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Review

Host poly(ADP-ribose) polymerases (PARPs) in acute and chronic bacterial infections

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ABSTRACT

Protein ADP-ribosylation is a reversible post-translational modification, which alters protein activity, localization, interactome or stability, leading to perturbation of cell signaling. This review summarizes the emerging data indicating that host cell ADP-ribosylating enzymes, poly(ADP-ribose) polymerases (PARPs), influence the course of a bacterial infection, in parallel to ADP-ribosylating bacterial toxins. Host cell PARP targeting could be an efficient therapeutic approach to treat certain bacterial infections, possibly by repurposing the approved or clinical trial PARP inhibitors developed for cancer therapy.

Post-translational modifications (PTMs) are mechanistically important to mount an appropriate phenotypic switch to an environmental change. Activity, localization, molecular interactions or stability of the modified protein are altered leading to dynamic perturbation of cell signaling. Eukaryotic cells express intracellular and extracellular enzymes catalysing protein ADP-ribosylation called ADP-ribosyltransferases (ARTs) – sirtuins, diphtheria toxin-like ADP-ribosyltransferases (ARTDs) and cholera toxin-like ADP-ribosyltransferases (ARTCs) [1,2]. ADP-ribosylation and the founding member of ARTDs, ARTD1, have been extensively studied in the context of genome stability [1]. ARTD1 is classically designated as poly(ADP-ribose) polymerase 1 (PARP1) [1,2]. Due to its central role in the DNA damage response, small-molecule inhibitors of PARP activity, e.g. Olaparib and Rucaparib, have entered clinical use in cancer treatments, in particular in patients with homologous recombination defects due to *BRCA1*/*BRCA2* mutations [1]. However, compelling evidence indicates that PARPs also regulate the sterile and infectious inflammatory response, even under conditions that are absent of apparent DNA damage [3]. A recent review summarized the functions of host cell PARPs in viral infections [4]. Here, we describe the current phenotypic data and the main underlying pro-inflammatory mechanisms of host cell PARPs in acute and chronic bacterial infections.

1. Protein ADP-ribosylation – reversible and dynamic PTM

Protein ADP-ribosylation refers to the covalent conjugation of an ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD^+) onto a substrate amino acid with simultaneous release of nicotinamide (Fig. 1) [1]. This PTM exists as mono-ADP-ribosylation (MARylation) and as linear or branched poly-ADP-ribosylation (PARylation) (Fig. 1) [1]. In human, there are two major ART enzyme families with homology to bacterial ART-toxins – intracellular, both nuclear and cytosolic, PARPs (n = 18) and extracellular, mainly membrane-bound ARTCs (n = 4) [2]. PARPs that catalyse protein MARylation (n = 12) are more common than PARPs that catalyse protein PARylation (n = 4) (Fig. 1) [1]. However, the cellular and physiological functions of MARylation are less well understood. In addition to these ARTs, certain sirtuin family deacetylases such as SIRT4 and SIRT6 are capable of MARylation [1]. In respect of the catalytic reaction it is noteworthy, in sharp contrast to bacterial ART-toxins [5], that PARPs modify a number of different target proteins onto a complex repertoire of amino acids (e.g. serine, glutamate, aspartate and lysine), even within the same target [1]. Also, PARPs typically modify themselves, in a process called auto-ADP-ribosylation [1]. All PARPs contain a single

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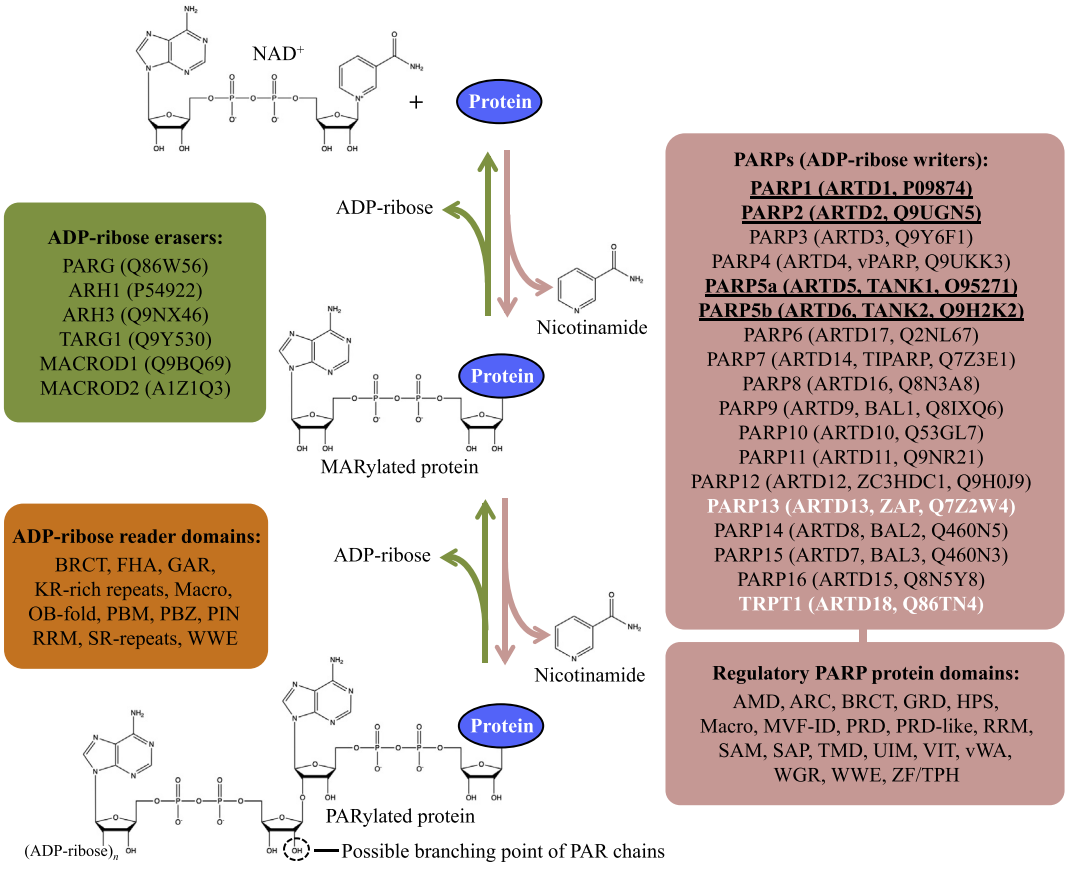

Fig. 1. ADP-ribosylation – a reversible and dynamic PTM. Protein ADP-ribosylation is catalyzed inside the cell by PARPs (ADP-ribose writers) and removed by specific enzymes (ADP-ribose erasers). UniProtKB (https://www.uniprot.org) accession numbers are given in parentheses for human ADP-ribose writers and erasers. ADP-ribosylation perturbs cell signaling frequently via interactions with various ADP-ribose reader domains present in hundreds of different proteins. 18 PARP homologs have been identified in human, and 16 of those are known to ADP-ribosylate proteins. PARylating enzymes are indicated with bold and underlined font. PARP-family members with no reported protein ADP-ribosylation activity are indicated with bold and white font. Designations of the main regulatory PARP protein domains and ADP-ribose reader domains – AMD, PARP1 auto-modification domain; ARC, ankyrin repeat cluster; BRCT, BRCA1 C-terminal; FHA, forkhead-associated; GAR, glycine-arginine-rich; GRD, glycine-rich domain; HPS, histidine-proline-serine region; KR-rich repeats, lysine-arginine-rich repeats; Macro, macrodomain; MVP-ID, major vault particle interaction domain; OB-fold, oligonucleotide/oligosaccharide-binding fold; PIN, PilT N-terminus; PBM, PAR-binding motif; PBZ, PAR-binding zinc finger; PRD, PARP regulatory domain; PRD-like, PARP regulatory domain similar to PRD; RRM, RNA recognition motif; SAM, sterile α motif; SAP, SAF-Acinus-PIAS DNA-binding domain; SR-repeats, serine–arginine repeats; TMD, trans-membrane domain; UIM, ubiquitin interaction motif; VIT, vault protein inter-α-trypsin; vWA, von Willebrand type A; WGR, tryptophan-glycine-arginine; WWE, tryptophan-tryptophan-glutamate; ZF, zinc finger domain; ZF/TPH, zing finger/Ti-PARP homologous domain.

conserved and primarily C-terminal ART catalytic domain but the rest of the protein is subject to considerable variation, including heterogenous protein domains with functional implications in the regulation of PARP activities (Fig. 1) [1]. No specific consensus sequence, similar e.g. to tyrosine phoshorylation, is known to predict how a given protein could be modified by PARPs. For a database of experimentally identified ADP-ribosylated proteins refer to ADPriboDB at http://adpribodb.leunglab.org.

ADP-ribosylation is involved in a multitude of cellular functions such as DNA damage repair, chromatin remodeling and transcription [1]. In part, ADP-ribosylation regulates these cellular functions via affecting the activity and stability of the modified protein [1]. However, downstream cell signaling responses are frequently mediated via scaffolding protein domains, also referred to as ADP-ribose readers, present in hundreds of different cellular proteins (Fig. 1) [1]. The different ADP-ribose reader domains have distinct binding modes being either directed to the terminal ADP-ribose moiety or internal ADP-ribose moieties, also including the branching points of PAR chains [1]. Most of the currently known ADP-ribose reader domains recognize PARylation [1].

ADP-ribosylation is a reversible PTM by the action of enzymes referred to as ADP-ribose erasers (Fig. 1) [1]. Poly(ADP-ribose) glycohydrolase (PARG), which belongs to the macrodomain family, is an enzyme cleaving ADP-ribose moieties of PAR chains via its exo(primary)- and endoglycosidase activities [1]. PARG also releases PAR chains [1]. However, PARG appears to be unable to remove the protein-bound proximal MAR [1]. ADP-ribose glycohydrolase 3 (ARH3) is another enzyme capable of cleaving ADP-ribose moieties of PAR chains, but, in contrast to PARG, apparently only from the terminal end [1]. In addition, ARH3 removes the protein-bound proximal MAR moiety if it is conjugated to serine [1]. ARH1 removes the MAR moiety if it is conjugated to arginine [1]. Some macrodomain family members (TARG1, MACROD1 and MACROD2) are also capable of removing the MAR moiety, but it has to be conjugated to acidic residues aspartate or glutamate [1]. It has also been proposed that TARG1 completely removes the PAR chain if it is conjugated to aspartate or glutamate [1]. ADP-ribose residues are also processed by Nudix hydrolases, which cleave the pyrophosphate bond [1]. Taken together, protein ADP-ribosylation is a reversible and dynamic PTM regulated by ADP-ribose writers, ADP-ribose readers and ADP-ribose erasers (Fig. 1).

2. Phenotypic data of PARP functions in bacterial infections

Studies on the functional role of mammalian PARPs in acute and chronic bacterial infections have almost exclusively focused on

PARP1. Two experimental strategies have yielded valuable information (Table 1), i.e. genetic PARP1 depletion and small molecule NAD^+ mimics, which inhibit the ART activity. Caution should be taken to interpret the ART activity inhibition results as many of the NAD^+ mimics, e.g. PJ34 and 3-aminobenzamide (3-AB) similar to the compounds in current clinical use in cancer treatments (e.g. Olaparib and Rucaparib), act as pan-PARP inhibitors rather than as specific PARP1 inhibitors [6]. Also, functionally relevant off-target effects on Pim kinases with PJ34 are plausible [7].

2.1. Phenotypic data with living bacteria

2.1.1. Helicobacter *gastric infection*

Helicobacter-caused chronic inflammation is a significant risk factor for the development of gastric cancer. It has been shown in an oral *Helicobacter felis* mouse model that PJ34 administration via drinking water not only prevents the formation of pre-malignant lesions, but also efficiently cures the pre-existing lesions when combined with an antibiotic therapy [8]. In this mouse model, development of pre-malignant lesions correlates with strong infiltration of T_H1-polarized IFN-γ-producing $CD4^+$ T cells into the gastric wall, which appears to be part of the physiological response to eradicate *Helicobacter* [8]. PJ34 treatment paralleled with a higher bacterial burden in the gastric wall, lower numbers of $CD4^+$ T cells in the gastric wall and lower numbers of IFN-γ-positive $CD4^+$ T cells in the mesenteric lymph nodes [8]. PJ34 inhibited the formation of IFN-γ-positive $CD4^+$ T cells from splenic $CD4^+$ T cells in an *in vitro* co-culture system with *Helicobacter*-primed dendritic cells (DCs) and secretion of IFN-γ by $CD4^+$ T cells upon CD3/CD28 cross-linking [8]. PJ34 also inhibited proliferation of $CD4^+$ T cells *in vitro* induced with CD3/CD28 cross-linking [8]. Interestingly, IFN-γ transcription *in vitro*, induced with CD3/CD28 cross-linking, took place equally well and was inhibited to similar extent by PJ34 in wild-type, $PARP1^{-/-}$ or $PARP2^{-/-}$ $CD4^+$ T cells [8]. The data imply that PJ34-sensitive PARPs other than PARP1 and PARP2 are intrinsically required for the T_H1 polarization, which appear to drive the formation of pre-malignant lesions in *Helicobacter*-infected gastric wall.

The role of PARP1 in *Helicobacter*-induced chronic gastric infection has also been studied in a conditional lysozyme-expressing myeloid cell-specific PARP1 knockout (PARP1ΔMyel) mouse model with oral *Helicobacter pylori* inoculation [9]. Gastric wall samples of PARP1ΔMyel mice contained more viable *H. pylori* bacteria as compared to the wild-type mice [9]. The infection induced infiltration of $CD4^+$ T cells into the gastric wall, similar to *H. felis* mouse model studies [8], but this phenotype was not affected by the myeloid lack of PARP1 [9]. However, the gastric wall population of $CD4^+$ T cells of infected PARP1ΔMyel mice contained significantly less IFN-γ-producing $CD4^+$ T cells [9]. Remarkably, *in vitro*-cultured and *H. pylori*-challenged bone marrow derived macrophages (BMDMs) of PARP1ΔMyel were impaired in their ability to express IL-1β and IL-12 [9]. This implies that myeloid PARP1 has a functional role to provide the key IL-12 cytokine stimulus of T_H1 polarization in chronic *Helicobacter* infection, but it does not appear to participate in the infiltration of $CD4^+$ T cells into the gastric wall.

Apart from the mouse-based *in vivo* and *in vitro* experimentation [8,9], it has been reported that *H. pylori* expresses an as-yet unidentified heat- and protease-sensitive factor, which activates auto-ADP-ribosylation activity of PARP1 *in vitro* [10,11]. Physiological significance of this PARP1-activatory factor, in particular in perturbation of immune signaling, has remained elusive. *H. pylori* is known to induce epithelial cell DNA-damage *in vitro* [12], which should be sufficient in itself to activate PARP1 (see Chapter 3.1. and Fig. 2). Also, *Helicobacter*-induced inflammation in the gastric wall [8,9] should amplify the DNA damage-induced activation of PARP1. In addition, *H. pylori* produces a pore-forming toxin VacA [13], which induces necrotic cell death *in vitro* associated with the

Table 1
Summary of the animal experimentation data on the functional role of mammalian PARPs in acute and chronic bacterial infections. The table includes those studies that have been conducted with living bacteria and two selected early studies with LPS exposure.

Animal model, perturbation	Bacterium or bacterial molecule	Major phenotypes due to genetic or pharmaceutical perturbation	Ref.
Mouse, PJ34 (oral)	*Helicobacter felis* (oral)	reduced T_H1 polarization and gastric wall infiltration of $CD4^+$ T cells, reduced formation of pre-malignant cancer lesions, reversal of existing pre-malignant cancer lesions, increased *H. felis* gastric colonization	[8]
Pig, PJ34 (intravenous)	*Escherichia coli* (intraperitoneal)	improved survival, improved hemodynamics, reduced concentration of circulatory TNF-α	[25]
Rabbit, PJ34 (intravenous)	*Pseudomonas aeruginosa* (intratracheal)	unaffected pulmonary edema, but reduced gut edema	[28]
Sheep, 3-AB (intravenous)	*Pseudomonas aeruginosa* (intrabronchial)	improved pulmonary function, less pulmonary edema, reduced lung leukocytic infiltration, reduced lung lipid peroxidation and protein nitrosylation	[27]
Rat, 3-AB (intraperitoneal)	*Streptococcus pneumonia* (intrabrain)	reduction of meningitis-associated central nervous system complications, lower leukocyte counts and reduced concentration of IL-6 in cerebrospinal fluid	[29]
Mouse, systemic $PARP1^{-/-}$	normal gut microbiota (cecal ligation and puncture)	improved survival, reduced concentration of circulatory TNF-α and IL-6, less leukocyte infiltration into the gut, lung and liver	[26]
Mouse, systemic $PARP1^{-/-}$	*Salmonella* Typhimurium (oral)	delayed cecal pro-inflammatory gene expression, delayed development of histological signs of cecal inflammation, unaffected *Salmonella* colonization	[14]
Mouse, systemic $PARP1^{-/-}$	*Streptococcus pneumonia* (intrabrain)	reduction of meningitis-associated central nervous system complications, lower leukocyte counts in cerebrospinal fluid, reduced concentration of IL-1β, IL-6 and TNF-α in brain homogenates	[29]
Mouse, systemic $PARP1^{-/-}$	*Chlamydophila abortus* (intraperitoneal)	more pronounced weight loss, less infective progeny in the liver, unaffected circulatory concentrations of IL-6, IFN-γ, MIP-2 and M-CSF	[30]
Mouse, systemic $PARP1^{-/-}$	no bacterial challenge	order- and family-level changes in the composition of gut microbiota, more gut lamina propria $CD4^+$ $CD25^+$ $Fox3P^+$ regulatory T cells	[21, 22]
Mouse, myeloid $PARP1^{-/-}$	*Helicobacter pylori* (oral)	reduced T_H1 polarization, unaffected gastric wall infiltration of $CD4^+$ T cells, increased *H. pylori* gastric colonization	[9]
Mouse, PJ34 (intraperitoneal)	LPS (intratracheal)	reduced protein content, concentration of TNF-α, MIP-1α and NO as well as myeloperoxidase activity in bronchoalveolar lavage fluid, reduced lung lipid peroxidation, decreased leukocyte extravasation into the alveolar spaces and less alveolar hemorrhages	[45]
Mouse, systemic $PARP1^{-/-}$	LPS (intraperitoneal)	improved survival, reduced circulatory concentration of TNF-α and IFN-γ	[47]

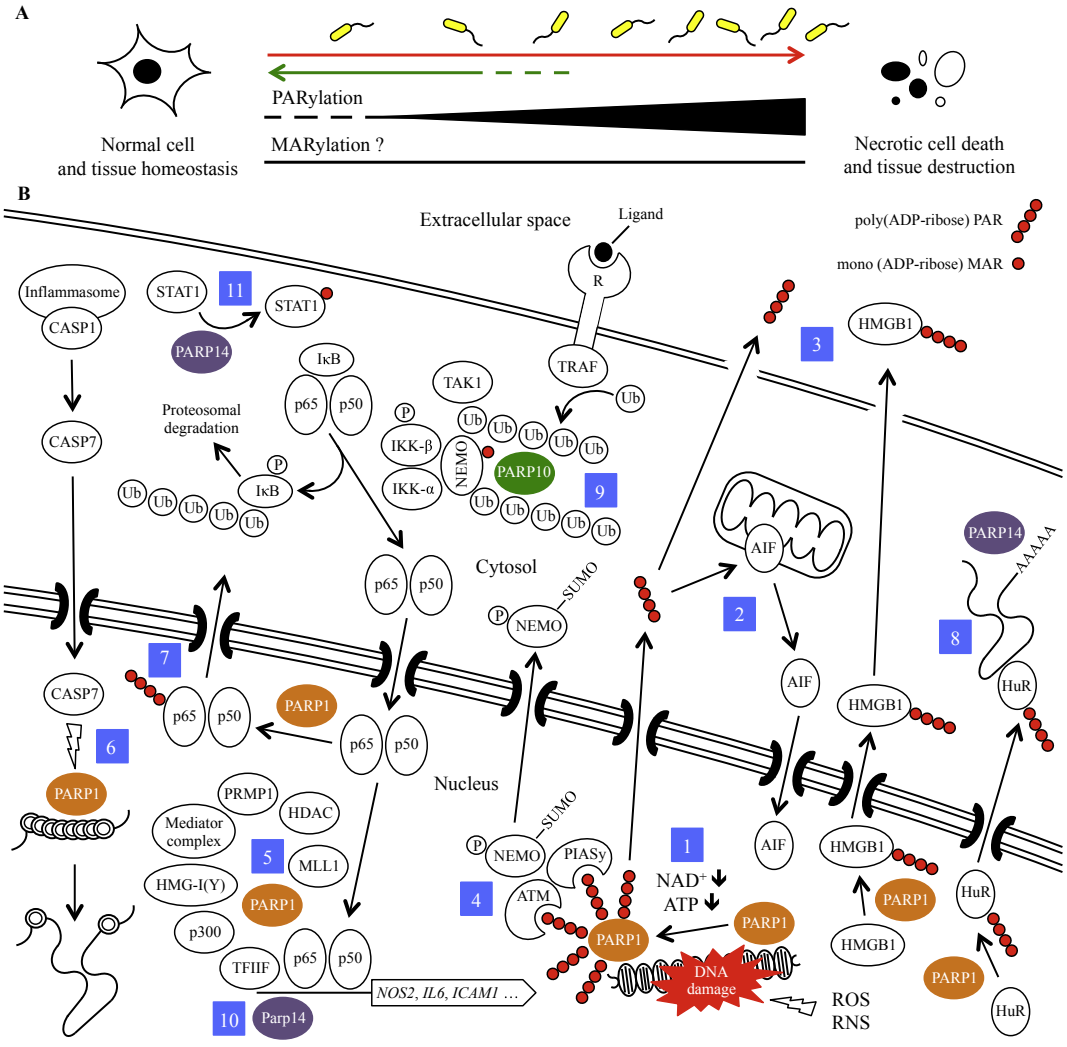

Fig. 2. Schematic representation of the pro-inflammatory functions of host cell PARPs in bacterial infections. A) PARylation regulates cellular responses to maintain tissue homeostasis in bacterial infection. Extensive PARylation, e.g. due to inflammation-induced DNA damage, may drive necrotic cell death and tissue destruction. The functional role of host cell PARP-catalyzed MARylation in bacterial infections remains elusive. **B)** Schematic summary of the mechanistic basis of host cell PARP functions in bacterial infections. 1, extensive DNA damage-induced PARP1-mediated PARylation in the nucleus depletes NAD^+ and ATP and causes cell death; 2, PAR is found in the cytosol upon extensive nuclear PARylation and induces a distinct type of cell death called parthanatos; 3, PARP1 regulates the release of immunomodulatory molecules PAR and HMGB1; 4, DNA damage-induced PARP1 activation regulates the atypical nuclear-to-cytoplasmic NF-κB activation; 5, PARP1 acts as a transcriptional co-regulator of NF-κB-dependent genes; 6, non-apoptotic caspase-7 activation degrades chromatin-associated PARP1 and reduces chromatin packaging to enhance expression of NF-κB-dependent genes; 7, PARP1-mediated PARylation of p65 inhibits nucleus-to-cytosol shuttling of p65 to prolong the NF-κB-dependent gene expression; 8, PARP1 and PARP14 regulate the stability of transcripts; 9, PARP10 inhibits signal propagation in the NF-κB pathway by interfering with ubiquitylation of NEMO via MARylation of NEMO; 10, PARP14 acts as a transcriptional co-regulator of STAT-dependent genes; 11, PARP14 MARylates STAT1 to inhibit STAT1 phosphorylation and downstream STAT1-dependent signaling. Refer to the main text for details and references.

activation of poly-ADP-ribosylation activity most likely via PARP1-mediated sensing of DNA damage.

2.1.2. Salmonella *gut infection*

It has been shown in an oral *Salmonella enterica* serovar Typhimurium mouse model that a systemic absence of PARP1 is associated with a delayed pro-inflammatory response [14]. This model where gut microbiota is perturbed prior to *Salmonella* infection with streptomycin recapitulates closely human enterocolitis [15]. The number of viable bacteria did not differ between cecum, mesenteric lymph nodes and spleen of wild-type and PARP1-deficient mice [14]. However, the typical histological signs of *Salmonella*-induced cecal inflammation such as infiltration of neutrophils and disruption of the crypt architecture developed more slowly in PARP1-deficient mice [14]. This phenotype correlated with a delayed onset of pro-inflammatory gene expression [14]. Gene ontology analysis of the whole genome microarray data revealed that many of the PARP1-dependent genes were known immune response genes, in particular involved in the IFN-γ signaling [14]. It therefore appears plausible that PARP1 is involved in the activation or recruitment of immune cells in *Salmonella*-infected gut with analogy to what happens in *Helicobacter* gastric infection (see Chapter 2.1.1.).

In vitro experimentation indicates that also PARP14, alternatively designated as ARTD8 [2], has a functional role in *Salmonella* gut infection, in particular in macrophages [16]. PARP14 was discovered as a Stat6 interacting protein [17], it has a protein MARylation activity [1], and it has been implicated in the regulation of lymphocyte and macrophage functions, e.g. Refs. [18–20]. PARP14-deficient RAW264.7 macrophages contained more viable intracellular *Salmonella* as compared to parental cells [16]. The same PARP14-deficient macrophages were impaired in nitric oxide (NO) production upon stimulation with either living *Salmonella* or with the bacterial surface molecule lipopolysaccharide (LPS) [16].

The same stimuli were unable to fully induce IFN regulatory transcription factor 3 (IRF3)-dependent genes such as *Ifnb1*, *Ccl5*, *Cxcl10* and *Ifit1* in PARP14-deficient RAW264.7 macrophages [16]. Interestingly, the authors found out that PARP14 expression is induced, in addition to IFN-β, IFN-γ, Poly(I:C) and LPS, with the bacterial DNA mimic ODN1826 and the bacterial lipopeptide mimic Pam3CSK4 in mouse BMDMs [16], further reinforcing the importance of PARP14 in the antibacterial response of macrophages.

2.1.3. Gut microbiota

There are no reports in the literature that PARP1-deficient mice, or other PARP-deficient mice (PARP2, PARP3, PARP4, PARP5a, PARP5b, PARP7, PARP9, PARP11 and PARP14), would be more sensitive to spontaneously develop microbial infections during breeding and mouse colony maintenance. However, differences in the gut microbiota composition have been documented between wild-type and systemic PARP1-deficient mice [21,22]. Clostridiales and Bacteroidales represented the most abundant bacterial orders in the duodenum, cecum and fecal samples as analysed after normal *ad libitum* housing conditions [21]. A significant order level difference was that the duodenum of PARP1-deficient mice had higher abundancy of Clostridiales [21]. This paralleled with a family level abundancy changes of Clostridiales in the fecal samples [21]. Family level abundancies of the order Bacteroidales were significantly different in PARP1-deficient mice as compared to wild-type mice throughout the gastrointestinal tract [21]. Another study found out that fecal samples of systemic PARP1-deficient mice under normal *ad libitum* housing conditions had lower abundancy of order Lactobacillales and also found abundancy changes within the order Clostridiales [22]. In particular, Clostridium clusters IV and XIVa were more abundant in PARP1-deficient mice [22]. These members of order Clostridiales fall into a class of bacteria with beneficial immunohomeostatic functions via induction of $CD4^+$ $Fox3P^+$ regulatory T cells [23]. Accordingly, it was found that the colon lamina propria of PARP1-deficient mice contained more $CD4^+$ $CD25^+$ $Fox3P^+$ regulatory T cells as compared to the wild-type mice [22]. However, increased numbers of $CD4^+$ $CD25^+$ $Fox3P^+$ regulatory T cells have been witnessed also in other peripheral tissues of PARP1-deficient mice [24]. More work is therefore needed to clarify causality in the PARP1 – gut microbiota – gut immune cell homeostasis triad. Physiological implications could be significant having relevance to the clinical trials and use of PARP inhibitors in the clinic.

2.1.4. Escherichia coli *and polymicrobial septic peritonitis*

The effect of PJ34 on the development of *Escherichia coli*-induced septic peritonitis has been studied in pigs by intraperitoneal implantation of *E. coli* in fibrin-thrombin clots [25]. Survival rate of the PJ34-treated group was significantly higher than the vehicle control group [25]. All vehicle controls were dead at 72 h post-infection whereas 60% of the PJ34-treated animals survived until the end of the follow-up (4 days) [25]. The better survival of PJ34-treated animals correlated with a higher cardiac output and with a more sustained cardiac contractility [25]. Peritonitis induced a rapid increase in circulatory TNF-α concentration, which was inhibited with the PJ34 treatment [25]. Histological sections of heart and lungs from septic pigs showed significant PAR staining, which was not detected in PJ34-treated animals [25].

Specific PARP1 function was studied in a systemic $PARP1^{-/-}$ mouse model of polymicrobial sepsis induced by cecal ligation and needle puncture (CLP) [26]. In this model bacteria from the cecum get access to the peritoneal cavity and induce peritonitis and subsequently sepsis [26]. There was a significant delay in the onset of death in systemic PARP1-deficient mice in response to CLP and improvement in survival rate at the end of the follow-up [26]. PARP1-deficient mice had significantly lower circulatory concentrations of TNF-α and IL-6, as well as less leukocyte infiltration into the gut, lung and liver as measured with tissue lysate myeloperoxidase activity [26]. Histological sections of the gut samples from wild-type mice showed significant PAR staining, which was not detected in the PARP1-deficient mice [26]. Taken together, the findings indicate a body-wide activation and functional involvement of PARP1 and possibly other PARPs in the induction of pro-inflammatory response in septic peritonitis.

2.1.5. Pseudomonas aeruginosa *pneumonia*

The effect of 3-AB on the development of *P. aeruginosa* pneumonia has been studied in a sheep model where *P. aeruginosa* was inoculated into the lungs intrabronchially [27]. Continuously infused 3-AB in the sheep model significantly reversed coagulation abnormality, a hallmark of sepsis, as measured by platelet counts and anti-thrombin activity [27]. 3-AB also lowered the lung wet/dry weight ratio, which is used as a marker of edema reflecting an increase in microvascular permeability [27]. Pulmonary function as measured e.g. by oxygen saturation was significantly improved with 3-AB [27]. Histological lung sections showed reduction of the cellular infiltrate, mostly composed of neutrophils, and hemorrhage with 3-AB [27]. 3-AB also lowered the level of lipid peroxidation and protein nitrosylation in the lung indicative of lower concentrations of nitrogen and/or oxygen-derived free radicals [27]. Bacterial infection induced PARylation in the lung tissues as assessed with histology, in particular in pulmonary epithelial cells, which was attenuated in the 3-AB treatment group [27]. Independent experimentation with an intratracheal injection of *P. aeruginosa* in a rabbit model indicates that also PJ34 elicits a protective effect [28]. Taken together, the findings indicate that PARPs are involved in the pathology of *P. aeruginosa* pneumonia.

2.1.6. Streptococcus pneumonia *meningitis*

The effect of 3-AB and genetic PARP1-depletion on the development of *S. pneumonia* meningitis has been studied in a rat and a mouse model, respectively, where *S. pneumonia* was directly injected into the brain [29]. Clinical examination revealed that systemic PARP1-deficient mice were protected against the meningitis-associated central nervous system complications such as the blood–brain barrier permeability increase and the intracranial pressure increase [29]. 3-AB treatment also protected rats against the meningitis-associated central nervous system complications, but the overall effect appeared milder as compared to the systemic PARP1-deficiency in mice [29]. This could relate to the blood–brain barrier permeability of 3-AB. Brain homogenates of PARP1-deficient mice as compared to wild-type mice contained less IL-1β, IL-6, TNFα and leukocyte infiltration marker myeloperoxidase activity, which was also paralleled with lower leukocyte counts in the cerebrospinal fluid [29]. In both mouse and rat brain homogenate Western analysis, it was found out that infection caused a significant increase of PARylation without evident changes in PARP1 protein level or its proteolytic processing [29]. *In vitro* modeling also supported the functional role of PARP1 activation in *S. pneumonia* meningitis. When rat brain-derived endothelial cells were co-cultured with RAW264.7 macrophages provided in Transwell-inserts, *S. pneumonia* induced endothelial cell PARylation with reduction in the cellular NAD^+ concentration and cell viability [29]. 3-AB treatment of the co-cultures strongly reversed the infection-associated increase of PARylation, depletion of NAD^+ and cytotoxicity [29]. In conclusion, PARP1 activation appears to play a functional role in the development of meningitis-associated central nervous system complications in *S. pneumonia* infection.

2.1.7. Chlamydia

The effect of genetic PARP1-depletion on the infection of strict intracellular bacteria *Chlamydia* has been studied in a mouse model where *Chlamydophila abortus* was injected into the intraperitoneal cavity [30]. Systemic PARP1-deficiency did not affect the highly elevated serum concentrations of IL-6, IFN-γ, MIP-2 or M-CSF [30]. However, a more severe weight loss was detected in PARP1-deficient mice [30]. Livers of PARP1-deficient mice contained less bacteria with the capacity to subsequently infect cells *in vitro* [30]. *In vitro* it was demonstrated that the *C. abortus*-infected fibroblasts of PARP1-deficient mice could produce less infective progeny [30]. McCoy cells also produced less infective progeny *in vitro* when treated with PJ34 [30]. Although further experimentation is needed to substantiate these findings, PARP1 appears to have a functional role in *C. abortus* infection.

High-mobility group box 1 protein (HMGB1) is a ubiquitous primarily nucleus-localized protein, which is both actively and passively released from stressed or damaged cells, e.g. under DNA-damaging conditions [31], to activate the immune response [32]. In this respect it is noteworthy that *Chlamydia trachomatis* expresses a protease-like activity factor (CPAF), which induces proteolysis of PARP1 [33,34] and also HMGB1 [34]. Kinetic analyses indicated that *C. trachomatis* prevents HMGB1 release via two mechanisms – i) degradation of PARP1 and thereby inhibition of HMGB1 translocation from the nucleus into the cytosol at early time points and ii) direct degradation of HMBG1 at late time points [34]. However, HMGB1-*Chlamydia* association appears more complex as it has been reported that genital tract secretions of mice experimentally infected with *C. trachomatis* contain higher rather than lower levels of HMGB1 [35], which also has been replicated in a number of *in vitro* cell culture experiments [35,36]. Therefore, further experimentation is required to substantiate the findings on *Chlamydia* – PARP1 – HMGB1 triad.

2.1.8. Streptococcus pyogenes *(group A streptococcus, GAS)*

GAS utilizes a pore-forming streptolysin O (SLO) protein to translocate an enzyme, NAD^+ glycohydrolase (NADase) known as SPN, into the host cell cytosol [37]. SPN-deficient GAS mutant is attenuated in virulence in mouse models of invasive GAS infection both via subcutaneous or intraperitoneal infection routes [38]. Although the cellular basis appears multifactorial, SPN affects host cell PARylation [39]. Infection of epithelial cell cultures with wild-type SPN (SPN^{pos})-expressing GAS induced a rapid and short duration PARylation, including PARP1 auto-modification, whereas infection with SPN-deficient strain or mutant NADase-negative SPN (SPN^{neg})-expressing strain resulted in a rapid and sustained PARylation [39]. Short duration SPN^{pos}-induced PARylation was paralleled with translocation of HMGB1 from nucleus into the cytosol, whereas sustained SPN^{neg}-induced PARylation was paralleled with pronounced IL-8 and TNF-α secretion without HMGB1 translocation [39]. HMGB1 translocation upon GAS infection was inhibited with PJ34 or PARP1 siRNA knockdown [39]. SPN^{pos}- and SPN^{neg}-expressing strains of GAS therefore induce kinetically very different cellular responses, although the final outcome from SLO/SPN function is cell death [39]. These findings may have clinical significance since SPN^{pos} and SPN^{neg} strains exist in natural populations of GAS with differences in tissue tropism [40].

2.2. Phenotypic data with LPS

LPS is a highly abundant molecule found on and shed from the outer membrane of Gram-negative bacteria. LPS activates a robust pro-inflammatory response [41], which may get over-amplified and cause fatal septic shock characterized by hypotension and acute failure of multiple organs. A large number of studies have been published on PARPs, in particular on PARP1, in LPS-induced inflammatory signaling and subsequent physiological response. First indications on the role of PARPs in LPS-induced inflammatory response came when LPS-induced secretion of NO was shown to be inhibited *in vitro* by nicotinamide, 3-AB and 3-methoxybenzamide in mouse BMDMs [42]. Northern blot and iNOS-reporter studies in RAW264.7 macrophages indicated that this effect took place at the level of transcription [43]. Nicotinamide and meta-iodobenzylguanidine (MIBG, guanidine analogue of the neurotransmitter norepinephrine), specific inhibitor of MARylation, were also reported to down-regulate mRNA levels and secretion of TNF-α and IL-6 *in vitro* in LPS-treated human PBMCs [44]. Early animal model studies also utilized PARP inhibitors. For example, PJ34 decreased the extent of alveolar hemorrhages and leukocyte extravasation into the alveolar spaces in mice after an intratracheal LPS exposure that paralleled with reduced concentrations of TNF-α, MIP-1α and NO in the bronchoalveolar lavage fluid [45]. More recently, it was reported that PARP inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) decreased lung neutrophil infiltration, lung vascular permeability and lung mRNA levels of *Il1b*, *Il6*, *Tnf*, *Cxcl1*, *Cxc2* and *Nos2* after mouse intraperitoneal LPS challenge [46]. mRNA results could be replicated *in vitro* with peritoneal macrophages indicating that PARPs have an intrinsic functional role in LPS-induced inflammatory response in macrophages [46].

Genetic perturbation studies have demonstrated that PARP1 plays an important role in the LPS-induced inflammatory response, e.g. Refs. [9,47,48]. Systemic PARP1-deficient mice were more resistant to lethal intraperitoneal LPS challenge [47,48] and experienced less liver damage [48]. Inflammatory parameters such as the amount of circulatory TNF-α and IFN-γ [47] as well as NO [48] were lower in the LPS-challenged systemic PARP1-deficient mice as compared to the wild-type control mice. PARP1 appears to have a macrophage-intrinsic functional role in LPS-induced inflammatory response because peritoneal macrophages of systemic PARP1-deficient mice were defective to secrete NO *in vitro* upon LPS challenge [47]. BMDMs of systemic PARP1-deficient mice were also defective *in vitro* in secreting IL-12p70, IL-18, IL-6 and TNF-α upon combinatorial treatment with LPS and IFN-γ [9]. RNA-Seq provided evidence that PARP1-deficiency caused up- and down-regulation of several hundred genes in LPS/IFN-γ-treated BMDMs [9]. In the same study, LPS responses of the conditional lysozyme-expressing myeloid cell-specific PARP1 knockout (PARP1ΔMyel) mouse were also reported [9]. mRNA levels of *Ifng*, *Il12b*, *Il18* and *Il6* were significantly lower in spleens of LPS-challenged myeloid cell-specific PARP1-deficient mice, which was paralleled with lower concentration of circulatory IFN-γ [9]. Interestingly, spleen NK cells from the myeloid cell-specific PARP1-deficient mice had a lower expression level of IFN-γ, although *in vitro* these cells were secreting similar amounts of IFN-γ as compared to the wild-type mice upon IL-12p70 stimulus [9]. Accordingly, the authors proposed that the lower amount of circulatory IFN-γ in PARP1-deficient mice is caused by the decreased production of IFN-γ by NK cells because PARP1-deficient macrophages are unable to provide the NK cell-activatory IL-12p70 stimulus [9]. Taken together, PARPs, and in particular PARP1, have a functional role in LPS-induced pro-inflammatory response.

3. Pro-inflammatory functions of PARPs

3.1. PARP1

Proposed pro-inflammatory functions of PARP1 are schematically represented in Fig. 2. Inflammation is characterized by DNA damage that is caused by reactive oxygen species (ROS) such as

hydrogen peroxide, superoxide and hydroxyl radical as well as reactive nitrogen species (RNS) such as NO and peroxynitrite [50]. On the one hand, PARP1 positively regulates NO secretion, e.g. upon LPS stimulation of primary mouse macrophages [47], and thereby contributes to the overall tissue burden of DNA-damaging oxidative stress [51]. On the other hand, DNA damage activates the PARP1-mediated PARylation and DNA damage repair pathways [1]. Above certain threshold PARylation leads into extensive depletion of cellular NAD^+ and ATP causing a loss of cell viability with necrotic cell death-like characteristics [51–53]. Caspase-mediated PARP1 cleavage has been proposed to counteract this energy depletion-induced mode of cell death, in addition of promoting apoptosis by preventing the DNA repair-induced survival [54]. Furthermore, upon excessive PARP1 activation in the nucleus, PAR is also found in the cytosol where it induces the release of apoptosis inducing factor (AIF) from mitochondria and subsequent necrotic cell death-like process designated as parthanatos [55–57]. Plasma membrane compromised cells may subsequently release molecules potentiating the inflammation. For example, PAR itself has been reported as an activator of the macrophage pro-inflammatory response via Toll-like receptors 2 and 4 [58]. HMGB1 is an important immunostimulant released from stressed or damaged cells (see also Chapters 2.1.7. and 2.1.8.) where PARP1 via PARylation regulates HMGB1 translocation from the basally dominating nuclear localization into the cytosol [31,59]. Taken together, PARP1-catalyzed PARylation contributes to cell death and tissue damage in bacterial infections, but also systemically regulates inflammation via release of immunomodulatory molecules such as PAR and HMGB1 (Fig. 2).

PARP1 has key functions in the regulation of the NF-κB-dependent signaling, and PARP1 also appears to influence the activity of other inflammatory transcription factors such as STAT1, AP-1 and SP-1 [60]. Activation of PARP1-mediated PARylation by DNA damage has been reported to regulate the atypical nuclear-to-cytoplasmic NF-κB activation [61,62]. The PAR chains of auto-PARylated PARP1 in the nucleus recruit protein inhibitor of activated STATy (PIASy) and ataxia telangiectasia (ATM) kinase [62]. This complex formation leads into SUMOylation and phosphorylation of the nucleus-localized NF-κB essential modulator (NEMO), followed by nucleus-to-cytosol translocation of NEMO and subsequent activation of the NF-κB signaling pathway [61,62].

PARP1 acts as a transcriptional co-regulator, mostly as a co-activator, at the promoters of a sub-set of NF-κB-dependent pro-inflammatory genes [9,46,47,60,63–78]. Direct DNA-PARP1 interaction at the promoter has been reported [73]. However, most of the studies provide evidence that PARP1 associates with nuclear proteins involved in the transcriptional activation, such as NF-κB transcription factor p65 [67], NF-κB transcription factor p50 [66], general transcription factor TFIIF [65], high mobility group protein HMG-I(Y) [79] and components of the Mediator complex [65]. Therefore, PARP1 appears either as an assembly factor, or it modulates the functions of individual protein components at the transcription initiation complex.

It has been reported that an LPS-activated non-apoptotic PARP1 cleavage by caspase-7 releases PARP1 from the chromatin, thereby reducing chromatin packaging and enhancing the expression of a subset of NF-κB-dependent genes [76]. In respect of the chromatin packaging effect, PARP1 also has synergy in the NF-κB-dependent transcription with a number of proteins known to post-translationally modify histones, such as histone acetyltransferase p300 [74] and histone methyltransferases PRMT1 [64] and MLL1 [77]. Interestingly, PARP1 is itself acetylated by p300 and de-acetylated with histone deacetylases HDACs, which appears to be functionally important for the NF-κB-dependent transcription [65]. PARylation of histones as catalyzed by PARP1 has also been proposed to facilitate NF-κB-dependent transcription via de-stabilizing DNA-histone interactions and thereby increasing the promoter accessibility in LPS-stimulated macrophages [78].

Another mechanistic proposal implies that PARP1-mediated PARylation of p65 under LPS stimulation prolongs the duration and strength of NF-κB-dependent transcription via a PAR-mediated steric blockage of the interaction between p65 and components of the nuclear export machinery [75]. PARP1 also appears to act at the post-transcriptional level by promoting the stability of LPS-induced transcripts via PARylation of the mRNA-binding protein Hu-antigen R (HuR) [80]. Taken together, a number of mechanisms have been proposed how PARP1 regulates the NF-κB-dependent transcription (Fig. 2). Controversy exists within the field on whether the ART activity of PARP1 is involved or not, in particular under conditions that apparently lack the PARP1-activatory DNA damage. Contradictory findings could relate to differences in cell types, specific genes under question and other experimental parameters such as the nature of the inflammatory stimulus and temporal kinetics of stimulation.

3.2. Other PARPs

Proposed pro-inflammatory functions of PARPs other than PARP1 are schematically represented in Fig. 2. Systems level transcriptomic studies have shown that several PARPs get up-regulated *in vitro* by stimulation with living bacteria or bacteria-derived molecules such as LPS, e.g. in human PBMCs [81] or mouse BMDMs [16], implying functional relevance. Also, PARP12 and PARP14 have been reported to be more abundant at the protein level in RAW264.7 macrophages upon LPS treatment *in vitro* [16,49]. The LPS-induced up-regulation applies also for PARP12 in mouse BMDMs and splenic DCs [49]. One of the transcriptionally up-regulated PARPs, PARP10, regulates IL-1β- and TNF-α-induced NF-κB signaling *in vitro* [82]. It was proposed that PARP10 inhibits the signal propagation in the cytosol by binding to NEMO and interfering with the K63-linked poly-ubiquitylation of NEMO via MARylation of NEMO [82]. In regards to ubiquitylation, it is noteworthy that PARP9 was shown to MARylate the C-terminus of free ubiquitin and thereby could affect cellular processes that are ubiquitin-dependent, including the inflammatory signaling [83]. PARP14 expression is up-regulated and it gets translocated into the nucleus in mouse BMDMs upon LPS stimulation *in vitro* [16]. PARP14-deficient mouse macrophages had difficulties to express a number of interferon-inducible genes such as *Ifnb1*, *Ccl5*, *Cxcl10* and *Ifit1* upon LPS-stimulation [16]. This was paralleled with a defective histone H3K27 acetylation in chromatin regions containing STAT/IRF DNA-binding motifs and an impaired RNA polymerase II recruitment to the promoters [16]. PARP14 has also been reported to suppress the pro-inflammatory gene expression in macrophages upon IFN-γ treatment [19]. Mechanistically, it was proposed that PARP14 ADP-ribosylates STAT1, and that this modification inhibits STAT1 phosphorylation and subsequent STAT1 activity [19]. However, these ADP-ribosylation data have been questioned mainly because the study omitted analysis of over-lapping SUMO-conjugation site known to regulate STAT1 phosphorylation [84]. Moreover, it has recently been reported that PARP14 positively affects STAT1 phosphorylation [16]. PARP14 also acts as a promoter-binding transcriptional co-factor in STAT-dependent signaling [17], e.g. regulating IL-6/STAT3-dependent differentiation of T cells into Th17 direction but also to follicular helper T cell [20]. PARP14 also appears to act at the post-transcriptional level. PARP14 was reported to suppress expression of the major coagulant initiator tissue factor (TF) in macrophages upon lipopolysaccharide (LPS) stimulation via binding and accelerating the decay of TF mRNA [18]. Taken together, PARP10 [82], PARP12 [49], PARP14 [16–20,84] and

possibly PARP9 [83], elicit regulatory functions that may influence the course of a bacterial infection (Fig. 2). As for now, however, an anti-bacterial cellular phenotype has only been reported for PARP14-deficiency, based on *in vitro* experimentation with macrophages [16].

4. Concluding remarks

ADP-ribosylation, as catalysed by the MARylating bacterial ART-toxins, is a centrally important PTM at the interface of bacterial pathogen-host interaction. Emerging data indicate that also the host cell MARylating and PARylating PARPs influence the course of a bacterial infection. A vast number of animal and cell culture-based experiments have demonstrated that the lack of PARP functions cause difficulties to mount a well-balanced anti-bacterial immune response, in particular the pro-inflammatory response. Key questions relate to the mechanisms how ART activity of PARPs is activated during the infection and to what extent ART activity is temporally required in or influences the bacteria-induced inflammation and resolution of the infection. Key future challenge in the field, therefore, is to identify the inflammation-associated protein MARylation and PARylation events, and to relate these to the perturbation of inflammatory signaling and subsequent physiological response.

Chemical PARP inhibition in animal experiments has demonstrated a therapeutic effect, e.g. in models of acute septic shock. It appears that this therapeutic effect mostly relates to the state of extensive level stress where DNA damage-mediated hyper-activation of PARP1 ART-activity drives tissue destruction. Repurposing of the approved or clinical trial PARP inhibitors developed for cancer therapy holds promise in the development of novel targeted antibacterial therapies. One caveat, at least in prolonged treatments, could be the simultaneous blockage of DNA damage repair pathways and thereby induction of DNA damage-induced malignancies. Second caveat relates to the inhibition of beneficial ART-dependent signaling. In both cases, combinatorial strategy with currently used antibiotics could allow lowering of the PARP inhibitor dosage. Pharmaceutical targeting of ADP-ribose readers and ADP-ribose erasers could provide alternative intervention strategies. Also, development of molecules targeting the interactions of PARPs with their downstream signaling components merits further investigation.

Conflict of interest

The authors declare that they have no conflicts of interest.

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