Regulation of poly(ADP-Ribose) polymerase 1 functions by post-translational modifications

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1. ABSTRACT
The poly(ADP-ribose) polymerases (PARPs) catalyze poly(ADP-ribose)lation, a post-translational modification of proteins. This consists of the attachment of mono- or poly-adenosine diphosphate (ADP)-ribose units from nicotinamide adenine dinucleotide (NAD+) to specific polar residues of target proteins. PARP1 is the most abundant and best-characterized member of the family of PARP enzymes. PARP1 plays key roles in DNA repair, as well as a wide variety of cellular processes, including transcriptional regulation, chromatin modulation, cellular signaling pathway, inflammation, cellular stress responses and so on. Hence, PARP1 inhibitors have become a promising therapeutic approach for human diseases including cancer. Recent studies indicate that post-translational modifications (PTMs) such as phosphorylation, acetylation, and methylation are crucial for the regulation of PARP1 activity, and dysregulation of modifications on PARP1 is observed in human cancer. In this review, we describe the importance of PTMs to regulate the activity of PARP1, and the involvement of dysregulation of PTMs in human cancer.

2. INTRODUCTION
The Poly(ADP-ribose) Polymerase (PARP) superfamily contains at least 17 enzymes and plays critical roles in multiple biological processes including DNA repair, transcriptional regulation, cell cycle regulation, inflammation, hypoxic response, spindle pole function, oncogene-related signaling and cell death (1–3). Poly(ADP-ribose) Polymerase-1 (PARP1) is the most abundant and ubiquitous member of the PARP family enzymes (4). PARP1 catalyzes the covalent attachment of poly(ADP-ribose) polymers on itself and other acceptor proteins such as histones, DNA repair proteins, transcription factors and chromatin modulators, using NAD+ as a donor of ADP-ribose units (4–6). Poly(ADP-ribose)lation is a dynamic process consuming substantial amounts of NAD+. The in vivo half-life of the polymer is less than 1 min with the steady-levels of poly(ADP-ribose) (PAR) being regulated by the catalytic reactions of poly(ADP-ribose) glycohydrolase (PARG) and possibly the ADP-ribose hydrolyase ARH3 (7). PARP1, which has many protein binding partners in the nucleus, has been identified as a component of a variety of protein complexes, including those that (1) repair DNA damage, (2) regulate transcription, (3) function as insulators, (4) methylate DNA, and (5) promote iPSC reprogramming (8–21). Many of these binding partners have been reported to be PARylated as targets of PARP1 (22–24). It is known that covalent attachment of PAR alters the activity of target proteins through both steric and charge effects, ultimately preventing protein-protein interactions, protein-nucleic acid interactions, enzymatic activity or subcellular localization (2, 6, 8).
PARP1 has been considered as an important target for the development of anti-cancer agents (25). In 1980, Durkacz and colleagues used the still immature, low-potency PARP inhibitor 3-aminobenzamide (3-AB) to derail DNA damage repair and to enhance the cytotoxicity of dimethyl sulfate, a DNA alkylating agent (26). From that time on, a large number of preclinical studies proved the importance of PARP inhibitors as sensitizing agents to DNA-damaging drugs and radiotherapy (1). In particular, cancer cells endowed with genetic defects in other DNA repair pathways like BRCA1 or BRCA2 deficient cells are extremely dependent on PARP1 activity (27–29), which proposes the concept of synthetic lethality as viable strategy in cancer drug development (25, 30). Recently several PARP inhibitors are in phase 1 and 2 clinical trials, and the combination of therapeutic efficacy with minimal to toxicity has finally led to the approval of olaparib (Lynparza™) by the US food and drug administration (FDA) and European commission for the treatment of advanced ovarian cancer in patients with BRCA mutations (31, 32). Furthermore, an extensive body of work has now revealed applicability of PARP inhibitors in cancer therapy beyond the presence of BRCA mutations, often referred to as “BRCAness”, and in order to develop useful anti-cancer drugs efficiently, it is important to analyze biological and physiological functions of PARP in more detail.

In this review article, we discuss the molecular mechanism of PARP1 functional regulation, focusing the significance of post-translational modifications on regulating PARP1 functions, and their dysregulation involved in human cancer.

3. PARP1 STRUCTURE AND CHARACTERISTICS

PARP1 (ARTD1) is the founding member of the PARP family, which is responsible for the synthesis of the majority of PAR in eukaryotic cells and after the histones, is the most abundant nuclear protein (7, 33). As well as many other chromatin- and transcription-related proteins, it has a modular structure constituting multiple independently folded, well-characterized domains (8). The major functional units of PARP1 are an amino-terminal DNA binding domain (DBD), a central automodification domain (AD) and a carboxyl-terminal catalytic domain (CAT) (2, 34) (Figure 1). The DNA binding domain contains two Cys-Cys-His-Cys (CCHC) zinc finger motifs (ZF1 and ZF2) that mediate binding to DNA and have pivotal roles in DNA-dependent activity, a newly discovered third zinc binding domain (ZF3) that mediates inter-domain contacts important for DNA-dependent enzyme activation, a nuclear localization signal (NLS), and a caspase-3 cleavage site (2, 34–36). An internal automodification domain contains a BRCT (BRCA1 C-terminus) fold involved in mediating protein-protein interactions and three lysines that are targeted for automodifications (35, 37, 38). The carboxyl-terminal catalytic domain is the most conserved domain among PARP family and contains a WGR motif, which is named after the conserved central motif of Trp (W), Gly (G), Arg (R), an α-helical PARP regulatory domain (PRD), which may be involved in regulation of the PARP-branching activity, and a catalytic PARP domain, which forms the NAD+ binding site (8).

PARP1 plays critical roles in DNA repair, chromatin modulation and transcription (8, 23, 39). It binds to nucleosomes containing intact DNA as well as to damaged DNA structures under conditions of genotoxic stress, leading to the activation of its enzymatic activity (5). PARP1 is enriched at the promoters of actively transcribed genes, where it plays an important role in modulating the chromatin environment to regulate gene expression, particularly in response to cellular signaling pathways (4, 40, 41). PARP1 knockout mice are viable and show only mild phenotype, but some interesting phenotypes have been revealed in response to certain chemical agents, in some genetic backgrounds and under certain physiological conditions (8, 42). For instance, Parp1−/− mice are more sensitive to chemically-induced genotoxic stress (42–44). They also show resistance in various models of inflammation, as well as increased tumor formation in some genetic backgrounds such as p53−/− and in chemically-induced models of cancer (45–48), indicating the importance of PARP1 as a target of anti-cancer treatment.
4. POST-TRANSLATIONAL MODIFICATIONS OF PARP1

4.1. Phosphorylation

Kauppinen et al. reported that extracellular signal-regulated kinases 1/2 (ERK1/2) promote PARP1 activation through direct phosphorylation of PARP1 at serine 372 and threonine 373 (Figure 2A) (49). These phosphorylation sites are located near the beginning of the BRCT portion of the automodification domain, which is known to modulate PARP1 activity (5). The S372A and T373A mutations to prevent phosphorylation generate reduced activation of PARP1 after DNA damage, whereas the S372E and T373E mutations to mimic constitutive phosphorylation enhance PARP1 activity, implying that phosphorylation at these sites is likely to be essential for maximal PARP1 activation, which is also confirmed by Cohen-Armon et al. (50). Inhibition of ERK1/2 phosphorylation with either the MEK1/2-ERK1/2 inhibitor 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98059), or ERK2-specific siRNA blocks the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-methyl-D-aspartate (NMDA), or peroxynitrite induced PARP1 activation, and clearly reduces neuron and astrocyte death due to extensive PARP1 activation (49). In addition, blockade of ERK1/2 phosphorylation during hypoglycemia prohibits PARP1 activation and also subsequent PARP1-mediated neuronal death in vivo.

In 2012, Wright et al. described a novel mechanism of DNA-independent activation of PARP1 (51). The hormone-activated kinase CDK2 in complex with Cyclin E and progesterone receptor (PR) phosphorylates Ser785 and Ser786 of PARP1, which are essential for the enhanced activity of PARP1, followed by remarkable increases in PAR and PARP1 levels in breast cancer cells upon progestin...
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stimulation (Figure 2B) (51). PARP1 and CDK2 specifically interact following hormone treatment via the BRCA1 C-terminal domain (BRCT) of PARP1 and the cyclin-binding domain (CBD) of CDK2, which facilitates the phosphorylation of PARP1 at S785/S786. Phosphorylation of these serines on PARP1 may result in a more open NAD-binding pocket within the catalytic domain, enhancing PARP1 activity, leading to auto-PARylation. Moreover, PARP1 and CDK2/Cyclin E appear to be coregulated to chromatin, resulting in PARylation of chromatin proteins and chromatin opening via the displacement of histone H1. Herein, the catalytic activities of PARP1 and CDK2 are indispensable for hormone-dependent recruitments of both enzymes as well as H1 displacement.

More recently, Yi et al. reported that the receptor tyrosine kinase c-Met associates with and phosphorylates PARP1 at Tyr907 (pTyr907 or pY907) (52). The enzyme activity of c-Met is essential for the interaction between PARP1 and c-Met enhanced with H2O2 treatment. C-Met-phosphorylated PARP1 at Tyr907 increases PARP1 enzymatic activity, and moreover, leads to the reduction of the binding to PARP inhibitors, thereby rendering cancer cells resistant to PARP inhibition (Figure 2C). Hence, cancer cells become more sensitive to PARP inhibitors by treatment with c-Met inhibitors as well as c-Met specific shRNA. The combination of c-Met and PARP1 inhibitors synergizes to substantial reduction of tumor growth in the breast and lung cancer xenograft tumor models. These results indicate that PARP1 pY907 may be a useful marker to predict tumor resistance to PARP inhibitors, and the combined treatments of c-Met and PARP inhibitors will benefit populations of patients with high c-Met expression who are resistant to PARP inhibition alone (52). Given that EGFR is also identified to possibly phosphorylate PARP1 by phospho-RTK antibody array analysis (52), and the combined EGFR and PARP1 inhibition achieves synthetic lethality in highly aggressive triple negative breast cancers (TNBCs) (53), EGFR may involve a similar mechanism.

In 2016, Fenqing et al. reported the first-line anti-diabetic drugs biguanides and angiotensin II receptor blockers (ARBs) confer a benefit on the vasculature by AMP-activated protein kinase (AMPK) mediated phosphorylation of PARP1 at Ser177 to inhibit PARP1 activity and attenuate protein poly(ADP-ribosylation) (54, 55).

Although phosphorylation sites are not determined, c-Jun N-terminal Kinase 1 (JNK1) is reported to directly associate and phosphorylate PARP1 to promote sustained PARP1 activation during H2O2-induced nonapoptotic cell death (56). Calmodulin kinase (CaMK)-dependent phosphorylation of PARP1 is crucial for PARP1 poly(ADP-ribosylation) activity required for dismissal of the Groucho/TLE1 corepressor complex and for MASH1 gene activation in differentiating NSCs (16). Txk, a member of the non-receptor tyrosine kinase of the Tec family, forms a phosphorylation-dependent trimolecular complex specifically binding to the IFN-γ gene promoter in vitro through direct phosphorylation of complex protein PARP1 and EF1α (57). Insulin-like growth factor-1 (IGF-1)-dependent phosphorylation of PARP1 inhibits PARP1 activity and results in enhanced VEGF expression (58). Furthermore, phosphorylation of PARP1 by protein kinase C (PKC) attenuates PARP1 DNA binding capacity and catalytic activity (4, 58–60). Although it is reported that DNA-dependent protein kinase (DNA-PK) phosphorylates PARP1 in a DNA-dependent manner, the biological roles of phosphorylated PARP1 are not well elucidated (61).

Combining bioinformatics-based prediction tools for generic and kinase-specific phosphorylation sites, in vitro phosphorylation assays and mass spectrometry analysis, Gagne et al. identified several phosphorylated serine/threonine residues within PARP1 (62). S257/T258 and S782 are revealed as phosphorylation sites on PARP1 by JNK1. Likewise, S257/T258 and S782/S785/S786 are observed as potential phosphorylation sites by CaMK-II, SS04, SS19, and T656 by PKCβ. However, these target phosphosites are still needed to be validated by further experimental analysis.

4.2. Methylation

Besides phosphorylation, protein methylation is one of the important post-translational modifications and in particular, its importance in epigenetic regulation via histone methylation has been elucidated (63–67). In addition, the biological and physiological importance of non-histone protein methylation has recently been clarified (68–73).

To examine the possibility of PARP1 methylation, we conducted in vitro methyltransferase assays using a number of protein methyltransferases and ultimately identified that the protein lysine methyltransferase SMYD2 methylates PARP1 (74). Based on three different methods that are liquid chromatography tandem mass spectrometry (LC-MS/MS), amino acid analysis and protein sequencing combined with Edman degradation, we demonstrate that lysine 528 of PARP1 (PARP1 K528) located in the automodification domain (Figure 3A and 3B), is methylated by SMYD2 (74). In order to evaluate the effect of PARP1 K528 methylation on its enzymatic activity, we measure PARP1 activities in the presence or absence SMYD2-mediated methylation and find that methylated PARP1 shows significantly higher enzymatic activities than unmethylated PARP1 (74). Moreover, we also confirm that SMYD2-depleted cells
interaction with p50, a component of NF-κB, and synergistic coactivation of NF-κB by p300 and the Mediator complex in response to inflammatory stimuli (Figure 3C). PARP1 physically interacts with the Mediator complex through the direct interaction with the Mediator subunits CDK8 and MED14. PARP1 also interacts with members of class I histone deacetylase HDACs 1–3 but not HDACs 4–6, and is potentially deacetylated by HDAC1, HDAC2 or HDAC3 in vivo (14). Moreover, PARP1-dependent transcriptional activation of NF-κB seems to be repressed by HDACs 1–3 in part through deacetylation of PARP1. Together, acetylation of PARP1 is important for the function as transcriptional coactivator.

Rajamohan et al. found that PARP1 is acetylated and activated after stress of cardiomyocytes and that acetylation-mediated activation of PARP1 is independent of DNA damage (76). The histone exhibit remarkable reduction of PAR signals compared with those in control cells under genotoxic stress conditions, and concordantly, SMYD2-overexpressing cells showed significantly higher PARP activity than control cells, indicating a novel mechanism for the regulation of PARP1 activity upon cellular stress.

Kassner et al. also reported PARP1 as a target protein of SET7/9. SET7/9 methylates PARP1 at Lys508, which is blocked by PARP1 auto-poly(ADP-ribosylation), moreover, SET7/9-dependent methylation stimulates PARP1 activity (75).

4.3. Acetylation

Hassa et al. demonstrated that p300/CBP acetylates lysines 498, 505, 508, 521 and 524 on PARP1 both in vitro and in vivo (14). Importantly, acetylation of these residues is required for the interaction with p50, a component of NF-κB, and synergistic coactivation of NF-κB by p300 and the Mediator complex in response to inflammatory stimuli (Figure 3C). PARP1 physically interacts with the Mediator complex through the direct interaction with the Mediator subunits CDK8 and MED14. PARP1 also interacts with members of class I histone deacetylase HDACs 1–3 but not HDACs 4–6, and is potentially deacetylated by HDAC1, HDAC2 or HDAC3 in vivo (14). Moreover, PARP1-dependent transcriptional activation of NF-κB seems to be repressed by HDACs 1–3 in part through deacetylation of PARP1. Together, acetylation of PARP1 is important for the function as transcriptional coactivator.

Rajamohan et al. found that PARP1 is acetylated and activated after stress of cardiomyocytes and that acetylation-mediated activation of PARP1 is independent of DNA damage (76). The histone
deacetylase SIRT1 could bind to PARP1 and play a major role in regulating deacetylation of PARP1 in vivo (Figure 3C). SIRT1 could deacetylate the 1 to 214 aa and 477 to 524 aa regions of PARP1, whereas HDAC1 is only effective for the 477 to 524 aa region. SIRT1-dependent deacetylation of PARP1 blocks its enzymatic activity, and cellular NAD levels play a role in the interplay between PARP1 and SIRT1 (76). SIRT1 overexpression also significantly reduces the level of PARP1 mRNA, suggesting that SIRT1 is capable of regulating the activity of PARP1 at the post-translational as well as at the transcriptional levels. Furthermore, SIRT1 appears to be capable of blocking cell death induced by PARP1 activation. Lysine acetyltransferase 2B (PCAF) is able to acetylate PARP1 at undetermined sites. PCAF-mediated PARP1 acetylation leads to enhancing its activity for auto-poly(ADP-ribosyl)ation as well as for transferring ADP-ribose units to other substrates.

### 4.4. ADP-ribosylation

Auto-ADP-ribosylation (automodification) of PARP1 may occur as an extensive addition of ADP-ribose in chains >200 units in length or as a more modest addition of a single unit or chains up to 20 units in length (43, 77). Previously it was published that PARP1 is both auto-mono and auto-poly(ADP-ribose) ated (38, 77, 78). Altmeyer et al. reported that three lysine residues, Lys498, Lys521, and Lys524 within the automodification loop (aa 466–525) and additional residues within the first 214 amino acids of PARP1 are target sites for enzymatic auto-ADP-ribosylation (Figure 4A) (8, 38). Extensive automodification of PARP1 inhibits its DNA binding and catalytic activities, and biochemical and cell-based assays demonstrate that auto-ADP-ribosylation of PARP1 results in its release from chromatin (5, 8, 79–82). The effect of less extensive auto-ADP-ribosylation of PARP1 still remains unclear; modestly modified PARP1 may have altered activities, but retain its association with chromatin (8). Recently, Muthurajan et al. reported that auto-ADP-ribosylation of PARP1 remarkably reduces PARP1 affinity for intact chromatin but not for nucleosomes with exposed free DNA ends (83). Auto-ADP-ribosylated PARP1 has a strikingly higher affinity for histones than unmodified PARP1 both in vitro and in cells, and acts as a nucleosome assembly factor in vitro (Figure 4A).

Sirtuin 6 (SIRT6), a chromatin associated protein with two enzymatic activities—deacetylase and mono-ADP-ribosyltransferase, directly associates with PARP1 and mono(ADP-ribosyl)ates PARP1 at Lys521 (84). This modification stimulates poly(ADP-ribose)ylation activity of PARP1 and enhances subsequent double-strand breaks (DSBs) repair under oxidative stress. SIRT6 point mutant possessing only deacetylase activity fails to stimulate PARP1, whereas point mutant possessing only mono(ADP-ribose)ylation activity strongly stimulates PARP1. Hence, SIRT6 promotes DSBs repair through stimulation of PARP1.

In addition to auto-ADP-ribosylation, it has been reported that other PARP family members can trans-modify PARP1 and vice versa. PARP1 and PARP2 can heterodimerize and ADP-ribosylate each other, which may be critical in efficient base excision DNA repair (4, 85, 86). The DNA binding domain and BRCT domain of PARP1 are responsible for this poly(ADP-ribosyl)ation. PARP3 also binds to PARP1, and activates PARP1 in the absence of DNA, although it is uncertain what role the mono(ADP-ribose) transferase activity of PARP3 plays in this process (87, 88). The cross-talk among different PARP members implies specific roles for individual PARP members that go beyond simple redundancy and vicarious functions (4).

### 4.5. SUMOylation and Ubiquitination

In eukaryotes, modification by the ubiquitin (Ub)-like SUMO proteins has been reported to regulate the activity of numerous transcription factors and cofactors (89–91). Martin et al. demonstrated that the SUMO E3 ligase, Protein Inhibitor of Activated STAT4 (PIAS4) interacts with PARP1, and mediates heat shock-induced poly-SUMOylation of PARP1 at lysines 486 and 203 (Figure 4B) (92). While, mutation of either one, or both fails to abrogate entire SUMOylation of PARP1, indicating that there might be other SUMOylation sites on PARP1. This PIAS4-mediated SUMOylation is essential for full HSP70.1-promoter activation. Chromatin immunoprecipitation (ChIP) analysis indicates that PARP1, PIAS4, SUMO-2 as well as Ubiquitin-Conjugating Enzyme E2 I (UBE2I) are rapidly recruited to the HSP70.1 promoter upon heat shock, and subsequently released with similar kinetics (92). Additionally, the SUMO-targeted ubiquitin ligase Ring Finger Protein 4 (RNF4) mediates heat-shock-inducible ubiquitination of PARP1, regulates the stability of PARP1, and is a positive regulator of HSP70.1. gene activity. RNF4 is crucial for the activation of HSP70.1 transcription through controlling degradation of SUMOylated PARP1. These results suggest a crosstalk between SUMOylation and ubiquitination on PARP1 in the transcriptional regulation of gene expression (92). Meanwhile, Messner et al. reported that PARP1 is preferably SUMOylated by SUMO3 and desumoylated by SENP1 and SENP3, and lysine 486, located nearby the previously described sites of acetylation and ADP ribosylation is the major SUMOylated site of PARP1(Figure 4B) (93). Although K486 SUMOylation does not affect ADP-ribosylation activity of PARP1, it completely attenuated p300-dependent acetylation of PARP1, which implies an intriguing crosstalk of SUMOylation and acetylation on PARP1. Moreover,
implying that CHFR-silenced cancer cells were sensitized to microtubule inhibitors. Additionally, mouse embryonic fibroblasts (MEFs) from Chfr knockout mice and CHFR-silenced primary gastric cancer tissues express high levels of PARP1 proteins. These results support that there is a significant advantage for use of combinational chemotherapy with PARP inhibitors for cancer cells resistant to microtubule inhibitors (94).

Binding of Ring Finger Protein 146 (RNF146 or Iduna), a PAR-dependent E3-ligase, to PARP1 is dependent on poly(ADP-ribosyl)ation of PARP1. RNF146 targets poly(ADP-ribosyl)ated PARP1 for ubiquitin proteasomal degradation, and mass spectrometry identified 24 different lysines on PARP1 as potential ubiquitinated residues via K11 and K48 ubiquitin linkages (95).

5. FUTURE PERSPECTIVE

Since the Human Genome Project was completed, the importance of PTMs has been widely recognized, and indeed, a large number of drugs...
targeting enzymes relevant to PTMs such as kinase have already been developed. Now PARP1 is considered as one of the key targets for anti-cancer therapy, and several clinical studies have already been started (31, 96–100). The model reviewed in this article integrates a fraction of the possible post-translational modifications of PARP1 (Figure 4C). In order to understand the mechanism of actions, it is crucial to deeply understand the PTMs of PARP1 though most of the functions may still remain unclear, and the accumulated information will contribute to efficient drug development. Importantly, PTMs of PARP1 are likely to affect the efficacy of PARP1 inhibitors and other anti-cancer drugs because PARP1 plays a critical role in DNA repair after genotoxic stress. Further functional analyses regarding PTMs of PARP1 may explore the availability of PARP1 inhibitors as anti-cancer drugs and provide novel strategy of anti-cancer treatment.

6. ACKNOWLEDGEMENT

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