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ADP-Ribosyltransferases and Poly ADP-Ribosylation

Chao Liu and Xiaochun Yu*

Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA

Abstract

Protein ADP-ribosylation is an important posttranslational modification that plays versatile roles in multiple biological processes. ADP-ribosylation is catalyzed by a group of enzymes known as ADP-ribosyltransferases (ARTs). Using nicotinamide adenine dinucleotide (NAD⁺) as the donor, ARTs covalently link single or multiple ADP-ribose moieties from NAD⁺ to the substrates, forming mono ADP-ribosylation or poly ADP-ribosylation (PARylation). Novel functions of ARTs and ADP-ribosylation have been revealed over the past few years. Here we summarize the current knowledge on ARTs and PARylation.

Keywords

ADP-ribosylation; ADP-ribosyltransferase; ART; ARTD; PAR; PARylation

1. INTRODUCTION

ADP-ribosylation has been identified for over 50 years. In early 1960s, Collier, Honjo and their colleagues found that the toxicity of diphtheria toxin required NAD⁺ to inhibit mammalian protein synthesis [1-3]. Subsequently, single ADP-ribose moiety from NAD⁺ was revealed to covalently bind to elongation factor 2 or aminoacyl transferase 2, which leads to the identification of mono ADP-ribosylation. In 1966, Mandel's group discovered a DNA-dependent NAD⁺ consuming reaction in which poly ADP-ribose (PAR) chains were synthesized [4, 5]. The identification of ADP-ribosylation opened a new avenue in the areas of DNA damage repair, cell proliferation, apoptosis, gene transcription, signal transduction and etc. [6]. The reactions are catalyzed by a group of enzymes, namely ADP-ribosyltransferases (ARTs), which transfer single or multiple ADP-ribose moieties from NAD⁺ to their targets, including proteins, nucleotides, antibiotics and other small molecules [6-13].

Among these reactions, ADP-ribosylation on proteins is well studied. Protein ADP-ribosylation is initiated with transferring one ADP-ribose moiety from NAD⁺ to the side chain of asparagine, aspartic acid, glutamic acid, arginine, lysine and cysteine residues

*Address correspondence to this author at the Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA; Tel: +1-734-615-4945; Fax: +1-734-936-6684; xiayu@umich.edu.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

[14-25]. Poly ADP-ribosyltransferases continue to add ADP-ribose moieties from NAD⁺ to the initial ADP-ribose through glycosidic bonds to form linear chain of PAR. Branched ADP-ribose chain is also generated by $\alpha(1'''-2'')$ -ADP-ribose linkage. Up to 200 ADP-ribose residues can be immediately linked to form a single PAR chain *in vitro* [26]. With phosphate moieties in each ADP-ribose, the polymer of ADP-ribose brings huge amount of negative charges to local environment and regulates different biological processes. Moreover, ADP-ribosylation is a reversible posttranslational modification. Mono ADP-ribose and PAR are recognized and degraded by ADP-ribosylhydrolases including Poly (ADP-ribose) glycohydrolase (PARG), Terminal ADP-ribose protein glycohydrolase 1 (TARG1), ADP-ribosylhydrolase 1 (ARH1), ARH3, Macro D1 and D2, and Nudix-Type Motif 9 and 16 (NUDT9 and NUDT16) [27-31]. Thus, this dynamically regulated posttranslational modification allows cells to respond to external stimuli as well as to participate in a variety of cellular activities.

In this review, we will focus on the protein ADP-ribosylation catalyzed by ARTs. ADP-ribosylation on other substrates, such as nucleotides, antibiotics and other small molecules will not be discussed here.

2. ADP-RIBOSYLTRANSFERASE

To date, there are 22 human gene products possessing ADP-ribosyltransferase activity [32]. These enzymes were previously named as poly ADP-ribose polymerases (PARPs) and ADP-ribosyltransferases (ARTs). However, since most PARPs only catalyze mono ADP-ribosylation, Hottiger *et al.* have suggested that ADP-ribosyltransferase should be subjected to a unified nomenclature and categorized into two sub-families [32]. First, the ADP-ribosyltransferases sharing homology with bacterial diphtheria toxin, are named as ARTDs. Second, the rest enzymes with homology to clostridial C2 and C3 toxins are named as ARTCs. Besides these ADP-ribosyltransferases, sirtuin family enzymes, known as the NAD⁺-dependent deacetylases, are also able to transfer mono ADP-ribose moiety [33-36], although the detailed catalytic mechanism is unclear.

ARTC family enzymes, aka ecto-ARTs, are extracellular, membrane-bound or secretory proteins that only mediate mono ADP-ribosylation. Although five ARTC genes have been cloned, only four of them are expressed in human due to a pseudo ARTC2 gene. These ARTCs catalyze protein mono ADP-ribosylation extracellularly or at cell surface, which regulates cell-cell communication and activates signaling transduction [37].

ARTD family members are intracellular enzymes with either mono or poly ADP-ribosyltransferase activities. Based on the domain architectures, ARTDs are categorized into five subgroups: DNA-dependent ARTDs, Tankyrases, CCCH Zn Finger ARTDs, ARTDs with the Macro domain, and other unclassified ARTDs (Table 1 and Fig. 1). Among DNA-dependent ARTDs, both ARTD1 (aka PARP1) and ARTD2 (aka PARP2) contain DNA-binding domain at the N-terminus. Upon binding DNA ends, the activation sites in their enzymatic domains are fully exposed, which activates PARylation [38]. Different from ARTD1 and 2, ARTD3 does not have an obvious DNA-binding domain. But ARTD3 is still able to be activated by DNA *in vitro* [39, 40], although the detailed activation mechanism is

unclear. The tankyrases contain several ankyrin repeats clusters, serving as protein-protein interaction platform, and facilitating substrate recognition [41]. The CCCH Zn Finger ARTDs contain both the Cys-Cys-Cys-His zinc fingers and the WWE domains. The zinc fingers are able to interact with RNA, while the WWE domains recognize PAR. The Macro domains of ARTDs also bind to ADP-ribose and mediate substrate ADP-ribosylation.

All the ARTs contain the catalytic domain (CAT) with similar secondary structure. These catalytic domains share a common NAD⁺ binding motif similar to those in bacterial exotoxins, such as diphtheria toxin and exotoxin A [32]. In these NAD⁺ binding motifs, a His residue and a Tyr residue are crucial for positioning the A-ribose moiety and the N-ribose moiety of NAD⁺ in a correct orientation [42, 43]. A conserved Glu residue in these motifs is responsible for transferring of ADP-ribose. However, not all the ARTs retain these critical residues. Substitution of the key NAD⁺-binding His residue in ARTD9 and ARTD13 abolish the activities ADP-ribose transferring. Substitution of the Glu residue to other residues may alter the activities of ARTs from poly to mono ADP-ribosyltransferase. Recent studies suggest that only ARTD1, 2, 5 and 6 possess PARylation activity, while the rest of ARTs are likely to be mono ADP-ribosyltransferases.

2.1. DNA-Dependent ARTDs

2.1.1. ARTD1—ARTD1 (PARP1) is a highly abundant chromatin-associated protein important for the maintenance of genomic integrity, chromatin remodeling and gene transcription. All these diverse functions of ARTD1 are carried out by its multiple functional domains, including three zinc fingers motifs (ZF1-3), a BRCT domain, a WGR domain, and a catalytic domain (CAT containing two sub-domains: helical domain-HD and ADP-ribose transferase domain-ART). The majority of ARTD1 exists in nucleus and associates with nucleosome with a very low activity [44]. However, upon DNA damage, ARTD1 is significantly activated in cells [45, 46]. Interaction between ARTD1 and DNA ends is mediated by the N-terminal three zinc finger motifs. Since the zinc finger motifs primarily contact the ribose-phosphate back-bone of DNA, ARTD1 can be activated by DNA breaks regardless the sequence of DNA fragments [38, 47]. It has been suggested that ZF1 and ZF2 function together to recognize DNA single-strand breaks (SSBs), whereas ZF1 and ZF3 mediate the interaction with DNA double-strand breaks (DSBs). In addition, ZF3 plays an important role for the homodimer formation of ARTD1 [48]. Besides DNA fragment, RNA could also be recognized by ZF3 and WGR domains of ARTD1, which induces the conformational change in CAT and activates CAT *in vitro* [49]. Thus, ARTD1 could recognize diverse oligonucleotide structures. Upon oligonucleotide binding, ARTD1 forms a network of interdomain contacts, which results a global conformational change in CAT. It distorts HD to significantly increase the flexibility of CAT and expose the activation sites to substrates [38]. This model has been further confirmed by mutations within the hydrophobic core of HD domain, which also increase the flexibility of catalytic domain and thus activate enzymatic activity in the absence of oligonucleotide-binding [38].

Besides oligonucleotide, ARTD1 could also be activated by nucleosomes *in vitro* [50, 51]. Moreover, posttranslational modifications on ARTD1, such as phosphorylation by

extracellular signal-regulated kinases 1/2 and acetylation by PCAF, may facilitate the activation of ARTD1 *in vitro* [52, 53].

2.1.2. ARTD2—Like ARTD1, ARTD2 (PARP2) has the C-terminal WGR domain and CAT domain. Different from ARTD1, ARTD2 has a very short DNA-binding domain (DBD) at the N-terminus. Due to the presence of basic residues in this region, the N-terminal DBD domain of ARTD2 has been proposed to bind nucleic acid. Indeed, like ARTD1, the activity of ARTD2 is largely increased in the presence of DNA and RNA *in vitro* [39, 54], especially with the phosphate group at 5' end [55]. However, compared to the essential role of the N-terminal zinc finger domains in ARTD1 for its activation, the N-terminal DNA-binding domain of ARTD2 only contributes partially for enzymatic activation. The WGR domain is also important for DNA-dependent activation of ARTD2 [55]. Due to lacking of structural analysis of ARTD2, the detailed mechanism of ARTD2 activation is still unclear.

2.1.3. ARTD3—Compared to ARTD1 and 2, ARTD3 (PARP3) also has a WRG domain and a CAT domain. However, the N-terminus of ARTD3 lacks a canonical DNA-binding domain, although it can also be activated by DNA [39, 40]. Different from ARTD1 and ARTD2, ARTD3 only has mono ADP-ribosyltransferase activity even with conserved catalytic motif (H-Y-E triad). It is probably because of the difference of donor site loop (D-loop, NAD⁺ binding site) in ARTD3 [56]. Vyas *et al.* swapped the D-loops of ARTD1 and ARTD3, and found that ARTD1 with ARTD3's D-loop could only catalyze mono ADP-ribosylation, while ARTD3 with the D-loop of ARTD1 was enzymatically inactivated, suggesting that D-loop in catalytic domain plays a key role for PARylation. However, simple replacement of D-loop is insufficient for PAR synthesis [39].

The current studies on ARTD3 are mainly focused on its role in DNA damage response. ARTD3 has been shown to associate with Ku70/80, DNA-PKcs and DNA ligase IV [57], which all participate in non-homologous end joining pathway (NHEJ) for DSB repair. Moreover, ARTD3 associates with both ARTD1 and DNA ligase III, and was found to mediate the recruitment of APLF to the site of DNA damage [40, 58]. ARTD1, DNA ligase III and APLF are crucial SSB repair factors. Thus, accumulated evidence suggests that ARTD3 participates in both DSB and SSB repair.

2.2. Tankyrases

In 1998, Smith and de Lange identified an ankyrin repeats-containing ADP-ribose transferase, named as Tankyrase 1 (ARTD5/TANK1), interacting with Telomeric Repeat-binding Factor1 (TRF1) [59]. ARTD5 contains N-terminal homopolymeric stretches of His, Pro and Ser residues (HPS domain), Ankyrin Repeat Clusters (ARCs), a sterile alpha module (SAM), and a C-terminal ADP-ribose transferase (CAT) domain. With similar searching for the binding partner of TRF1, ARTD6 (TANK2) was identified [60]. It shares 85 % primary sequence identity with ARTD5 in the ARCs, SAM and CAT but has a unique N-terminal domain. Deletion of either *Artd5* or *Artd6* gene does not affect development in mice. However, lacking both Tankyrases cause early embryonic lethality, suggesting that they have redundant function during prenatal development [61].

At each end of a chromosome, a telomere shelterin complex is responsible for chromosome end protection. This complex consists of TRF1, TRF2, protection of telomeres protein 1 (POT1), TERF-interacting nuclear factor 2 (TINF2), tripeptidyl peptidase 1 (TPP1) and TRF2-interacting protein (TERF2IP, aka RAP1). Both ARTD5 and 6 recognize TRF1 and TRF2 via their ARCs and poly ADP-ribosylate TRF1 and TRF2, which facilitates proteasome-dependent degradation of TRF1 and TRF2 [62, 63].

In addition, Tankyrase-dependent PARylation of DNA-PKcs may also play a role in telomere maintenance as DNA-PKcs-dependent NHEJ is involved in telomere capping. In contrast to TRF1, PARylation of DNA-PKcs stabilizes itself from proteasome-mediated degradation [64].

Another pathway regulated by Tankyrases is the WNT signaling pathway. In this pathway, β -catenine, the key transcription factor, is regulated by the cytoplasmic β -catenine destruction complex. This complex consists of adenomatous polyposis coli (APC), GSK3 β and AXIN1/2. Interestingly, AXIN1/2 are poly ADP-ribosylated by Tankyrases. Poly ADP-ribosylated AXIN1/2 is recognized by the WWE domain of E3 ligase RNF146, which facilitates RNF146-dependent ubiquitination and degradation of AXINs. Therefore, the suppression of Tankyrases rescues AXIN from proteasome-dependent degradation, and promotes the destruction of β -catenine [65].

2.3. CCCH Zn Finger ARTDs

ARTD12 (PARP12) is a putative anti-viral factor, whose expression is induced during viral infections [66, 67]. Five CCCH-type Zn-fingers at N-terminus were known to bind viral RNA for degradation [68, 69]. Recently, ARTD12 is reported to accumulate in cytoplasmic stress-granules in the presence of oxidative stress, and inhibits the mRNA translation [70]. Also, in response to LPS, ARTD12 relocates to autophagosomes by interacting with p62/SQSTM1, and facilitates NF- κ B-dependent signaling, suggesting a role of ARTD12 in inflammation [70].

ARTD13 (PARP13/ZAP/ZC3HAV1) is another anti-viral factor, actively against specific RNA viruses such as murine leukemia virus, Sindbis virus, human immunodeficiency virus, Epstein–Barr virus as well as the RNA intermediate of hepatitis B DNA virus [71–76]. During viral infection, ARTD13 binds viral RNA via its four CCCH-type zinc finger motifs and targets viral RNA for degradation by recruiting RNA decay factors [68, 77]. ARTD12 and ARTD13 co-localize in the stress granules [78]. Recently, ARTD13 has been shown to regulate mRNA degradation without viral infection [79].

ARTD14 (PARP7/tiPARP) contains a TPH domain, a WWE domain and a CAT. Inside of the TPH domain, it contains a CCCH-type zinc finger that is a putative RNA binding module. Moreover, ARTD14 shares 27.5% and 26.0% primary sequence identity with ARTD12 and ARTD13, respectively [80]. The WWE domain is known as an ADP-ribose-binding motif. Thus, it is likely that ARTD14 recognizes ADP-ribosylated targets and catalyzes additional ADP-ribosylation. ARTD14 was identified as a gene product that was strongly up-regulated upon exposure to halogenated aromatic hydrocarbons, a type of industrial contaminant [81]. ARTD14 exhibits auto mono ADP-ribosyltransferase activity

and mono ADP-ribosylates core histones as well [81, 82]. In addition, ARTD14 may function together with ARTD1 for maintaining the naive state of pluripotency of ES cells [83].

2.4. Macro Domain ARTs

Macro domain ARTDs contain multiple macro domains at the N-terminus and an ADP-ribosyltransferase domain at the C-terminus. The Macro domain is known as an ADP-ribose binding module [84]. Thus, it is likely that this type of ARTD recognizes ADP-ribosylated substrates and catalyzes additional ADP-ribosylation. Whereas ARTD7 (PARP15) remains largely uncharacterized, both ARTD8 (PARP14) and ARTD9 (PARP9) have been reported to regulate gene transcription [85, 86].

ARTD8 has three tandem Macro domains at the N-terminus and exists in both cytoplasm and nucleus [87]. It has been shown that ARTD8 regulates gene transcription following the stimulation of IL-4. ARTD8 mono ADP-ribosylates HDAC2 and HDAC3 at gene promoters for reprogramming histone codes and facilitating transcription [85]. Recent study also shows that ARTD8 is involved in mRNA stability. ARTD8 is found to form a complex with tristetraprolin (TTP) and 3' untranslated region of mRNA, which suppresses the degradation of mRNA [88].

ARTD9 is originally identified as a transcription modulator that is overexpressed in chemoresistant, diffuse large B-cell lymphomas [86]. Although it has two tandem Macro domains for binding ADP-ribose, the CAT domain of ARTD9 lacks key residues for NAD⁺-binding and transferring ADP-ribose [89]. The tandem Macro domains of ARTD9 have been shown to mediate the recruitment of ARTD9 to the sites of DNA damage [90, 91]. Since ARTD9 is an enzymatically inactive enzyme, it is likely to behave as a negative regulator for DNA damage-induced PARylation.

2.5. Other ARTs

ARTD4 (PARP4/vPARP) has been identified as a protein subunit in the vault ribonucleoprotein particle that might be involved in mRNA transportation [92]. ARTD4 contains multiple functional domains including a BRCT domain, a CAT domain, a VIT (vault inter- α -trypsin) domain, a vWA (von Willebrand factor type A) domain and a MVP-BD (major vault protein binding domain). However, the function of each domain is unclear. Since ARTD4 has been shown to localize at mitotic spindle, ARTD4 may regulate mitosis [92]. ARTD4-deficient mice have been generated. However, no obvious developmental defect has been observed. It has been shown that ARTD4-deficient mice were prone to carcinogen-induced tumors, suggesting ARTD4 may protect genomic stability from chemically induced neoplasia [93].

ARTD16 (PARP8) and ARTD17 (PARP6) have not been well characterized. Both of them contain a mono ADP-ribosyltransferase domain and an uncharacterized N-terminal region. Over expression of ARTD16 suppresses cell proliferation. Moreover, in human tumor samples, ARTD17 expression is negatively correlated with the Ki-67 proliferation index, suggesting ARTD17 is a possible negative regulator for cell growth [94].

ARTD15 (PARP16) is the only ARTD family member with a C-terminal transmembrane domain. It localizes at nuclear envelope and the membrane of endoplasmic reticulum (ER) [97]. It has mono ADP-ribosylation activity. Recently, ARTD15 was reported to bind and ADP-ribosylate Karyopherin- β 1/importin- β 1 for nuclear protein transportation [97]. It has also been found that the enzymatic activity of ARTD15 is upregulated during ER stress when it is auto ADP-ribosylated or ADP-ribosylates PERK and IRE1 α [98]. The C-terminal luminal tail of ARTD15 is required for its function during ER stress, suggesting that ARTD15 transduces stress signals to the cytoplasmic catalytic domain [98].

ARTD10 (PARP10) contains an N-terminal RRM (RNA recognition motif), a NES (nuclear export sequence), two tandem UIMs (ubiquitin interaction motifs) and a C-terminal CAT domain. It is conceivable that some of these domains may mediate the ADP-ribosylation of specific substrates in different compartments, which renders ARTD10 suitable for wide set of processes in cells. Interestingly, the UIMs of ARTD10 bind to K63-linked poly-ubiquitin, a modification that is essential for NF- κ B signaling. ARTD10 has been shown to inhibit the activation of NF- κ B and downstream target genes in response to interleukin-1 β and tumor necrosis factor- α [99]. The molecular mechanism of the activation of ARTD10 remains elusive as oligonucleotide is not required for its activation. As ARTD10 shuttles between cytoplasm and nucleus, multiple substrates have already been revealed, such as histones, c-MYC, GSK3 β , NEMO and PCNA [99-102].

3. FUNCTION OF PARYLATION IN DNA DAMAGE

Although ADP-ribosylation has been identified for more than half a century, the biological function of ADP-ribosylation, especially mono ADP-ribosylation, remains elusive. Compared to mono ADP-ribosylation, PARYlation is a bulky and highly negatively charged posttranslational modification. PAR has been shown to regulate protein-protein or protein-nucleic acid interaction, function as a scaffold to mediate the recruitment of DNA damage response factors, and regulate protein degradation via PAR-binding E3 ligases.

PARYlation has been shown to participate in DNA damage response, gene transcription, DNA replication, cell cycle regulation, ageing, intercellular transport and apoptosis/ necrosis [6]. Especially upon DNA binding, PAR synthesis could be magnified to 1700 fold above basal level *in vitro* [48]. Thus, the role of PARYlation has been most extensively studied. Here, we summarize the identified function of PARYlation in DNA damage response, including chromatin remodeling surround the sites of DNA damage, recruiting DNA damage repair machineries, and crosstalk with other DNA damage-induced posttranslational modifications.

3.1. Decondensation of Chromatin

As a founder member of ARTDs, ARTD1 is an abundant protein and associated with chromatin. Enzymatically silent ARTD1 suppresses gene transcription by contributing to the condensation of chromatin, which creates a barrier against gene transcription. This is supported by the evidence from an *in vitro* assay in which purified chromatin become more compacted in the presence of recombinant ARTD1 [103]. Moreover, the presence of inactive ARTD1 in heterochromatin regions, such as telomeres, facilitates the maintenance

of the repressive DNA state [104]. However, immediately following DNA damage, ARTD1 and ARTD2 are activated by DNA ends, and could use up to 90% cellular NAD⁺ to generate a huge amount of PAR at the sites of DNA damage [105, 106]. The highly negatively charged PAR chains repulse nucleotide and relax chromatin surround DNA lesions, mimicking the effects of linker histone H1 depletion [107]. Electron microscopic studies further demonstrated that PAR results in opening and relaxation of the tightly coiled nucleosome structure in the isolated chromatin [107]. Similar to DNA damage response, activation of ARTD1 also relaxes chromatin during gene transcription. In the study of stress-induced gene activation in *Drosophila*, it has been reported that ARTD1 was activated by steroids and environmental stress [108]. Synthesized PAR facilitates the removal of transcription barriers and induces chromatin remodeling for active gene transcription during development [108].

3.2. Recruitment of DNA Damage Response Factors

Besides directly inducing chromatin remodeling through negative charges, PARylation mediates the recruitment of DNA damage response factors to DNA lesions via PAR-binding domains. To date, 7 PAR recognition modules have been found to mediate PAR-dependent protein interaction: the Macro domain, the PAR-binding zinc finger (PBZ), the WWE domain, the BRCT domain, the FHA domain, the OB-fold domain and the RRM domain. Although a putative PAR-binding motif (PBM) has been suggested to interact with PAR, the PBM with a short putative peptide sequence is much degenerated and is unlikely to form a folded structure to interact with PAR. Moreover, the putative PBM in XRCC1 is actually a part of the BRCT domain. The short peptide alone does not fold well into any secondary structure. Additional study suggests that the BRCT domain of XRCC as a whole is a PAR-binding module [109]. Thus, in this review, we do not include PBM as a PAR-binding module.

3.2.1. The Macro Domain—The Macro domain is the first identified PAR-binding module. It contains 140–190 residues and is able to recognize one ADP-ribose residue [84]. The Macro domain was initially identified in histone variant macroH2A [110]. Other proteins containing the Macro domain include ARTD7, 8, 9, MacroD1, MacroD2, MacroD3, ALC1 and TARG1 [111]. ALC1 (aka CHD1L) is a SNF2-like ATPase, and is rapidly recruited to the sites of DNA damage following PARylation. As an ATP-dependent chromatin remodeler, ALC1 forms nucleosomal intermediate with ARTD1 and histones, and promotes PAR-dependent chromatin relaxation at DNA lesions [112]. Depletion of ALC1 sensitizes cells to DNA damage reagents [113]. MacroD1/2 and TARG1 have terminal ADP-ribose glycohydrolase activity and are recruited to the sites of DNA damage rapidly [29, 30]. Although the molecular mechanism of these enzymes in DNA damage repair have not been extensively studied, it is likely that these enzymes participate in PAR removal from DNA lesions.

3.2.2. The PAR-Binding Zinc Finger (PBZ)—The PBZ domain is another small PAR-binding module, which recognizes tandem ADP-ribose residues [114]. It has been found in various proteins, such as DNA damage repair machineries APLF and CHFR [114, 115]. APLF (Aprataxin-PNK-like factor) is an apurinic-apyrimidinic (AP) endonuclease with a

high binding affinity for PAR [116]. Depletion of APLF impaired DNA damage repair following ionizing radiation [116]. Checkpoint with Forkhead-associated and Ring finger domain protein (CHFR), is an E3 ubiquitin ligase and found to be recruited to DSBs by PAR [114, 117]. Via PAR recognition, CHFR ubiquitinates ARTD1 for its displacement from the sites of DNA damage. Thus, CHFR is an important E3 ligase which links PARylation and ubiquitination pathways during DNA damage response [117].

3.2.3. The WWE Domain—Recently, a set of WWE domain-containing proteins have been identified to bind the linker region between two ADP-ribose residues [118-120]. One of them is an E3 ubiquitin ligase RNF146/Iduna. RNF146 recognizes Tankyrase-induced PARylation on AXIN1/2 and ubiquitinates AXIN1/2 for proteasome-dependent degradation [65]. Moreover, RNF146 is recruited to the site of DNA damage and participate in DNA damage repair [121, 122].

3.2.4. The BRCT Domain and the FHA Domain—The BRCT domain and FHA domain are originally known as phospho-protein binding domain. It has been suggested that the FHA domains recognize phosphor-Thr motifs [123-125] and BRCT domains recognize phosphor-Ser motifs [126, 127].

Unexpectedly, we found that the BRCT domains of BARD1 recognize ADP-ribose [128]. Upon DNA damage, the interaction between the BARD1 BRCT domain and PAR mediates the fast recruitment of the BRCA1/BARD1 complex to the sites of DNA damage thus promoting the homologous recombination repair. Similarly, we found that the BRCT domains in Ligase IV can also bind to ADP-ribose and mediate the fast recruitment of Ligase IV to DNA lesions, which is likely to promote NHEJ [109]. Another BRCT domain containing protein, NBS1 is a subunit in the MRN complex that activates ATM in response to DSBs. We find that the BRCT domain of NBS1 binds to DNA damage-induced PAR, which mediates the fast recruitment of the MRN complex to DSBs and facilitates the early activation of ATM-dependent signal transduction as well as cell-cycle checkpoints [109].

Besides BRCT domains, we also find that the FHA domains in PNKP and APTX bind to PAR, which is important for their early recruitment to DNA lesions. Interestingly, these two FHA domains recognize the iso-ADP-ribose, the linker of PAR, instead of ADP-ribose [109]. A chemical feature of iso-ADP-ribose is that it has phosphate group on each site. Since the BRCT domain and FHA domain are originally known as phospho-amino acid binding domains, it is likely that the BRCT domain and the FHA domain recognize the phosphate group in ADP-ribose and iso-ADP-ribose respectively.

3.2.5. The OB-Fold Domain and the RRM Domain—Since chemical structure of PAR is similar to oligonucleotide, it is possible that oligonucleotide-binding domains may recognize PAR. The oligonucleotide/oligosaccharide-binding (OB) fold is a single-strand DNA/RNA binding motif in prokaryotes and eukaryotes [129]. We find that the OB-fold domain of hSSB1 recognizes iso-ADP-ribose and mediates the fast recruitment of hSSB1 to the DNA lesions. We also screened other OB-folds and found that several of them could also recognize PAR, suggesting a set of OB-fold domains serve as a PAR-binding domain [130].

Recently, a RNA-binding protein NONO was identified as a novel PAR-binding protein [131]. It has two tandem RNA recognition motifs (RRM) at the N-terminus, while only the first RRM has strong affinity for PAR. Its fast recruitment to the DNA damage sites through PAR-binding is essential for its function in NHEJ, although the detailed mechanism is still unclear. Another example of PAR-binding RRM is the RRM in Alternative Splicing Factor/ Splicing Factor 2 (ASF/SF2) [132]. It also contains two tandem RRM at the N-terminus, but only the first RRM is able to bind PAR. ASF is a RNA splicing factor, which is regulated by the kinase activity of DNA topoisomerase I. When binding PAR, ASF becomes a poor substrate of DNA topoisomerase I, which suppresses its function in RNA splicing.

3.3. Crosstalk Between ADP-Ribosylation and Other Posttranslational Modifications

ARTs are able to transfer ADP-ribose to lysine, arginine, glutamate, aspartate, cysteine, and serine residues [14-25]. However, besides ADP-ribosylation, other post-translational modifications, including phosphorylation, ubiquitination, sumoylation, methylation, and acetylation may also modify the same sites or adjacent sites on the same targets. Thus, crosstalk between these post-translational modifications may occur during different biological processes.

Different modifications carry out different functions during gene transcription, DNA damage repair and chromatin remodeling. With improved mass spectrometry, more and more ADP-ribosylation sites have been revealed. Recent studies showed that H2AK13, H2BK30, H3K27, K37, and H4K16 could be ADP-ribosylated [21]. Interestingly, H4K16 could also be acetylated, which induces chromatin relaxation. Since acetylation competes with ADP-ribosylation at H4K16 [21], deacetylation by SIRT1 [133] may facilitate ARTD1-dependent PARylation of H4K16.

As mentioned above, PARylation also regulates protein ubiquitination and proteasome-dependent degradation. Both CHFR and RNF146 are PAR-binding E3 ubiquitin ligases. Both E3 ligases recognize poly ADP-ribosylated substrates and catalyze ubiquitination following PARylation. Thus, PARylation is an activator for protein ubiquitination and degradation.

4. SUMMARY

Studies over the past few years have gradually uncovered the molecular mechanisms and biological functions of ARTs in multiple biologic processes. ADP-ribosylation changes the chemical and physical characters of the substrate, and regulates protein-protein or protein-DNA interaction. The function of PARylation is relatively better understood, but the function of mono ADP-ribosylation is still largely unknown. Studies on writers, readers and erasers of mono ADP-ribosylation are required for elucidate its role in a variety of biological processes.

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Fig. (1).

The classification and domain architecture of human ARTDs. X: CAT domain without ADP-ribosyltransferase activity; ZF: zinc finger; BRCT: BRCA1 C-terminus domain; WGR: Trp-Gly-Arg domain; CAT: catalytic ADP-ribosyltransferase domain; DBD: DNA-binding domain; HPS: His-Pro-Ser domain; ARC: ankyrin repeats cluster; SAM: sterile alpha motif; TPH: TIPARP homologous domain; VIT: vault inter- α -trypsin domain; vWA: von Willebrand factor type A domain; MVP-BD: major vault protein binding domain; RRM: RNA recognition motif; NES: nuclear exporting sequence; UIM: ubiquitin interaction motif.

Table 1

Summary of human ARTs.

Subfamily	Name	Aliases	Size (aa)	Subcellular Localization	Triad Motif	Key Domains	Activity	
DNA dependent	ARTD1	PARP1	1014	Nucleus	H-Y-E	zinc fingers, BRCT, WGR WGR, DBD WGR	P	
	ARTD2	PARP2	583	Nucleus and cytoplasm	H-Y-E		P	
	ARTD3	PARP3	533	Nucleus and cytoplasm	H-Y-E		M	
Tankyrase	ARTD5	PARP5a, TANK1	1327	cytoplasm cytoplasm	H-Y-E	Ankyrin repeat Ankyrin repeat	P	
	ARTD6	PARP5b, TANK2	1166		H-Y-E		P	
CCCH Zn Finger	ARTD14	PARP7, tiPARP	657	Nucleus and cytoplasm cytoplasm cytoplasm	H-Y-I	zinc fingers, WWE zinc fingers, WWE zinc fingers, WWE	M	
	ARTD12	PARP12 PARP13, ZC3HAV1	701		H-Y-I		M	
	ARTD13		902		Y-Y-V		I	
Macro	ARTD9	PARP9, BAL1	854	Nucleus and cytoplasm Nucleus and cytoplasm ND	Q-Y-T	macro domain macro domain, WWE macro domain	I	
	ARTD8	PARP14, BAL2	1801		H-Y-L		M	
	ARTD7	PARP15, BAL3	678		H-Y-L		M	
unclassified	ARTD4	PARP4	1724	Nucleus and cytoplasm cytoplasm	H-Y-E	BRCT RRM, UIM WWE	M	
	ARTD17	PARP6	630		H-Y-Y		M	
	ARTD16	PARP8	854	cytoplasm	H-Y-I		M	
	ARTD10	PARP10	1025	Nucleus and cytoplasm	H-Y-I		M	
	ARTD11	PARP11	331	Nucleus and cytoplasm	H-Y-I		M	
	ARTD15	PARP16	322	cytoplasm	H-Y-I		M	
tRNA phosphotransferase	ARTD18	TRPT1, TpT1	253	cytoplasm	H-H-V			
ecto-ARTs	ARTC1	ART1	327	ER, plasma membrane plasma membrane, ER plasma membrane extracellular	R-S-E		M	
	ARTC2P	ART2P	389		K-L-V		M	
	ARTC3	ART3	314		G-S-E		M	
	ARTC4	ART4	291		R-S-E			M
	ARTC5	ART5						M

Note: P, PARylation; M, mono ADP-ribosylation; I, inactive enzyme; ND, not determined.