agents, such as UV, anisomycin, H2O2 and MMS (methyl methanesulfonate) also induced its expression [111,112]. It is a monomeric Ser/Thr phosphatase with monogenic activity. Its phosphatase activity requires the presence of Mg2+ or Mn2+, and it is relatively insensitive to OA treatment [113]. Overexpression of PPM1D has been reported in multiple human cancers, including neuroblastomas, pancreatic adenocarcinomas, and medulloblastomas, as well as ovarian and gastric carcinomas [114–117]. Its oncogenic properties have been elucidated in several tumor suppressor pathways [118]. Overexpression of PPM1D in cells removes the IR- or UV-induced intra-S and G2-M cell cycle checkpoints, whereas repression of PPM1D expression prolongs these checkpoints [119,120]. Identification of its substrates, which share a p(S/T)Q motif, has also revealed the critical function of PPM1D as a regulator of DDR [113,121]. Upon UV-irradiation, p53 is phosphorylated by the stress-responsive kinase p38 mitogen-activated protein kinase, at Ser33 and Ser46, which enhances the activity of p53 and ultimately results in cell cycle arrest or apoptosis [122]. The activated p53 then induces the expression of PPM1D, which in turn attenuates the phosphorylation of p53 as well as the conserved active site of p38, to suppress its transcriptional activity and UV-induced apoptosis. Furthermore, PPM1D facilitates inactivation of the BER by dephosphorylating and deactivating the crucial regulator UNG2, which is a nuclear isozyme of uracil-DNA glycosylase activated upon UV damage [123]. Thus, p-38 MAPK-p35-PPM1D forms a negative feedback loop in the UV-induced DNA damage response. The ATR-CHK1-p53 pathway also occurs upon UV-induced DDR. Upon UV damage, ATR phosphorylates and activates CHK1 at Ser345, as well as p53 at Ser15. At the same time, the activated CHK1 also phosphorylates p53 at Ser20 to stop the interaction between p53 and MDM2, which is an E3 ligase and expressed in a p53 dependent manner, thus stabilizing p53 after DNA damage [124]. In response to DSBs, ATM turns into an active form via auto-
phosphorylation and monomerization [125], and then phosphatase catalytic subunit may have many targets in- volved in many molecular pathways. To achieve pathway-specific dephosphorylation by protein phosphatase is a major pathway that deactivates phosphoproteins is important role in determining its substrate specificity. It is a challenging and important task to determine physiological substrates and their corresponding specific holo-phosphatases in the near future.

Reversible protein phosphorylation plays a critical role in tumorigenesis. Protein kinases have become the second most important target in the development of anticancer drugs, and it is logical to expect that protein phosphatases are also potential anticancer drug targets. Indeed, a number of natural compounds have been identified to target the catalytic subunit and block its phosphatase activity, however, these compounds have either not been tested in a clinical trial or they failed in the early stage of a trial because of their side effects. These side effects result from the fact that a phosphatase catalytic subunit may have many targets involved in many molecular pathways. To achieve pathway- or substrate-specific blockade, rational design of small molecules targeting the catalytic-substrate interaction or the regulatory subunit-substrate interaction is important.

3 Phosphatases for γH2AX

H2AX is rapidly phosphorylated by ATM/ATR/DNA-PK on Ser139 (γH2AX) surrounding sites of DNA damage [130], which serve as a platform for recruitment and enrichment of DDR factors [131,132]. γH2AX is a marker for DSBs. It has been puzzling when and how γH2AX is removed. Thus far, several protein phosphatases have been reported to dephosphorylate γH2AX, these include PP2A, PP4C, PP6C, PPM1D and PPM1G [83,92,99,128,133,134]. We and our collaborators have shown that PP4C forms complexes with PP4R2, and PP4R3β, and specifically dephosphorylates ATR-mediated γH2AX within the mono-nucleosomes generated during DNA replication. PP2A is mainly responsible for γH2AX in response to exogenous DNA damage [83]. We and our collaborators have also revealed that γH2AX is dephosphorylated by PP6 both in vitro and in vivo [99,102]. Silencing of either PP6c or PP6R1 led to sustained γH2AX levels after IR, whereas inhibition of PP6c or PP6R2 expression, but not PP6R1 expression, resulted in sustained γH2AX levels after CPT treatment [102]. It is very interesting that γH2AX generated under different circumstances is dephosphorylated by different phosphatases, and if by the same phosphatase, the requirement of regulatory subunits is different. These studies indicate that PPM1D is an important negative regulator of DDR.

4 Perspective

OA-mediated inhibition effects have been attributed to PP2A phosphatase. In fact, OA also blocks phosphatase activity of PP4 and PP6. Therefore, it is necessary to re-examine if PP4 or PP6 participates in or regulates these "assumed" PP2A-mediated cellular activities reported in the literature.

Proteasome-mediated degradation of phosphoproteins has been validated in a significant number of phospho-targets, however, degradation and protein resynthesis are extremely costly in terms of adenosine triphosphate consumption. Given the smartness of both normal cells and cancer cells, the advantage of using this costly approach to deactivate a phosphoprotein is not clear. Though protein phosphatemediated in situ dephosphorylation of phosphoproteins has been only identified to date in significantly fewer phospho-targets, we hypothesize that in situ dephosphorylation by protein phosphatases is a major pathway that deactivates phosphoproteins.

One third of eukaryotic proteins are phosphorylated and more than 98% of phosphorylation events occur on Ser/Thr residues. The human genome only encodes about 140 catalytic subunits of protein phosphatases, about 40 of which are Ser/Thr phosphatases. Therefore, it would be very delicate and complex for a phosphatase to exhibit its specificity for substrate recognition and recruitment. As discussed above, complex formation of a holoenzyme plays an important role in determining its substrate specificity. It is a challenging and important task to determine physiological substrates and their corresponding specific holo-phosphatases in the near future.

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