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Review Article

The function and regulation of ADP-ribosylation in the DNA damage response

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ADP-ribosylation is a post-translational modification involved in DNA damage response (DDR). In higher organisms it is synthesised by PARP 1–3, DNA strand break sensors. Recent advances have identified serine residues as the most common targets for ADP-ribosylation during DDR. To ADP-ribosylate serine, PARPs require an accessory factor, HPF1 which completes the catalytic domain. Through ADP-ribosylation, PARPs recruit a variety of factors to the break site and control their activities. However, the timely removal of ADP-ribosylation is also key for genome stability and is mostly performed by two hydrolases: PARG and ARH3. Here, we describe the key writers, readers and erasers of ADP-ribosylation and their contribution to the mounting of the DDR. We also discuss the use of PARP inhibitors in cancer therapy and the ways to tackle PARPi treatment resistance.

Introduction

DNA plays an essential role in cell information storage. It contains the blueprints for the synthesis of the molecules necessary for life. Therefore, maintenance of this molecule is essential for organisms' survival. DNA's unique role means that it cannot be replaced when damaged, as other macromolecules are. Instead, it needs to be repaired. To this end, multiple mechanisms have evolved to repair the different types of damage that DNA can sustain. As with most cellular functions, DNA damage repair is regulated through post-translational modifications (PTMs), which allow for tight temporal control of the response. Many different PTMs are involved in the DNA damage response (DDR) and understanding their dynamics helps our understanding of how the cell maintains genomic stability. Failure to successfully maintain the genome integrity can lead to the development of various disease states. Deficiencies in DDR have been indicated to be involved in carcinogenesis, immunodeficiency and neurodegeneration [1,2]. Thus, understanding the players involved in mounting a successful DDR is recognised to be of great importance in the biomedical sciences community.

A PTM important in the DDR is ADP-ribosylation, a reversible covalent attachment of ADP-ribose (ADPr) moieties to proteins. In humans, the largest protein family that can synthesise ADPr are Poly (ADP-ribose) Polymerases (PARPs) [3]. ADPr can be added in chains (PAR) or as a single molecule (MAR). The PAR chains are more transient and the hydrolytic activity of the Poly(ADP-ribose) Glycohydrolase (PARG) enzyme swiftly turns them back into mono-ADPr tags that can stay on chromatin for longer [4,5]. The ADPr modification can be placed on different amino acids on the protein. While serine is the most common target in DDR [6–10], glutamate/aspartate is also modified [11,12] and some studies suggest tyrosines as possible sites [13–15]. The PAR chains can be branched and there is some evidence that the length and branching of the chain have implications for the functionality of the cell, suggesting the existence of a 'PAR code' [16–18]. ADPr modification could be also remodelled by the addition of an ubiquitin moiety which is catalysed by the deltex family of E3 ligases [19]. Altogether, ADPr is a very diverse modification that regulates many different processes including transcription, metabolism, immune response and DDR [20–23]. ADPr has been suggested to play a role in almost all DDR pathways. In fact, it has been demonstrated that upon DNA damage, one-third

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of the proteins present in the nucleus are ADP-ribosylated, mostly on serine residues [24]. All of this taken together makes understanding ADPr signalling critical in understanding way in which cells maintain genome integrity.

DNA damage response PARPs

Of all the 17 PARP family proteins, only PARP1–3 act as DNA damage sensors. PARP1 and PARP2 can catalyse the formation of PAR chains on proteins and have a partially redundant function [25,26]. PARP1 is thought to be the most robust ADPr writer in response to DNA damage. It is a very abundant nuclear protein and is responsible for the majority of ADPr upon exposure to DNA-damaging agents [27,28]. PARP1 is the founding member of the family as it was the first one to be successfully purified and characterised [29–31]. The protein contains a variety of domains: three zinc finger domains on the N-terminal which allow for binding to DNA breaks; a WGR domain which also interacts with the DNA; a BRCT domain which can interact with DNA, PAR or other proteins; an automodification loop and a catalytic domain on the C-terminal (Figure 1) [32–35]. The catalytic domain contains an autoinhibitory domain which prohibits PARP1 enzymatic activity unless it is bound to DNA breaks [36–39]. PARP1 is a DNA damage sensor which is quickly recruited to the site of damage and PARylates histones, itself and other surrounding proteins to create a scaffold to recruit DDR factors. PARP1 recognises the breaks in the DNA backbone and binds to them [40]. Since the DNA backbone is disrupted in a variety of situations, PARP1 can be involved in different DDR pathways [41].

PARP1–3 are activated by both single- and double-stranded DNA breaks [38,42–44]. The breaks may arise directly, as intermediates in various DDR pathways such as Base Excision Repair (BER) or as intermediates in replication. In all cases, PARP1 binds to the DNA which triggers a conformational change in its autoinhibitory HD domain [32,45]. This change uncovers the NAD^+ binding site, allowing for efficient NAD^+ binding and hydrolysis [46]. After binding to the DNA, PARPs can effectively PARylate themselves and neighbouring histones to recruit DDR factors to the site. The PAR chains can be recognised by a variety of reader domains. One of the key readers of PAR in the context of DDR is X-ray repair cross-complementing protein 1 (XRCC1), a scaffold protein which recruits DNA repair ligase and DNA end processing factors to DNA breaks [47]. Another aspect of PARP-regulated activity during DDR is regulation of chromatin structure. Upon DNA damage, chromatin relaxes in a PARP1-dependent manner. This is thought to occur through the activity of ALC1, a helicase, and Aprataxin-and-PNK-like factor (APLF), a histone chaperone, amongst other actors [48–52].

However, loss of PARP1 does not result in a dramatic phenotype of genomic instability [53]. This is likely due to the presence of PARP2 activity in the cells. Double knockout of PARP1 and PARP2 leads to embryonic lethality in mice, demonstrating that the enzymes have somewhat redundant activities [54]. PARP2 is a PARP1 homologue that lacks the N-terminal Zinc finger domains of PARP1 (Figure 1). Instead, it contains an unstructured N-terminal region (NTR) whose function in DDR is not fully defined, though it has been suggested that NTR plays a role in PARP2 recruitment to PAR [55,56]. However, PARP2 is still able to bind to DNA breaks through its WGR domain [44,55]. The dynamics of PARP2 association with DNA breaks are different from those of PARP1. PARP2 recruitment to the breaks is slower and it is retained on the damage site longer than

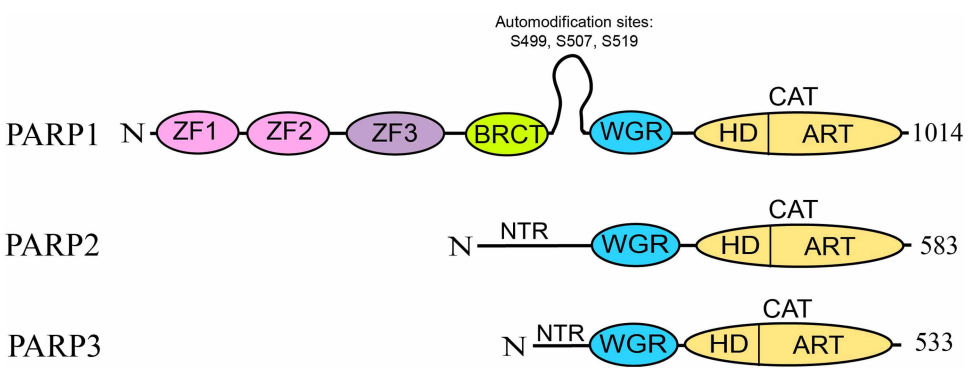

Figure 1. Domains of PARP1-3.

The number of amino acids indicated on the right. ZF, Zinc-finger; BRCT, BRCA1 C-terminal domain; WGR, nucleic acid binding domain; HD, helical domain; ART, ADP-transferase domain. Adapted from Luscher et al. [3].

PARP1 [57]. These subtle differences suggest that, while PARP1 and PARP2 roles in signalling overlap they contribute differently to the synthesis of PAR chains. PAR synthesised by PARP1 has been demonstrated to activate PARP2 which then synthesises branched polymers on the linear PARP1-derived PAR chains [56]. Currently, very little is known about how the branching is controlled and how it contributes to the recruitment of different DNA damage factors [17]. Additionally, cryo-EM structural studies suggest that PARP2, together with its accessory protein HPF1, is able to bridge the double-stranded break and hold the two strands together acting not only as the sensor of the break but also contributing to the first stage of the repair process [58]. This might explain why PARP2 is reported to stay longer on the chromatin after DNA damage.

Similarly to PARP2, PARP3 contains only the NTR, WGR and the catalytic domain (Figure 1) but unlike PARP2, it is only capable of attaching a single ADP-ribose moiety to proteins [44,59]. The role it plays in DDR is not clear but it has been reported to act in both single-stranded break (SSB) repair and Non-Homologous End Joining (NHEJ) [60,61]. A potential function in gene regulation has also been proposed [62]. In DDR, it is known to MARylate some of the key proteins such as Ku70 and PARP1, as well as histones [59,60,63]. Its interactions with Ku-proteins and PARP1 seem to be important for the regulation of the c-NHEJ pathway for repairing DSBs [61,64]. The exact mechanisms by which PARP3 participates in those pathways remain to be elucidated.

Serine is the most common ADP-ribosylation site

Historically, PARP1-3 were thought to primarily modify aspartate and glutamate residues, though Glu/Asp-linked ADPr has been mechanistically not well understood [12,26,65,66]. In the last few years, it has been demonstrated that ADP-ribosylation of serine residues in proteins is the most common form of ADPr upon DNA damage [6] and it predominantly targets proteins relevant for genome stability. It was likely unnoticed throughout the years because *in vitro* PARP1/2 almost exclusively PARylate glutamate and aspartate, and therefore serine-ADPr was not considered a possibility during earlier mass spectrometry studies. This changed with the discovery of Ser-ADPr in cells and the characterisation of the Histone Parylation Factor 1 (HPF1), an accessory protein which interacts with PARP1/2 [67,68]. The HPF1 makes a composite active site together with PARP1 or PARP2 which dramatically increases the efficiency of DNA damage induced ADP-ribosylation and changes PARP specificity to allow for PARylation of serine residues [8,67,69]. This change of specificity is possible because HPF1 completes the catalytic domain of PARP1 providing an additional glutamate residue which allows for deprotonation of serine. This is necessary for serine modification but does not happen during aspartate/glutamate ADP-ribosylation [69,70]. Through this, HPF1 interacts with PARP1/2 to promote PARylation of histones and facilitate DDR [8,9]. In line with its function, loss of HPF1 is associated with sensitivity to DNA-damaging agents such as methyl methanesulfonate (MMS) as well as PARP inhibitors [35,67]. As well as switching PARP 1/2 specificity towards serine, HPF1 limits the enzyme's ability to form PAR chains through steric hindrance of the binding pocket necessary for the recognition of the new acceptor NAD^+ during chain elongation [67,69]. This results in two distinct steps in the PARylation process in DDR: HPF1-dependent initiation of the chain; and HPF1-independent elongation [71]. This model is supported by data showing that once histones have been primed by HPF1/PARP1 complex, PARP1 is sufficient to create PAR chains [71,72]. New data suggests that by promoting shorter chain synthesis on the histones, HPF1 promotes PARP1-dependent chromatin relaxation during DDR [73]. Finally, HPF1 restricts PARP1 NAD glycohydrolase activity [74].

In addition to PARylating histones and other proteins in trans, PARP 1/2 also heavily automodify themselves upon DNA damage [8,75]. This activity can act partly as further scaffolding for the recruitment of DDR factors but it is mainly a part of negative feedback loop. AutoPARylation is key for PARP1/2 dissociation from the DNA which allows the repair factors to reach the break site. There are three serine residues on PARP1 that are key targets of automodification [8,35]. This mechanism is exploited by PARP inhibitors which, by blocking PARP 1/2 activity, take away its ability to dissociate from the break site — effectively 'trapping' it on the DNA [35,76–78]. This prevents the repair factors from successfully repairing DNA and leads to further breaks upon the collapse of the replication and transcription machinery with the trapped PARPs [77–80].

PARP/HPF1 also targets for Ser-ADPr many other proteins involved in genome stability, including various DNA repair factors, and proteins involved in chromatin structure regulation, transcription, RNA metabolism and mitosis [9,81]. It has been shown that HPF1-dependent ADPr is necessary for the recruitment of DNA Ligase III to Okazaki fragments [82]. And since many other proteins have been reported to be ADP-ribosylated on serine residues [8,9,14], it is likely that HPF1 and Ser-ADPr play an important role in different repair pathways and

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various aspects of genome stability. It is unclear how the PARP1/2 interaction with HPF1 is regulated. One proposed mechanism is through HPF1-dependent ADP-ribosylation by PARP1 [15]. Also, other factors could modulate PARP1/HPF1 interaction or activity such as the mitotic factor TPX2 and CARM1 [83,84].

Additionally, there is an emerging new target for ADPr — nucleic acids [85,86]. *In vitro* studies have shown that mammalian DDR PARPs are capable of modifying DNA breaks at phosphate groups [87–91]. Additionally, low levels of adenosine PARylation have been reported in human cells. This modification is likely catalysed on the bases in single-stranded DNA rather at the terminal phosphates as reported previously [92]. It has also been demonstrated that TARG1, a mammalian ADPr hydrolase, is capable of cleaving DNA–ADPr in cells [93]. It is currently unclear whether this activity of PARPs has any physiological roles or if it is an off-target, pathological event. However, the fact that a variety of mammalian hydrolases have been observed to be capable of removing ADPr from DNA [87] and that reversible DNA–ADPr signalling has been characterised in bacteria [94] suggests a potential for DNA–ADPr to play a role in DDR signalling. Suggested roles for this process include the protection of the DNA ends from nuclease activity or the inhibition DNA repair during the apoptotic response [87]. Further studies are necessary to determine whether DNA–ADPr is a lesion or a normal part of the DDR.

Readers of ADPr

As explained above, PARP1 acts as a sensor of DNA damage and it ADP-ribosylates histones to recruit DDR factors. Apart from better characterised ADPr/PAR-binding domains; PBM, PBZ, WWE and macrodomain [95], domains such as Forkhead-associated (FHA) or BRCA1 C-terminal (BRCT) can also interact with ADPr [96,97]. Many of these domains were originally discovered as readers of protein phosphorylation or nucleic acids and have also now been confirmed to interact with PAR. This variety of ADPr-recognizing domains allows for the recruitment of numerous factors important for DDR.

XRCC1 is a scaffold for DNA repair ligase III complex and is involved in single-strand break repair, BER and Nucleotide Excision Repair (NER) [98]. It interacts with DNA ligase via its C-terminal BRCT domain [99,100] and with DNA polymerase beta via its N-terminus [101]. It has a phosphorylated epitope that binds to either Aprataxin, APLF or polynucleotide phosphatase/kinase (PNKP) [98]. XRCC1 is capable of binding ADPr through its first BRCT domain [102,103]. Consequently, XRCC1 is quickly recruited to the DNA damage site in a PARP1/2-dependent manner [104].

APLF is an ADPr reader with a PBZ domain on the C-terminus [105]. It accumulates on the DSB sites in a PARP1-dependent manner [106]. While some studies report that it primarily binds PARP2-dependent branched ADPr, others have observed preferential binding to linear chains [17,56]. It is suggested to work with PARP3 to resolve γH2AX foci and thus aid in the repair of DSBs [61]. APLF can interact with both XRCC1 and XRCC4 [61,107]. It promotes the retention of the XRCC4/DNA ligase IV complex in chromatin thereby promoting repair by non-homologous end joining proteins [61]. In addition to being involved in the recruitment of DDR factors, APLF has been observed to act as a histone chaperone [50,108,109], although the exact role of this activity in DDR is not fully understood.

ALC1 (also referred to as Chromodomain-helicase-DNA-binding-protein-1 like (CHD1L) in the literature) is a chromatin remodelling enzyme that requires adenosine triphosphate (ATP) to induce chromatin relaxation in response to DNA damage [48]. It contains a macrodomain and has been demonstrated to be recruited by PARP1/2 in response to laser irradiation and UV radiation *in vivo* [48,110,111]. ALC1 binds to PARylated nucleosomes and its chromatin remodelling activity is crucial for DDR: the relaxation of chromatin is key to allowing DDR factors to gain access to the DNA damage site [49,112,113]. ALC1 loss is synthetically lethal with the defects in repair by HR, as ALC1 loss is associated with chromosome instability caused by unrepaired DNA gaps at replication forks [114]. ALC1 overexpression leads to sensitivity to X-irradiation and has been associated with cancer progression [115]. ADP-ribosylation can be recognised by a variety of other DDR factors and detailing all of them is beyond the scope of this review [96,116].

Erasers of ADPr

ADPr in response to DNA damage is under tight temporal control. The PAR chains only stay on the chromatin long enough to recruit the downstream factors to the site. Afterwards, they need to be removed from the area so that the factors can interact with the damaged DNA and repair it [117]. This fine control requires specific factors that can remove the ADPr signal in a timely manner. This is also important because long PAR chains are toxic to the cell long term, as it progresses through the cell cycle [71]. The production of ADPr is also

metabolically costly because of the consumption of NAD^+ associated with the process. The energetic cost can be compensated by the quick degradation of the chains which releases free ADP-ribose that can be converted into ATP [118–120]. A variety of ADPr hydrolases present in the cells for that purpose. The hydrolases can be divided into two groups: macrodomain hydrolases and ARH-type hydrolases [121]. PARG contains a macrodomain which can recognise the ribose-ribose linkage between two ADP-ribose units and cleave the bond [122–124]. This way, it can degrade the PAR chains with high efficiency. However, it is not able to remove the final ADPr that anchors the chain to the target protein [117,122,125]. This step can be performed by (ADP-ribosyl) hydrolase 3 (ARH3) [4]. That enzyme is evolutionary unrelated to PARG [126,127] and cleaves PAR chains with lower efficiency than PARG [128] but can also remove the final ADPr from the protein [4]. ARH3 is especially relevant for DDR as it is the only ADPr hydrolase that can ADPr from serine residues, where the majority of ADPr is placed upon DNA damage. Through these differing activities, the two enzymes work together to remove the ADPr chains from proteins. PARG degrades the majority of the poly-ADPr but its activity becomes lower as the chains become shorter [125]. ARH3 becomes more prominent on short chains and it removes the final unit from the protein [71,125]. This two-step degradation process of Ser-linked PAR fits together with the two-step synthesis process that was detailed earlier (Figure 2) [71]. Other hydrolyses are involved in removal of ADPr from other amino acids, for example, glutamate ADPr is reversed by terminal ADP-ribose glycohydrolase (TARG1), MACROD1 and MACROD2 [129–131].

PARG activity is essential for development in both mice and flies [132,133]. *ARH3* is not an essential gene but loss of ARH3 activity is associated with stress-induced childhood-onset neurodegeneration with variable ataxia and seizures (CONDSIAS) disorder [134–138]. The neurodegeneration is usually triggered by periods of stress or an infection. This suggests that PAR-degrading potential of ARH3 becomes particularly needed upon additional stress which triggers increased PARP activity and therefore increased PAR levels [136,137]. ARH3 deficiency in cells leads to oxidative stress sensitivity and telomere defects [71,139]. Mechanistically, it was proposed that ARH3 protects the cells from parthanatos — a PAR-associated programmed cell death. This is characterised by the overactivation of PARP1/2 upon significant DNA damage leading to the synthesis of long chains of PAR. These chains are then cleaved by PARG and released into the cytoplasm where they act as a death signal leading to the release of apoptosis-inducing factor (AIF) from the mitochondria [138,140]. ARH3 present in the cytoplasm is thought to counteract this by degrading the free-PAR that was released from the nucleus [139]. Without ARH3 present in the cell, lower levels of free-PAR are necessary to trigger parthanatos which would explain the CONDSAIS phenotype [141]. ARH3 is also located in mitochondria but the neurodegeneration phenotype appears to be due to its nuclear/cytoplasmic function [134,142]. Loss of another ADPr hydrolase, TARG1, has also been associated with neurodegeneration, highlighting the importance of proper ADPr homeostasis for cell viability [129]. TARG1 is a macrodomain type hydrolase related to PARG which is

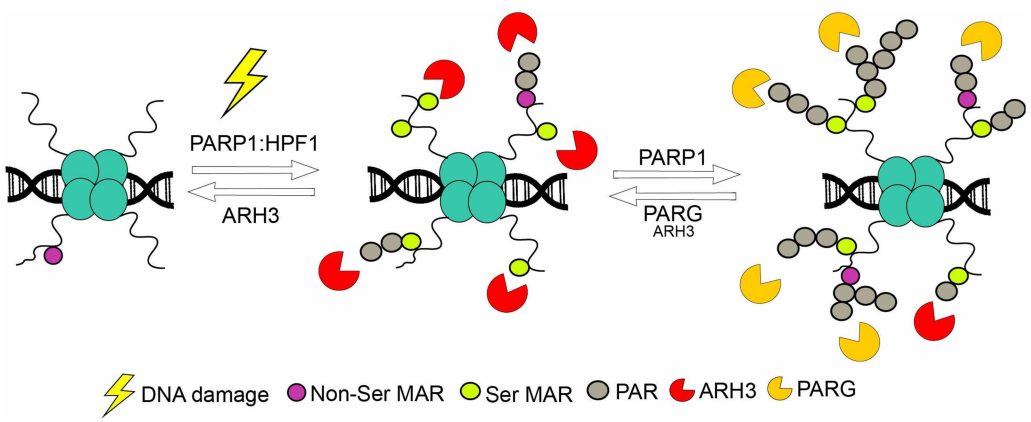

Figure 2. ADPr homeostasis.

Synthesis of ADPr during DDR occurs in two steps. First, HPF1/PARP1 complex MARylates serines. Then, PARP1 on its own creates long chains that recruit DDR factors. Degradation of PAR follows a similar pattern, long chains are degraded primarily by PARG with assistance of ARH3. However, as the chains become shorter ARH3 takes over. Only ARH3 is capable of removing MAR from serine. Adapted from Prokhorova et al. [71].

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capable of cleaving ADPr linked to glutamate [129]. Through this activity it can also release long PAR chains into the cytoplasm, possibly contributing to parthanatos.

Targeting PARP signalling in cancer therapy

Since PARP activity is so important for initiating a successful DDR, inhibitors of PARPs have been developed to selectively target Breast Cancer gene (BRCA)-deficient tumours [143–148]. This is possible because the inhibition of PARP activity decreases PARP's ability to dissociate from the DNA, resulting in DNA–PARP complexes which induce DSBs upon replication [76,149]. BRCA-deficient cells do not possess efficient Homologous Recombination (HR) that would be able to repair the breaks which explains the selectivity of the drug [78]. The trapping occurs because autoPARylation is necessary for PARP dissociation from the DNA, hence the inhibition of PARP causes the persistence of PARP on the DNA [35,76]. These observations have led to the development of numerous PARP inhibitors (PARPi) which inhibit PARP activity by competitive binding to the NAD^+ binding pocket [78,147,150,151]. However, structural studies also suggest a mechanism of reverse allostery that contributes to trapping, suggesting an additional mechanism of action for certain PARPi [46,152,153].

Originally PARPi have been shown to selectively affect BRCA1/2-deficient cells and they are now used for treatment of such cancers [154–156]. BRCA1/2 are not the only HR factors whose loss confers sensitivity to treatment. Indeed, studies demonstrate that Olaparib treatment can be effective in BRCA wild-type cancers [157]. This has been reflected in approvals for use of PARPi treatment in cancers that are platinum-sensitive, regardless of their BRCA status. Current research aims to better understand PARP signalling to further identify sensitivity markers for PARPi treatment in order to increase its therapeutic potential [158]. Indeed, the loss of some of the downstream factors of PARP1 signalling such as XRCC1 and ALC1 has already been demonstrated to sensitise cells to PARPi treatment [114,159,160] (Table 1); however, this has not been yet confirmed by any clinical trials to our knowledge. Interestingly, loss of PARP1 accessory factor HPF1 has also been observed to confer PARPi sensitivity [35,67,69]. It is important for autoPARylation of three serine residues which are key for PARP dissociation from the DNA [35]. Currently, it is unclear if HPF1 is a clinically relevant marker but it could serve as a potential target for combination therapy.

While the development of PARPi has been a big success, most tumours eventually develop resistance to the treatment. This can occur through multiple mechanisms, including increased drug efflux, the restoration of HR through BRCA reversion or the suppression of NHEJ [161,162]. As the toxicity of PARPi is mostly attributed to the trapping of PARPs, the loss of PARP1 and PARP2 is another major resistance mechanism [163–165]. Recently, it was suggested that modulation of other ADPr signalling enzymes, such as ALC1 could lead to changes in the response to PARPi and thus resistance [160]. Loss of PARG activity and resistance to PARPi

Table 1 Effects of changes in activity of ADPr regulators or binders on response to PARPi

Gene	Change	Effect	Clinical value	References
PARG	Loss of protein activity	Resistance	Can be targeted through ARH3 inhibition	(Gogola et al. [179]), (Gomez et al. [166])
ARH3	Loss of protein activity	Resistance	Can be targeted through PARGi inhibition	(Prokhorova et al. [71]), (Prokhorova et al. [35]), (Ipsen et al. [164])
PARP1	Loss or mutations of sites	Resistance	-	(Pettitt et al. [163]), (Ipsen et al. [164])
PARP2	Loss	Resistance	-	(Blessing et al. [165])
ALC1	Overexpression	Resistance	Possible marker	(Juhasz et al. [160])
HPF1	Loss	Sensitivity	Possible marker	(Gibbs-Seymour et al. [67]), (Prokhorova et al. [35])
ARH3	Overexpression	Sensitivity	Possible marker	(Prokhorova et al. [71])
ALC1	Loss of protein activity	Sensitivity	Possible marker	(Juhasz et al. [160]), (Hewitt et al. [114]), (Verma et al. [159])
XRCC1	Loss of protein activity	Sensitivity	Possible marker	(Demin et al. [180])

has been observed in cells exposed to chronic doses of PARPi [164,166]. More recently, the loss of ARH3 was shown to confer PARPi resistance in cell line models [35,71,164].

Loss of PARG activity in response to treatment with PARPi makes ARH3 a possible target in tumours and ARH3 inhibitors are currently being developed for this purpose [71,121,167,168]. Conversely, in theory PARG inhibitors are another emerging treatment that can selectively target cells that lose ARH3 activity in response to PARPi [71,169]. They could also possibly be utilised as a primary therapy as it has been demonstrated that certain replication defects confer PARGi sensitivity [170–173]. The exact mechanism of this sensitivity is being elucidated and possible markers are being identified [174,175]. This is a promising avenue of cancer therapy that can hopefully fill the therapeutic gaps left by PARPi. PARG inhibitors have recently entered clinical trials (IDEAYA Biosciences).

Future directions

The discovery of HPF1 and the importance of serine as a target of ADPr were significant advancements in our understanding of ADPr signalling in the DDR. However, many unanswered questions remain. What are the functional consequences of specific serine ADP-ribosylation modifications on hundreds of different proteins/sites? What is different about the glutamate ADP-ribosylation modifications? While we have a general picture of the canonical ADPr response, there are still a lot of details that remain elusive. Does DNA–ADPr occur in a physiological manner in mammalian cells or is it a pathological lesion [176]? There is also emerging evidence suggesting that other members of the PARP family are involved in the DDR [177,178]. Altogether, there is still a lot to uncover in the world of ADP-ribosylation. A variety of new protein players and potential drug targets are coming into view. Identification of new markers brings hope for expanding the therapeutic potential of already available PARPi treatments. Better understanding of PARPi resistance can aid clinicians in devising better treatment plans that address the issue. Since ADPr signalling is so fundamental to genome stability, targeting other factors in the pathway has potential to have therapeutic benefit. PARG inhibitors have recently entered clinical trials and ARH3 inhibitors are being developed. The clinical implications reach beyond cancer therapy as the role of ADPr in neurodegeneration is being better understood. The stage has been set for discovering the full picture of ADP-ribosylation signalling in DDR.

Perspectives

- DNA repair PARPs act as sensors of DNA breaks and regulate different aspects of the DDR to maintain genome stability. PARPs are involved in the recruitment of DDR factors, chromatin remodelling and can mediate cell death upon overactivation.
- Our understanding of ADPr in the DDR has been expanded by the discovery of serine ADPr and the recent identification of HPF1 as an important factor that changes the specificity of PARP1/2 towards serine residues both on histones and PARPs. However, the exact mechanisms and roles of Ser-ADPr remain elusive and need to be further studied.
- It is emerging that modulation of ADP-ribosylation signalling can alter the efficacy of clinical PARPi therapy. Identification of biomarkers which would modulate PARPi sensitivity will be essential for efficient treatment with PARPi but development of new inhibitors for ADP-ribosylation factors eg. ARH3, ALC1, PARG or HPF1 could have big clinical implications.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
ADPr, ADP-ribosylation; AIF, apoptosis-inducing factor; APFL, Aprataxin-and-PNK-like factor; ARH3, (ADP-ribosyl)hydrolase 3; ART, ADP-ribose transferase; ATP, adenosine triphosphate; BER, Base Excision Repair; BRCA, Breast cancer gene; BRCT, BRCA1 C-terminal domain; CONDSIAS, childhood-onset neurodegeneration with variable ataxia and seizures; Cryo-EM, cryogenic electron microscopy; DDR, DNA damage response; FHA, forkhead-associated; HPF1, histone parylation factor 1; HR, homologous recombination; MAR, mono-ADP-ribose; MMEJ, microhomology-mediated end joining; MMS, methyl methanesulfonate; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NTR, N-terminal region; PAR, poly-ADP-ribose; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; PARPi, PARP inhibitors; PNKP, polynucleotide phosphatase/kinase; PTM, post-translational modification; SSB, single-stranded break; TARG1, terminal ADP-ribose glycohydrolase; XRCC1, X-ray repair cross-complementing protein 1.

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