Journal of Molecular Cell Biology (2024), 16(11), mjae050 https://doi.org/10.1093/jmcb/mjae050
Published online December 12, 2024

Review

Prospects for PARG inhibitors in cancer therapy

Yangchan Hu[1,2], Yuxin Meng[1,2], Zirui Zhuang[2,3], Yuancong Li[1,2], Junjun Nan[1,2], Ning Xu[4], Zu Ye[5,6,*], and Ji Jing[2,6,*]

[1] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, China
[2] Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou 310022, China
[3] School of Molecular Medicine, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences (UCAS), Hangzhou 310024, China
[4] Institute of Drug Development & Chemical Biology, College of Pharmaceutical Science, Zhejiang University of Technology, Huzhou 313200, China
[5] Gastric Cancer Center, Zhejiang Cancer Hospital, Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou 310022, China
[6] Zhejiang Key Laboratory of Prevention, Diagnosis and Therapy of Upper Gastrointestinal Cancer, Hangzhou 310022, China
* Correspondence to: Ji Jing, E-mail: jingji@him.cas.cn; Zu Ye, E-mail: yezuqscx@zju.edu.cn

Edited by Zhiyuan Shen

Poly(ADP-ribose) glycosylhydrolase (PARG) is an enzyme involved in hydrolyzing the ribose–ribose bonds present in poly(ADP-ribose) (PAR), which are primarily found in the nucleus. Along with poly(ADP-ribose) polymerase, PARG regulates the level of PAR in cells, playing a crucial role in DNA maintenance and repair processes. Recent studies have revealed elevated levels of PARG in various cancers, such as breast, liver, prostate, and esophageal cancers, indicating a link to unfavorable cancer outcomes. PARG is a significant molecular target for treating PAR-related cancers. This review provides a comprehensive overview of the physiological role of PARG and the development of its inhibitors, highlighting its potential as an innovative target for cancer treatment.

Keywords: PARG, PARG inhibitor, cancer therapy

Introduction

ADP-ribosylation, a dynamic and reversible post-translational modification (PTM) similar to methylation, phosphorylation, and acetylation, occurs in two main forms: mono-ADP-ribosylation (MARylation) and poly-ADP-ribosylation (PARylation) (Li and Chen, 2014). PARylation affects numerous proteins across cellular compartments. It plays a critical role in DNA damage repair, transcriptional control, chromatin remodeling, genome stability, cell proliferation, apoptosis, and tumorigenesis (Miwa and Masutani, 2007; Frizzell et al., 2009).

Poly(ADP-ribose) polymerase (PARP) serves as the post-translational modifying enzyme found in the majority of eukaryotes and prokaryotes (Langelier et al., 2018). It facilitates the ADP-ribose (ADPr) modifications of numerous nuclear proteins (Huang and Kraus, 2022). PARP1, the archetypal member of the ADP-ribosylase superfamily, belongs to a group comprising at least 17 PARP-related enzymes that share homology with PARP1 (Schreiber et al., 2006). Among these, PARP1, PARP2, PARP5A, and PARP5B are capable of synthesizing poly(ADP-ribose) (PAR) chains, while others mainly generate single ADPr units (Richard et al., 2021; van Beek et al., 2021). Structurally, PARP1 consists of three primary domains: a zinc-binding domain (ZnI, ZnII, ZnIII), an auto-modification domain, and a catalytic domain (Curtin and Szabo, 2020; Sefer et al., 2022). PARP1 is activated upon the recognition of structurally damaged DNA fragments and has been proposed to function as a DNA damage sensor (Zhang et al., 2019; Figure 1).

PARylation, a prevalent cellular process, is transient and largely degraded by poly(ADP-ribose) glycosylhydrolase (PARG) at sites of DNA damage. Therefore, the dynamic regulation of the formation and dissociation of PARylation is crucial for maintaining cellular functions. PARG, identified as a PAR hydrolase in the nuclei of rat liver cells, is responsible for degrading ~90% of PAR polymers (Fisher et al., 2007; Min and Wang, 2009; Wei and Yu, 2016).

In this process, PARP attaches ADPr polymers, sometimes exceeding 200 units, to protein receptors, thereby facilitating PARylation (D'Amours et al., 1999; Alemasova and Lavrik, 2019). PARG exhibits both endoglycosidic and exoglycosidic activities, specifically targeting the PAR (1″–2′) ribose–ribose bond, yielding substantial free ADPr quantities (Barkauskaite et al., 2013; Pourfarjam et al., 2020). The exoglycosidase activity of PARG at the end of the PAR chain releases ADPr, whereas the

Received August 27, 2024. Revised November 22, 2024. Accepted December 11, 2024.

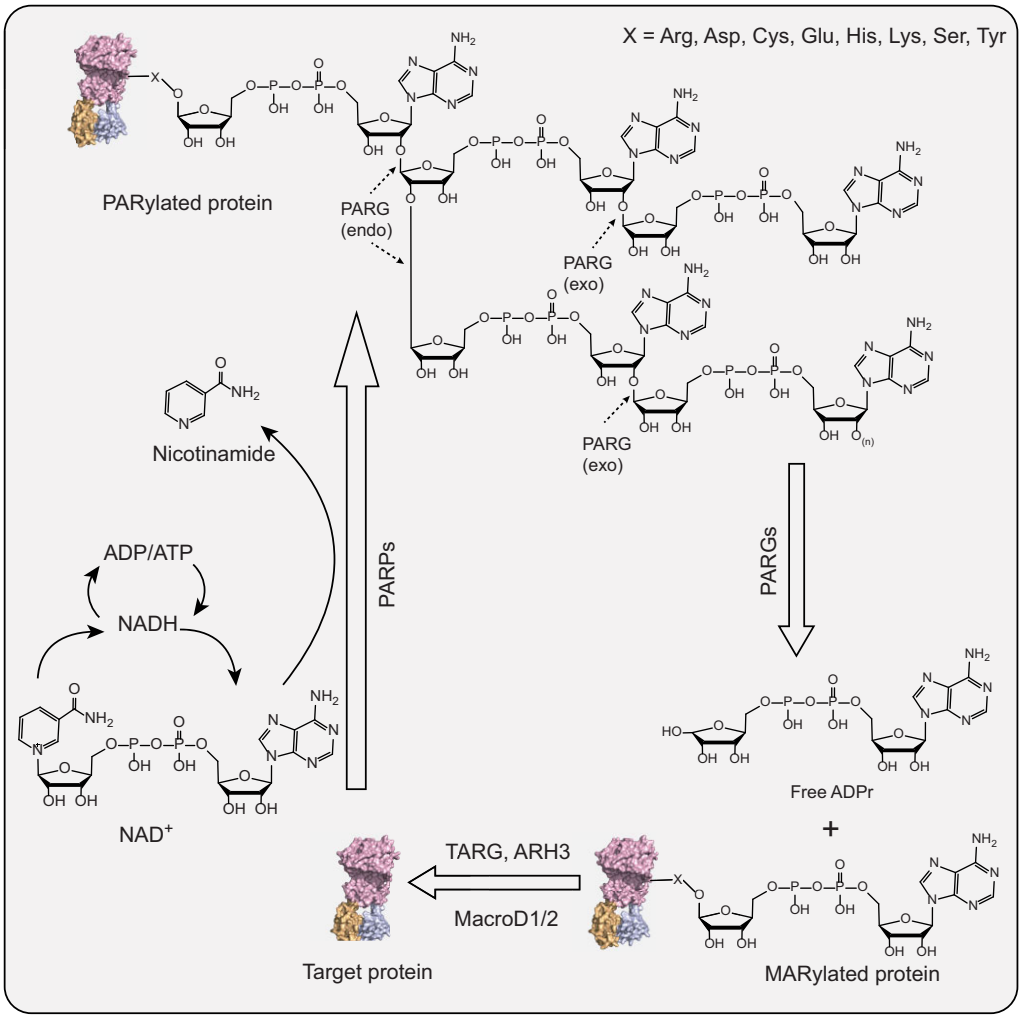
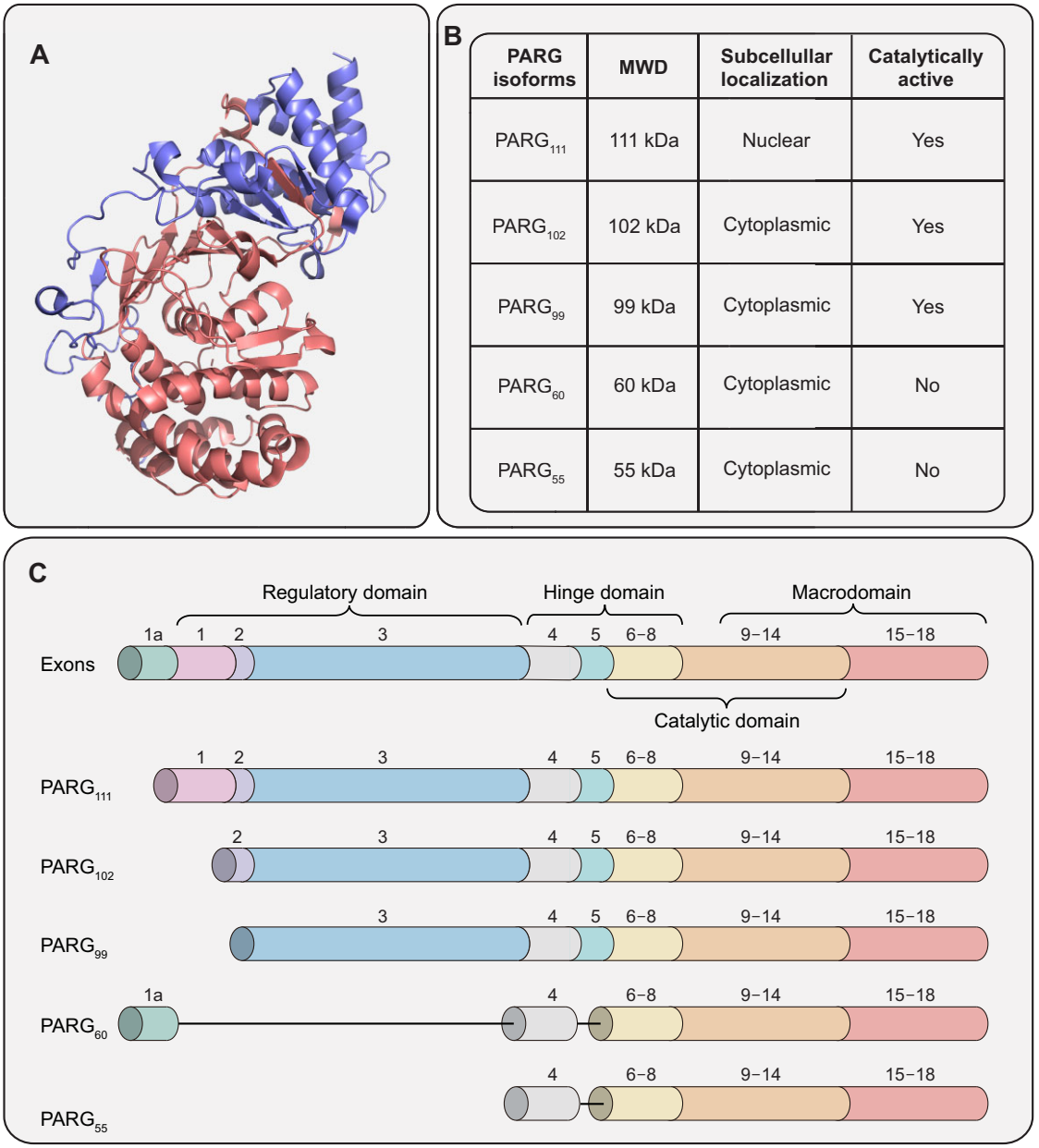


Figure 1 Formation and removal of PARylation. PARPs catalyze the cleavage of NAD^+ to nicotinamide and ADPr and partially catalyze the transfer of the ADPr to the target protein via unique 2′–1″ ribose–ribose glycosidic bonds repeatedly of arginine, aspartate, cysteine, glutamate, histidine, lysine, serine, or tyrosine residues to the sense carboxylate group, ultimately generating a PAR chain, which is introduced into the PAR branched chain via 2″–1‴ ribose–ribose glycosidic bonds. The PARylated proteins are hydrolyzed to free ADPr and MARylated proteins by PARGs via endonuclease and exonuclease activities. Human PARG is unable to cleave the proximal ADPr moiety, which is removed by TARG, ARH3, and MacroD1/2.

endoglycosidase activity of PARG releases the intact PAR chain (Figure 1; Barkauskaite et al., 2013). However, due to the spatial steric hindrance of the amino acid side chains of hydrolyzed proteins, PARG is unable to remove the final ADPr unit bound to proteins. Thus, other ADP-hydrolyzing enzymes, such as ADP-ribosylhydrolase 3 (ARH3), MACRO domain containing 1/2 (MacroD1/2), and terminal ADPr protein glycohydrolase (TARG1), fulfill this role (Niere et al., 2012; Rack et al., 2021; Groslambert et al., 2023). Together with PARP, PARG sustains the dynamic balance of PARylation, which is essential for effective DNA damage repair. PARG activity has been associated with processes involving inflammation, ischemia, and the progression and sustenance of tumors (Patel et al., 2005; Cuzzocrea et al., 2007; Marques et al., 2019). Inhibiting PARG activity can improve the efficacy of cancer treatments, including chemotherapy and radiotherapy. Thus, PARG inhibitors represent a promising strategy in cancer therapy and immunotherapy (Amé et al., 2009; Nagashima et al., 2020; Yu et al., 2022).

At present, PARP inhibitors, including olaparib, niraparib, rucaparib, and talazoparib, have been clinically approved for the treatment of diverse cancers, such as breast, ovarian, pancreatic, and prostate cancer (Litton et al., 2018; Golan et al., 2019; Patsouris et al., 2021; Turner et al., 2021; Dillon et al., 2022). Although the development of PARG inhibitors has advanced with successful validation in *in vitro* studies, they are still awaiting clinical approval. This review summarizes the structure and function of PARG in DNA damage repair. It also discusses the mechanisms and limitations of current PARG



PARG isoforms	MWD	Subcellular localization	Catalytically active
$PARG_{111}$	111 kDa	Nuclear	Yes
$PARG_{102}$	102 kDa	Cytoplasmic	Yes
$PARG_{99}$	99 kDa	Cytoplasmic	Yes
$PARG_{60}$	60 kDa	Cytoplasmic	No
$PARG_{55}$	55 kDa	Cytoplasmic	No

Figure 2 The PARG isoforms. (**A**) The 3D structure of PARG (PDB:6OA1). (**B**) Subcellular localization and activity of PARG isoforms. (**C**) Schematic diagram of PARG and its isoforms.

inhibitors in clinical trials and explores the potential of PARG as a novel target for cancer therapy.

The structure and function of human PARG

The structure of human PARG

The human PARG gene, which is highly conserved and located at chromosomal locus 10q11.23-21, is composed of 18 exons and 17 introns and encodes a protein comprising 976 amino acids with a molecular weight of 111.1 kDa (Figure 2A; Shimokawa et al., 1998; Amé et al., 1999; O'Sullivan et al., 2019). The PARG protein consists of four distinct structural components: a disordered regulatory region covering exons 1–3, a hinge domain spanning exons 4–8, a catalytic domain encompassing exons 9–14 containing the active site and PARG signature motif, and a macrodomain formed by the catalytic domain and exons 15–18. Numerous nuclear export signals (NESs) and nuclear localization signals (NLSs) are distributed throughout the PARG sequence (O'Sullivan et al., 2019). The PARG transcript undergoes selective splicing, leading to the diverse subcellular localization of its isoforms (Meyer-Ficca et al., 2004). The largest isoform, $PARG_{111}$, which contains all exons, primarily localizes to the nucleus, where it degrades PAR from

$PARP_{1/2}$ following genotoxic stress, with potential cytoplasmic translocation (Min et al., 2010; Žaja et al., 2013). $PARG_{102}$ and $PARG_{99}$, which lack portions of the N-terminal domain, are found in the cytoplasm and perinuclear region and translocate to the nucleus upon DNA damage (Meyer-Ficca et al., 2004; Haince et al., 2006; Mortusewicz et al., 2011). The nucleocytoplasmic shuttling mechanism of these isoforms and their role in efficient PAR degradation have still not been fully elucidated (Harrision et al., 2020). $PARG_{55}$ and $PARG_{60}$ are located in mitochondria with distinct exon compositions. $PARG_{60}$ lacks exons 1, 4, and 5, altering its N-terminal sequence, while $PARG_{55}$ similarly lacks these exons and additionally lacks exon 1a (Meyer et al., 2007; Rack et al., 2020). Due to the absence of exon 5, both $PARG_{55}$ and $PARG_{60}$ lack catalytic activity (Figure 2B and C; Whatcott et al., 2009). Further investigation is necessary to understand the potential significance of the quantity of NLSs and NESs in determining the localization patterns of these subtypes (Hassa and Hottiger, 2008).

PARG plays a crucial role in DNA damage repair

PARP-mediated PARylation recruits DNA damage response (DDR) proteins to sites of damage. PARG-mediated dePARylation then facilitates the loading of these proteins onto damaged areas to initiate repair (Figure 3A; Matanes et al., 2021).

Upon detecting single-strand DNA breaks (SSBs), PARP undergoes self-PARylation, attracting X-ray repair cross complementing 1 (XRCC1) to the site (Fisher et al., 2007). PARG then regulates the dissociation of XRCC1 from PAR chains, facilitating the recruitment of other factors involved in SSB repair to complete the process (Figure 3B; Chen and Yu, 2019; Kassab et al., 2020). Inhibited or depleted PARG results in the accumulation of automodified PARP1 at SSB sites, preventing XRCC1 translocation to these sites (Wei et al., 2013; Gogola et al., 2019).

The PARG inhibitor COH34 disrupts the repair of SSBs and double-strand breaks (DSBs) induced by ionizing radiation. This suggests its impact on key DSB repair pathways, including canonical non-homologous end joining (c-NHEJ), alternative NHEJ (a-NHEJ), and homologous recombination (HR) (Chen and Yu, 2019). PARG inhibition specifically hampers dePARylation at DNA damage sites, prolonging the retention of PAR-bound DNA repair factors.

Mortusewicz et al. (2011) elucidated a PAR-independent mechanism for PARG recruitment, wherein the interaction between PARG and proliferating cell nuclear antigen (PCNA) facilitates its localization at sites of damage. Kaufmann et al. (2017) identified a non-classical PARG PIP box within the disordered regulatory region, enabling interaction with PCNA. PARG plays a critical role in regulating PAR levels during replication stress to prevent excessive PAR accumulation, which could lead to replication fork collapse and DSBs (Illuzzi et al., 2014). Currently, there is no evidence suggesting that the interaction between PARG and PCNA is exclusive to replication stress.

PARP inhibition accelerates replication forks (Maya-Mendoza et al., 2018), disrupts fork reversal (Ray Chaudhuri et al., 2012), and triggers fork restarts (Ray Chaudhuri and Nussenzweig, 2017). Conversely, PARG inhibition slows fork progression (Ray Chaudhuri et al., 2015; Gravells et al., 2017; Houl et al., 2019), potentially inducing replication catastrophe (Pillay et al., 2019). In summary, PARG primarily facilitates DNA repair by hydrolyzing PAR chains, thereby preventing excessive PARP activation and ensuring the efficient progression of repair processes.

PARG regulates the transcriptional activity of cells

The role of PARP in transcriptional gene regulation is widely acknowledged, as is its involvement in DNA damage repair (Luo et al., 2017; Bamgbose and Tulin, 2024). Recent studies have revealed that PARG, traditionally recognized for its DNA repair function, also acts as an adjunct regulator of cellular transcriptional activities (Figure 3C). Inhibiting PARG, notably with gallic acid, leads to the nuclear accumulation of PAR, subsequently enhancing the expression of crucial enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in a PAR-dependent manner. The observed increases in iNOS and COX-2 were not associated with the activation of transcription factors or alterations in mRNA stability. However, the accumulation of nuclear PAR may lead to changes in chromatin structure and chromatin modifications, impacting gene accessibility and transcriptional activity. These alterations could further contribute to the upregulation of the iNOS and COX-2 genes. The significance of PARG in transcriptional regulation is emphasized by its recruitment and subsequent chromatin modification.

Moreover, the influence of PARG extends to chromatin dynamics, impacting both chromatin activation and silencing. This dual role underscores its complexity in transcriptional regulation (Tulin et al., 2006). Frizzell et al. (2009) shed light on how PARG, similar to PARP, localizes to specific gene promoters. It not only governs gene expression but also affects binding dynamics at these promoters in a gene-specific manner. This study further confirmed that PARP1 and PARG play complementary roles in gene regulation, often co-regulating the expression of the same sets of genes in similar directions and to comparable extents, rather than acting antagonistically.

In the context of gene transactivation, PARG plays a critical role in retinoic acid receptor (RAR)-mediated gene expression (Le May et al., 2012). It achieves this through its PAR degradation activity and is recruited to RAR-dependent gene promoters in a ligand-dependent manner, thereby creating a conducive environment for transcription. This recruitment and subsequent chromatin modification underscore the importance of PARG in transcriptional regulation.

Importantly, the absence of a DNA binding domain in PARG suggests that its regulatory functions are independent of direct chromatin binding, unlike those of PARP1 (Frizzell et al., 2009). This independence likely involves interactions with histones or other chromatin-associated proteins, indicating a more indirect but equally crucial role in transcriptional modulation. Additionally, PTMs of PARP1 and PARG are likely to further influence their roles in gene regulation. These modifications could alter their activity, interaction with other

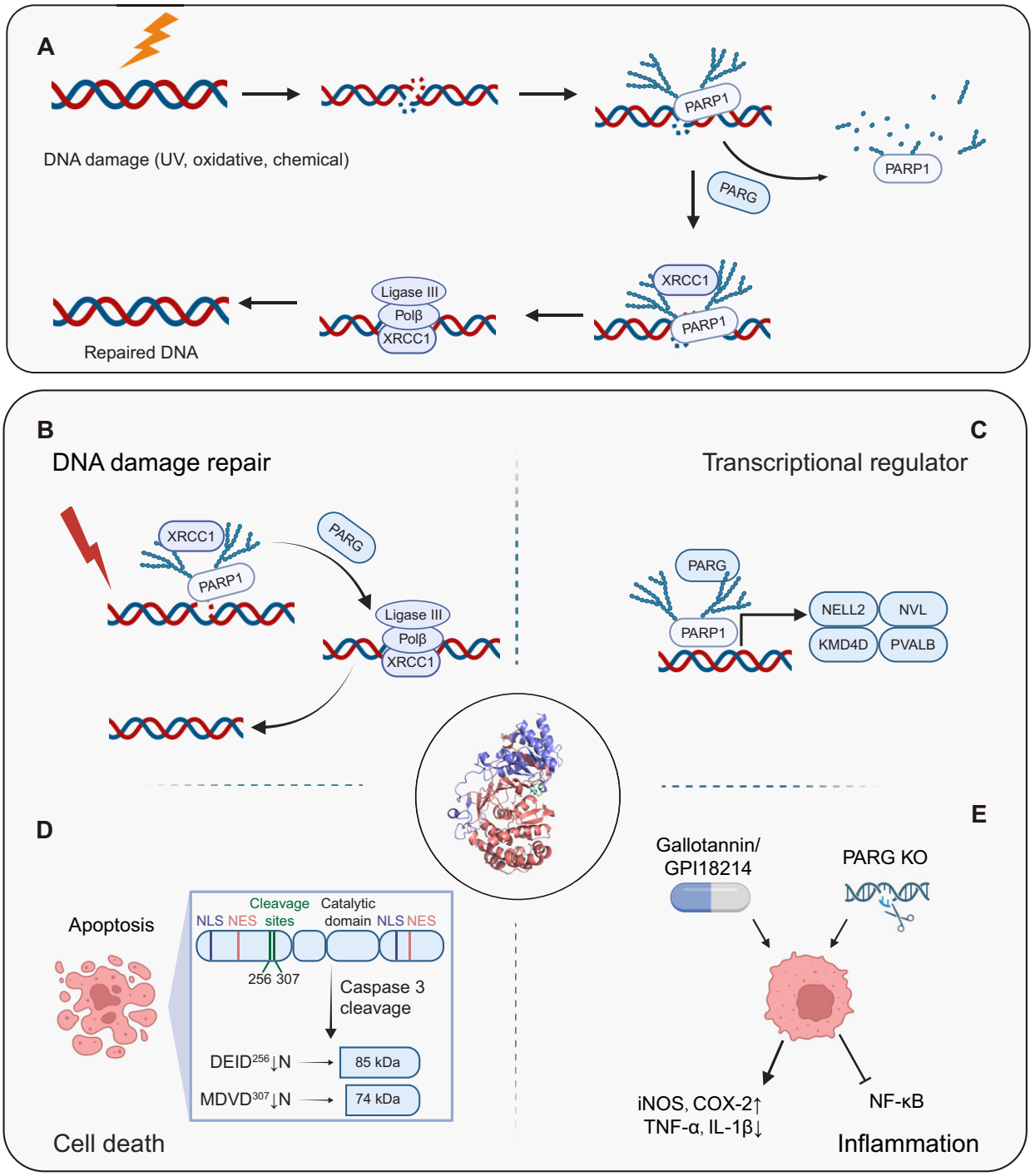


Figure 3 Schematic diagram of the function of PARG. (**A**) Mechanism of action of PARP and PARG in DNA damage repair. After DNA damage, PARP is recruited to the damage site and activated to recruit XRCC1 to the damaged DNA through its own PARylation modification. XRCC1 acts as a scaffold protein and subsequently recruits DNA ligase III and DNA polymerase β (polβ) to complete DNA repair. (**B**) PARG is involved in DNA damage repair. (**C**) PARG is recruited to the promoters of RAR-dependent genes to regulate gene transcription. (**D**) PARG is cleaved by caspase 3 during apoptosis. (**E**) Pharmacological and genetic inhibition of PARG upregulates iNOS and COX-2, inhibits NF-κB, and reduces the secretion of the pro-inflammatory cytokines TNF-α and IL-1β to regulate inflammatory processes.

proteins, and overall impact on gene expression, adding another layer of complexity to their function in the cell. For example, monomethylation of PARP1 at Lys23 contributes to DNA damage repair via the NHEJ pathway (Wang et al., 2022) and affects the binding of PARP1 to restarted replication fork structures. The receptor tyrosine kinase c-Met binds to the PARP1 Tyr907 site, phosphorylating PARP1 and increasing its enzyme activity while decreasing its affinity for PARP inhibitors. This phosphorylation at Tyr907 renders cancer cells resistant to PARP inhibition (Du et al., 2016). Additionally, USP1 removes the ubiquitin chain from PARP1 at Lys197, preventing its proteasomal degradation (Zhang et al., 2023). Stabilization of PARP1, however, promotes cholangiocarcinoma growth and metastasis *in vitro* and *in vivo*. SUMOylation of PARP1 at Lys486 fully abolishes p300-mediated PARP1 acetylation, inhibiting its transcriptional co-activator role and reducing the expression of PARP1 target genes (Messner et al., 2009). Furthermore, SIRT2-mediated deacetylation of PARP1 at Lys249 increases PARP1 ubiquitination, which attenuates oxidative stress-induced vascular injury and remodeling (Zhang et al., 2021). Recently, modification sites on PARG have been identified via proteomic discovery mass spectrometry (Mertins et al., 2016); however, the functional roles of these PTMs remain to be further investigated.

Other functions of PARG

PARP cleavage is a well-recognized marker of apoptosis and Caspase 3 activation. In humans, caspases cleave PARP between Asp124 and Gly215, separating the catalytic structural domain at the carboxyl terminus (89 kDa) from the DNA-binding structural domain at the amino terminus (24 kDa), resulting in the loss of PARP enzymatic activity (Chaitanya et al., 2010; Mashimo et al., 2021). However, what about PARG during cell death? Affar et al. (2001) investigated the cleavage of PARG by caspase-3 during Fas receptor-, astrocytein-, and VP-16-induced apoptosis. Their study revealed that PARG is cleaved into a 74-kDa fragment across all cell types, with human and mouse PARG exhibiting additional fragments (85 and 66 kDa, respectively). Importantly, the enzymatic activity of PARG remained unaffected during this process. Both the 85-kDa and 74-kDa fragments retained the ability to hydrolyze PAR, similar to the full-length enzyme (Figure 3D). The 85-kDa fragment is produced exclusively in human cells, indicating that its production is not essential in other mammalian species. The 74-kDa fragment is located within the cytoplasm of apoptotic cells, and it remains unclear whether this fragment plays a specific role in human apoptosis. Further investigations are needed to elucidate the role of PARG cleavage in apoptosis.

Cuzzocrea et al. (2007) induced inflammatory responses via dinitrobenzene sulfonic acid (DNBS). They investigated the genetic (PARG KO) and pharmacological (GPI18214 or GPI16552) effects of PARG inhibition on mitigating the inflammatory process by reducing the secretion of the pro-inflammatory cytokines TNF-α and IL-1β (Figure 3E). Given the significant role that PARG plays in cells, researchers have focused on its potential in treating inflammatory diseases.

Additionally, PARG deletion results in the accumulation of PARylation, impairing the proteasomal capacity to bind and degrade PARylated proteins (Sun et al., 2021). PARylation also facilitates the recruitment of the deubiquitinating enzyme USP7, which reverses the ubiquitination of PARylated topoisomerase I DNA–protein crosslinks (TOP1-DPC). Thus, PARG-driven dePARylation is essential for the proteasomal degradation of TOP1 in response to TOP1-DPC induction, underscoring the role of PARG in repairing TOP1-DPCs.

Furthermore, the E3 ligase RNF146 is accountable for ADP-ribosylation-dependent ubiquitination (Zhang et al., 2024). RNF146 is comprised of two conserved structural domains: a RING structural domain, which may function as an E3 ubiquitin ligase, and a WWE structural domain, which is a PAR-binding structural domain. The inhibition of PARG proteins results in an elevation of PARylation of targeted proteins. Consequently, in the context of PARG knockdown or inhibition, RNF146 is capable of interacting with and facilitating the degradation of PARylated proteins.

In summary, the evolving understanding of PARG's roles in DNA damage repair, transcriptional regulation, proteostasis, apoptosis and inflammation offers a nuanced perspective on its function. This highlights the importance of considering PARG not only within the context of DNA repair but also as a key contributor to a broader spectrum of cellular regulatory processes. Future research in this field is poised to uncover deeper insights into PARG's mechanisms and potential therapeutic implications. Recently, PARG inhibitors have shown promise in preclinical studies, demonstrating efficacy in alleviating ischemic brain injury (Lu et al., 2003) and renal ischemia/reperfusion injury (Cuzzocrea and Wang, 2005), as well as in protecting against astrocyte death (Ying and Swanson, 2000) and colitis (Cuzzocrea et al., 2007). This review focuses on exploring the therapeutic potential of PARG inhibitors in the context of cancer treatment.

PARG inhibition in cancer therapy

Analysis of The Cancer Genome Atlas (TCGA) database revealed the upregulation of PARG in numerous cancers, suggesting its potential involvement as a carcinogenic factor in tumor cells (Houl et al., 2019; Marques et al., 2019). Recent studies have demonstrated that inhibiting PARG can impede the progression of various cancers, such as hepatocellular carcinoma (HCC) (Yu et al., 2022), lung cancer (Dai et al., 2019; Sasaki et al., 2019), ovarian cancer (Pillay et al., 2021; Martincuks et al., 2024), breast cancer (Marques et al., 2019), pancreatic cancer (Jain et al., 2019), esophageal squamous cell carcinoma (ESCC) (Yan et al., 2023), colorectal cancer (Wang et al., 2019), and prostate cancer (Zhang et al., 2020), resulting in antitumor effects.

Pillay et al. (2019) observed varied responses of ovarian cancer cells to PARP and PARG inhibitors, with sensitivity to PARG inhibitors associated with persistent fork stalling and replication catastrophe due to potential DNA replication defects. Given their distinct sensitivities to PARP and PARG inhibitors, PARG inhibitors can complement PARP

inhibitors in ovarian cancer treatment. Additionally, research has revealed that combining PARG inhibition with DDR checkpoint kinase 1 results in synthetic lethality. Gravells et al. (2017) established a synthetic lethal interaction between PARG and genes such as breast cancer gene 1 (BRCA1), BRCA2, PALB2, ABRAXAS, and BARD1. This is based on the principle that PARG inhibition exacerbates endogenous DNA damage, leading to fork stalling and creating a synthetic lethal mechanism with HR-defective genes. Furthermore, Jain et al. (2019) discovered that PARG inhibitors are particularly effective in treating pancreatic ductal adenocarcinoma cells with HR defects. Moreover, Sasaki et al. (2019) identified dual specificity phosphatase 22 (DUSP22) as a novel synthetic lethal target for PARG inhibition, demonstrating that the increase in p38 phosphorylation caused by DUSP22 knockdown and PAR accumulation due to PARG knockdown synergizes to induce apoptosis in lung cancer cells. Andronikou et al. (2024) identified EXO1 and FEN1 as the major synthetic lethal interactors of PARG loss through whole-genome CRISPR/Cas9 drop-out screens. Targeting EXO1/FEN1 may enhance the efficacy of PARG inhibitors in HR-deficient tumors. These findings underscore the potential of PARG inhibitors as targeted treatments in specific cancer subtypes where genetic vulnerabilities can be therapeutically exploited. Therefore, PARG inhibitors represent a promising therapeutic approach for cancer patients with HR defects, and identifying new synthetic lethal targets of PARG is crucial for advancing cancer treatment.

The targeted inhibition of PARG has significant therapeutic potential in cancer and potentially impacts tumor growth by modulating various signaling pathways in cancer cells. Marques et al. (2019) reported a strong correlation between high PARG expression and poor prognosis in invasive breast cancer patients. *In vitro* studies indicated that PARG overexpression promotes tumor cell transformation and invasion. *In vivo*, PARG has been shown to synergize with the human epidermal growth factor receptor 2 (HER2) oncogene, enhancing tumor initiation and growth. Further investigations revealed that PARG amplifies the transcription of epithelial–mesenchymal transition (EMT)-related genes by increasing hypoPARylated SMAD2/3 binding to chromatin, suggesting that PARG is a promising therapeutic target for breast cancer.

Similarly, Yu et al. (2022) reported a notable association between elevated PARG expression and adverse outcomes in patients with HCC. PARG appears to facilitate liver cancer development and could serve as a diagnostic biomarker for HCC. Moreover, their study revealed a novel mechanism by which PARG, through dePARylation of damage-specific DNA binding protein 1, stabilizes the oncogenic protein c-Myc. In HCC patients receiving anti-PD-1 immunotherapy, PARG downregulation diminishes the expression of mismatch repair proteins induced by c-Myc. Collectively, these findings underscore the role of PARG as a viable target for cancer therapy and support the continued clinical advancement of PARG inhibitors.

Coulson-Gilmer et al. (2024) demonstrated that repression of Timeless increases cellular sensitivity to PARG inhibitors. This sensitivity associated with low Timeless levels can be partially mitigated by nucleoside supplementation, indicating a critical role for nucleosides in cellular energy metabolism and repair. These findings suggest that nucleotide imbalance may contribute to PARG inhibitor sensitivity in tumor cells. This study offers valuable insights into differential cellular drug responses under specific gene expression conditions and proposes that modulating nucleoside availability could enhance tumor sensitivity to PARG inhibitors.

The development of PARG inhibitors

PARP enzymes, due to their broad family of 17 related members, can often compensate for PARP function during therapy and remain unaffected by PARP inhibitors (Rose et al., 2020). In contrast, PARG is monogenic, implying that PARG inhibitors might have fewer off-target effects and greater potency and specificity (Antolin et al., 2020; Bejan and Cohen, 2022). Clinically, resistance to PARP inhibitors has arisen through mechanisms including the restoration of the HR repair (HRR) pathway, replication fork stabilization, PARP mutation, and drug efflux (Meghani et al., 2018; Pettitt et al., 2018; Lin et al., 2019; Marzio et al., 2019; Garg and Oza, 2023). Unlike PARP inhibitors, PARG inhibitors retain efficacy even if HRR function is restored, as persistent PAR accumulation disrupts both DNA replication and DNA damage repair, leading to cell toxicity (Fu et al., 2024). Additionally, while PARP inhibitor resistance can occur through the restoration of replication fork stability, PARG inhibitors counter this by inducing replication fork collapse and cell death, as they prevent PAR strand resolution at the replication fork, even in cells with enhanced fork stability (Ray Chaudhuri et al., 2016). This underscores the necessity of exploring novel therapeutic targets for PARP inhibitor-resistant tumors, with PARG emerging as a promising candidate for indirect PARP inhibition.

Current PARG inhibitors are categorized into natural product derivatives and small-molecule inhibitors (Table 1 and Figure 4A). The development of small-molecule PARG inhibitors focuses on targeting the high specificity of the adenine binding pocket (Waszkowycz et al., 2018). These inhibitors typically occupy the adenine binding site with ADPr, competitively binding to PAR and thereby inhibiting PARG activity (Figure 4B; James et al., 2016; Houl et al., 2019; Slade, 2020).

Natural PARG inhibitor—tannin

Tannins are ubiquitous in various plant parts, including the xylem, bark, leaves, fruits, and roots. They exhibit pharmacological properties that render them effective in treating a wide array of diseases, including anti-inflammatory, antioxidant, anticonvulsant, and antitumor effects (Hussain et al., 2019). Tannins are classified as condensed tannins or hydrolyzable tannins (HTs), which are further classified into ellagitannins (ETs) and gallotannins (GTs) according to their hydrolyzed products (Jaiswal et al., 2018). Only HTs serve as PARG inhibitors. The IC_{50} of ETs and GTs were 8.3–12.5 μM and 16.8–28.9 μM,

Table 1 PARG inhibitors.

PARG inhibitor	IC_{50}	Activity	Feature	Drug type	Organization	Highest phase	Reference
Tannin	16.8 μM	ADPr analogs and resisting PARG	Low cell permeability, off-target effects	Natural product	/	Preclinical	Tsai et al. (1991)
ADP-HPD	120 nM	ADPr analogs and resisting PARG	Specific, not cell-permeable	Small molecule	/	Preclinical	Slama et al. (1995a)
GPI16552	1.7 μM	Binding to PAR and resisting PARG-mediated hydrolysis	Low activity, not effective *in vivo*	Small molecule	/	Preclinical	Falsig et al. (2004)
RBPIs	1–6 μM	Blocking PARG-mediated PAR hydrolysis	Low specific, not cell-permeable	Small molecule	/	Preclinical	Finch et al. (2012)
PDD0017273	26 nM	Replication fork stalling and low DNA DSB repair	Specific, potent, cell-permeable, lacking bioavailability	Small molecule	/	Preclinical	James et al. (2016)
COH34	0.37 nM	Binding to PARG catalytic site and targeting dePARylation	Specific, potent, cell-permeable, effective *in vivo*	Small molecule	/	Preclinical	Chen and Yu (2019)
JA2131	0.4 μM	Binding to PARG active site and competing with the adenine moiety of PAR substrates for active site binding	Specific, cell-permeable	Small molecule	/	Preclinical	Houl et al. (2019)
IDE161	/	/	Potent, selective	Small molecule	IDEAYA Biosciences	Phase I	NCT05787587
ETX-19477	/	/	Potent, selective	Small molecule	858 Therapeutics, Inc.	Phase I	NCT06395519
DAT2645	/	/	High selectivity	Small molecule	Danatlas Pharmaceuticals Co.	Phase I	NCT06614751

respectively. The data indicated an increase in the degree of PARG inhibition by GTs as the amount of galloyl increased (Tsai et al., 1991). The higher the molecular complexity of ETs, the greater the degree of PARG inhibition. Compared with their dimeric or trimeric counterparts, monomeric ETs presented a lower level of inhibition.

In vitro experiments with HaCaT cells revealed that gallic acid pretreatment inhibits the depletion of the nicotinamide adenine dinucleotide (NAD^+) pool, resulting in PAR accumulation and reduced PARP activity following exposure to hydrogen peroxide or peroxynitrite (Bakondi et al., 2004). Keil et al. (2004) investigated the impact of tannins on PAR levels in HeLa nuclear extracts and reported an increase in PAR levels upon the addition of tannins, which was attributed to the inhibition of PARG catalytic activity. However, PARG silencing with siRNA attenuated the GT-induced expression of iNOS and COX-2, suggesting that pharmacological PARG inhibition alters macrophage gene expression profiles in a PAR-dependent manner (Rapizzi et al., 2004). Together, these studies underscore the efficacy of gallic acid as a PARG inhibitor.

González-Barrio et al. (2010) conducted research on the metabolism of anthocyanins, tannic acid, and tannins in healthy human volunteers and ileostomy subjects after ingesting 300 g raspberries. The study indicated that the low bioavailability of tannins, attributed to their solubility and membrane permeability, significantly limits their effectiveness as PARG inhibitors, thereby limiting their practical application. To address the challenge of low oral bioavailability, researchers have utilized parenteral delivery methods, including intraperitoneal and intranasal administration, in animal studies (Wei et al., 2007; Chandak et al., 2009).

Small-molecule PARG inhibitors

ADP-HPD, a chemical derivative of ADPr, serves as an amino analog synthesized through chemical methods and acts as a small-molecule inhibitor (Slama et al., 1995b). This compound displays high efficiency, noncompetence, and specific inhibition of PARG, with an IC_{50} of 0.12 μM (Slama et al., 1995a). Notably, ADP-HPD does not inhibit PARG at inhibitor concentrations up to 1 mM. At lower concentrations, ADP-HPD selectively inhibits PARG. However, achieving complete inhibition of PARG activity with ADP-HPD is challenging (Slama et al., 1995b). Its inability to penetrate the cell membrane restricts its utility in cellular and *in vivo* applications. Furthermore, ADP-HPD inhibits ARH3, thereby enhancing its effectiveness as an inhibitor of both PAR glycohydrolases (Finch et al., 2012; Rack et al., 2018). In summary,

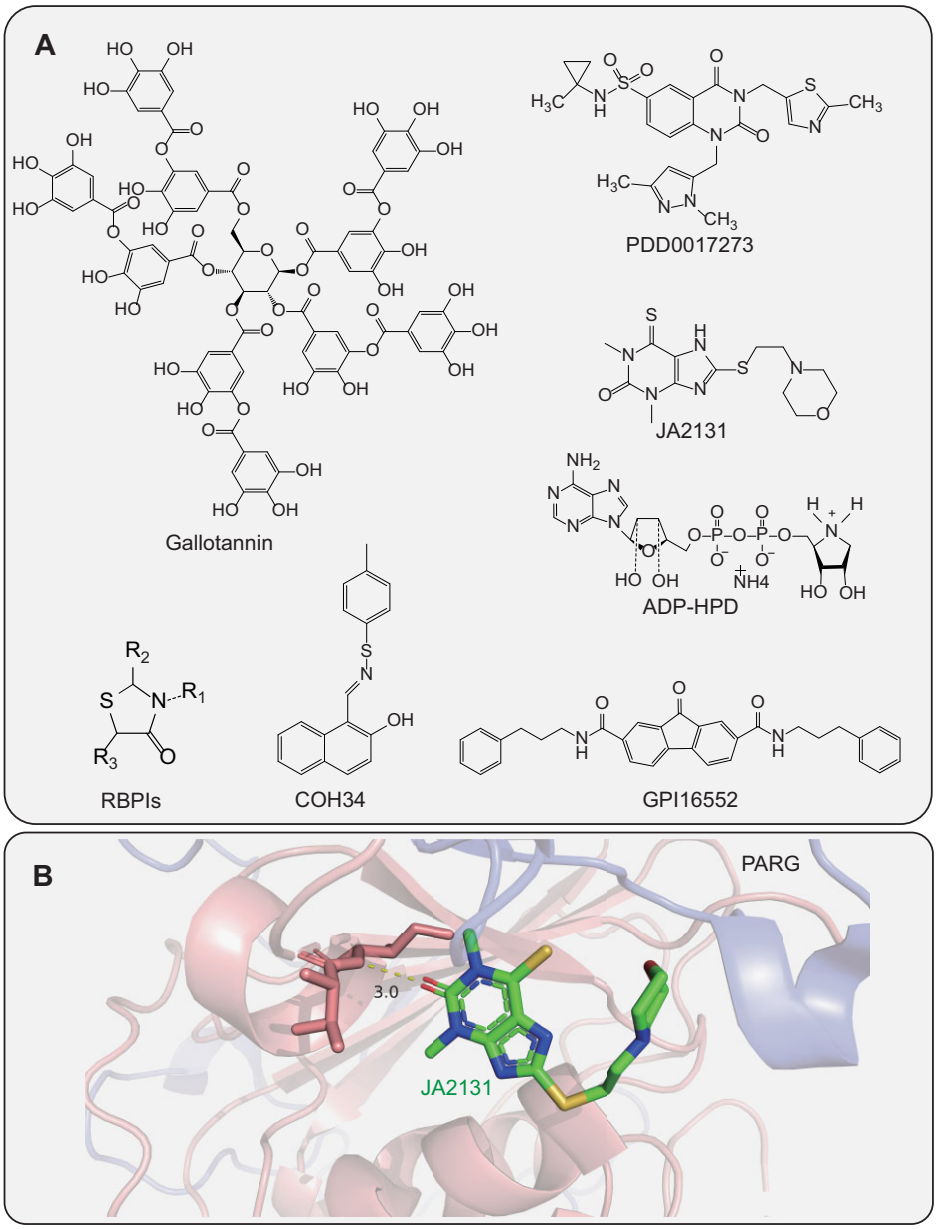


Figure 4 The PARG Inhibitors. (**A**) Chemical structures of PARG inhibitors. (**B**) The PARG inhibitor JA2131 occupies the adenine-binding pocket of PARG.

while ADP-HPD has proven valuable for studying the structure and function of PARG both *in vitro* and in cellular extracts, its high cost, complex synthesis, limited cell permeability, and partial PARG inhibition have restricted its broader research applications.

N-Bis-(3-phenylpropyl)-9-oxofluorene-2,7-diamide (also termed GPI16552) is a potential specific PARG inhibitor. When combined with temozolomide, it significantly reduces melanoma growth. Furthermore, it extends the lifespan of tumor-bearing mice and decreases the propensity of melanoma cells to form lung metastases and invade the extracellular matrix (Falsig et al., 2004; Tentori et al., 2005). Another novel PARG inhibitor, GPI18214, alleviated zymosan-induced non-infectious shock in mice by disrupting the cycle of RAR turnover, highlighting PARG as an alternative therapeutic target for shock treatment (Genovese et al., 2004). Treatment with GPI16552 and GPI18214 has notable protective effects on DNBS-induced colitis in wild-type mice (Cuzzocrea et al., 2007). However, GPI16552 exhibited an IC_{50} of 50 μM and limited maximal inhibition in *in vitro* PARG activity assays, achieving only 40% inhibition at 80 μM and showing no effect in cellular activity assays (Falsig et al., 2004). Consequently, this compound class is unsuitable for evaluating PARG inhibition effects in cellular and *in vivo* animal models.

Subsequently, Finch et al. (2012) developed rhodamine-based PARG inhibitors (RBPIs), which are effective and selective small-molecule inhibitors created via targeted screening and chemical optimization. RBPIs, which effectively target PARG at low micromolar concentrations, have potent inhibitory effects both *in vitro* and in cell lysates, effectively targeting PARG at low micromolar concentrations, with an IC_{50} ranging from 1 μM to 6 μM. However, the practical application of RBPIs is limited by issues related to cell membrane permeability.

To address the specificity and cell permeability constraints of current PARG inhibitors, James et al. (2016) conducted high-throughput screening to develop a novel, potent, and specific PARG inhibitor. Among the identified compounds, PDD00017238, a benzimidazolone derivative, displayed an IC_{50} of 40 nM and demonstrated moderate cytotoxicity in cells (James et al., 2016; Gravells et al., 2018). Notably, PDD00017273, a quinazolinone-based compound, emerged as the most effective and practical inhibitor, exhibiting an IC_{50} of 26 nM. It displayed cell permeability without cellular toxicity, leading to dose-dependent PARG inhibition and significant effects at low concentrations (0.3 μM) (Kassab et al., 2020). However, this compound underwent rapid degradation in *in vitro* particle assays and showed a short half-life in pharmacokinetic studies, limiting its viability for *in vivo* cancer therapy due to poor metabolic stability (Tanuma et al., 2019).

Recently, Chen and Yu (2019) identified a novel small-molecule PARG inhibitor, COH34, demonstrating cell membrane permeability through the site-mapping (siMMap) computational strategy via the National Cancer Institute (NCI) database. COH34, with an IC_{50} of 0.37 nM, efficiently and specifically inhibits PARG both *in vitro* and *in vivo*. It binds to the catalytic domain of PARG at a 1:1 ratio, competitively interacting with the substrate PAR. COH34 minimally affects other PARylation enzymes (TARG1 and ARH3), underscoring its specificity as a PARG inhibitor and its strong binding affinity for the active site of PARG. Notably, COH34 exhibits synthetic lethality in BRCA1/2 mutant tumor cells and enhances the efficacy of DNA-damaging agents such as cisplatin, doxorubicin, temozolomide, and camptothecin in inducing tumor cell death. Furthermore, its combination with these agents markedly increases lethality in PARP inhibitor-resistant cells, suggesting that PARG targeting is a potential therapeutic approach for such cancers. Additionally, COH34 at a dose of 20 mg/kg via intraperitoneal injection has been shown to have antitumor effects on mice without toxicity. Pharmacokinetic and pharmacodynamic studies have demonstrated the *in vivo* stability and efficacy of COH34 in targeting PARylation removal, highlighting its promise as a potential cancer therapeutic agent.

In the same year, Houl et al. (2019) developed selective PARG inhibitors through high-throughput screening of the National Cancer Institute (NCI) Diversity Set II library, focusing on drug structure design. The pharmacophore of these inhibitors is based on thio analogs of guanine derivatives. JA2131, a 6′-thio analog of a methylguanine derivative with an IC_{50} of 400 nM, specifically interacts with the active site of PARG and competitively binds to the adenine portion of the PAR substrate. JA2131 exhibits cell membrane permeability, selectively inhibits PARG, impedes PAR removal by PARG, and induces cancer cell death comparable to that of olaparib. Moreover, JA2131 increases PAR accumulation and γH2AX levels in ionizing radiation-treated cells, leading to replication defects in HeLa cells and reduced cancer cell survival.

IDE161 is a highly efficient and specific small-molecule PARG inhibitor discovered by IDEAYA Biosciences via its proprietary drug discovery system, with a particular focus on enhancing treatment for tumors with HR deficiency (HRD) (Abed et al., 2023). In various cell line-derived xenograft and patient-derived tumor xenograft models, including those of ovarian, gastric, and breast cancers, IDE161 has demonstrated antitumor efficacy as a standalone treatment. IDE161 also targets PARP inhibitor-resistant ovarian and breast cancers with HRD. In the first quarter of 2023, IDEAYA launched a phase I clinical trial (NCT05787587) to assess the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary efficacy of IDE161, both alone and in combination with pembrolizumab, in patients with advanced solid tumors. This trial is actively recruiting participants, and the results have not yet been reported. The estimated study completion date is May 2027.

ETX-19477, a novel and potent PARG small-molecule inhibitor, accumulates PAR chains through pharmacological inhibition of PARG, resulting in targeted activity at low nM levels within cells (Holleran et al., 2024). By exploiting the inherent vulnerability of cancer cells to replication stress, ETX-19477 has been shown to have effective and specific antiproliferative effects on various tumors, including ER + HER2−breast cancer, serous and mucinous ovarian cancer, lung cancer, and gastric cancer (Holleran et al., 2024). Preclinical studies have shown that ETX-19477 has oral bioavailability and favorable pharmacokinetics. 858 Therapeutics, Inc. initiated clinical trials (NCT06395519) in May 2024 to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and anti-tumor activity of ETX-19477. This trial is currently recruiting patients, with an estimated completion date of December 2026.

DAT-2645 is a novel, highly selective, oral small-molecule inhibitor of PARG developed by Danatlas Pharmaceuticals Co., Ltd. This compound has demonstrated selective cytotoxicity against tumor cells with specific DDR defects through a synthetic lethal mechanism. This mechanism may enable DAT-2645 to function as a potential broad-spectrum therapeutic. DAT-2645 targets tumor cells with particular DDR abnormalities. In June 2024, the US Food and Drug Administration approved an investigational new drug application for DAT-2645 tablets. Clinical trials associated with DAT-2645 (NCT066614751 and CTR20243912) have not yet been conducted. The purpose of the phase I trial is to evaluate the safety and tolerability of DAT-2645 in patients with advanced or metastatic solid tumors exhibiting BRCA1/2 loss-of-function alterations or other defects in the DDR pathway.

The development of small-molecule, cell-permeable PARG inhibitors that are strategically engineered to increase specificity and biological activity is highly important for cancer

therapy. This advancement presents a novel and clinically impactful therapeutic approach for cancer patients.

Conclusion and future prospects

PARP and PARG coordinate reversible PARylation at sites of DNA breaks and replication forks, facilitating the assembly and disassembly of complexes involved in DNA damage repair (Gibson and Kraus, 2012; Qi et al., 2019). As the primary hydrolase of PAR chains, PARG plays a crucial role in DNA damage repair mechanisms within cancer cells (Janisiw et al., 2020; Geng et al., 2023).

The present investigations into PARG predominantly focus on its involvement in DNA damage repair responses, primarily focusing on elucidating its intracellular functions in isolation. Although PARP significantly contributes to genome stabilization, few studies have directly explored the interplay between the biological functions regulated by PARP1 and PARG in various cellular contexts (Azarm and Smith, 2020; Bordet et al., 2022; Rouleau-Turcotte and Pascal, 2023). Consequently, the mechanisms underlying the collaborative modulation of genome-wide gene expression by PARP1 and PARG remain incompletely understood. Future research may explore the role of PARG in counteracting the gene regulatory effects exerted by PARP1 through the degradation of PAR chains synthesized by PARP1, thus revealing the antagonistic enzymatic functions of PARP1 and PARG.

Nuclear and cytoplasm-localized isoforms of PARG, notably $PARG_{111}$, $PARG_{102}$, and $PARG_{99}$, play pivotal roles in DNA damage repair (Hassa and Hottiger, 2008; Burns et al., 2009). However, the functions of mitochondrion-localized isoforms, such as $PARG_{55}$ and $PARG_{60}$, remain poorly understood and merit further investigation (Harrision et al., 2020). Moreover, disruption of the mouse PARG gene results in early embryonic lethality and heightened sensitivity to genotoxic stress, posing challenges for *in vivo* functional studies of PARG (Koh et al., 2004). Conditional knockout mice offer a promising avenue for exploring the function of PARG in specific tissues. In summary, numerous aspects of the role and mechanisms of PARG remain to be elucidated.

PARG has garnered increasing recognition as a promising therapeutic target for various diseases, including cancer, ischemia-reperfusion injuries, and neurodegenerative disorders (Cuzzocrea and Wang, 2005; Hussain et al., 2019; Slade, 2020). Researchers may utilize mouse models that either overexpress or lack PARG to investigate its role in tumorigenesis and other diseases. Additionally, organoid models derived from patient cells offer a more physiologically relevant platform to study PARG function. Screening for PARG synthetic lethal genes via a whole-genome CRISPR/Cas9 library can facilitate the development of personalized treatment regimens tailored to each patient's unique genetic mutational background, potentially leading to more effective and less toxic cancer therapies. Furthermore, the development of PARG inhibitors has become a focal point of biomedical research in recent years. Over the past few decades, extensive research into PARG inhibitors has led to significant improvements in key pharmacological aspects. These advancements include enhanced specificity, reduced off-target effects, increased membrane permeability, improved cell and tissue penetration, improved selectivity, more effective targeting of PARG without affecting similar enzymes, and overall greater efficacy in inhibiting PARG activity. Despite these promising developments, the journey toward clinical application faces obstacles. Currently, no PARG inhibitors have received clinical approval, indicating a gap between preclinical research findings and therapeutic translation. This delay may stem from various challenges, such as the complexity of PARG interactions in the cellular environment, potential toxicity, and difficulty in balancing efficacy and safety in human subjects. Additionally, given the involvement of PARG in multiple cellular pathways, a deeper understanding of its biological functions in different diseases is essential. Such insight could pave the way for expanding the use of PARG inhibitors beyond current focus areas, potentially leading to breakthroughs in treating other diseases where PARylation plays a crucial role.

Therefore, future research should prioritize refining PARG inhibitors, with a focus on overcoming these challenges. This includes optimizing pharmacokinetic properties to ensure safe and effective drug delivery to target sites within the body. Additionally, the clinical application of PARG inhibitors requires comprehensive clinical trials to assess their therapeutic potential and safety profile in diverse patient populations.

Acknowledgements

We express our appreciation to all researchers for their valuable contributions to the study of PARG and PARG inhibitors.

Funding

This work was supported by grants from the National Natural Science Foundation of China (32271485) and the Natural Science Foundation of Zhejiang Province (YXD23H0302) to J.J., as well as grants from the National Natural Science Foundation of China (82473195), the Natural Science Foundation of Zhejiang Province (LTGY23H160018), the Zhejiang Medical and Health Science and Technology Program (2024KY789), and the Beijing Science and Technology Innovation Medical Development Foundation (KC2023-JX-0270-07) to Z.Y.

Conflict of interest: none declared.

Author contributions: conceptualization: J.J., Z.Y., and Y.H.; writing—original draft preparation: Y.H., Y.M., Z.Z.; writing—review and editing: Y.H., Y.M., Z.Z., Y.L., J.N., and N.X.; supervision: J.J. and Z.Y.; funding acquisition: J.J. and Z.Y.; all authors read and agreed to the published version of the manuscript.

References

Abed, M., Muñoz, D., Seshadri, V., et al. (2023). Abstract 6093: IDE161, a potential first-in-class clinical candidate PARG inhibitor, selectively targets homologous-recombination-deficient and PARP inhibitor resistant breast and ovarian tumors. Cancer Res. *83*, 6093.

Affar, E.B., Germain, M., Winstall, E., et al. (2001). Caspase-3-mediated processing of poly(ADP-ribose) glycohydrolase during apoptosis. J. Biol. Chem. *276*, 2935–2942.

Alemasova, E.E., and Lavrik, O.I. (2019). Poly(ADP-ribosyl)ation by PARP1: reaction mechanism and regulatory proteins. Nucleic Acids Res. *47*, 3811–3827.

Amé, J.C., Apiou, F., Jacobson, E.L., et al. (1999). Assignment of the poly(ADP-ribose) glycohydrolase gene (PARG) to human chromosome 10q11.23 and mouse chromosome 14B by in situ hybridization. Cytogenet. Cell Genet. *85*, 269–270.

Amé, J.C., Fouquerel, E., Gauthier, L.R., et al. (2009). Radiation-induced mitotic catastrophe in PARG-deficient cells. J. Cell Sci. *122*, 1990–2002.

Andronikou, C., Burdova, K., Dibitetto, D., et al. (2024). PARG-deficient tumor cells have an increased dependence on EXO1/FEN1-mediated DNA repair. EMBO J. *43*, 1015–1042.

Antolin, A.A., Ameratunga, M., Banerji, U., et al. (2020). The kinase polypharmacology landscape of clinical PARP inhibitors. Sci. Rep. *10*, 2585.

Azarm, K., and Smith, S. (2020). Nuclear PARPs and genome integrity. Genes Dev. *34*, 285–301.

Bakondi, E., Bai, P., Erdélyi, K., et al. (2004). Cytoprotective effect of gallotannin in oxidatively stressed HaCaT keratinocytes: the role of poly(ADP-ribose) metabolism. Exp. Dermatol. *13*, 170–178.

Bamgbose, G., and Tulin, A. (2024). PARP-1 is a transcriptional rheostat of metabolic and bivalent genes during development. Life Sci. Alliance *7*, e202302369.

Barkauskaite, E., Brassington, A., Tan, E.S., et al. (2013). Visualization of poly(ADP-ribose) bound to PARG reveals inherent balance between exo- and endo-glycohydrolase activities. Nat. Commun. *4*, 2164.

Bejan, D.S., and Cohen, M.S. (2022). Methods for profiling the target and off-target landscape of PARP inhibitors. Curr. Res. Chem. Biol. *2*, 100027.

Bordet, G., Karpova, I., and Tulin, A.V. (2022). Poly(ADP-ribosyl)ating enzymes cooperate to coordinate development. Sci. Rep. *12*, 22120.

Burns, D.M., Ying, W., Kauppinen, T.M., et al. (2009). Selective down-regulation of nuclear poly(ADP-ribose) glycohydrolase. PLoS One *4*, e4896.

Chaitanya, G.V., Alexander, J.S., and Babu, P.P. (2010). PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. Cell Commun. Signal. *8*, 31.

Chandak, P.G., Gaikwad, A.B., and Tikoo, K. (2009). Gallotannin ameliorates the development of streptozotocin-induced diabetic nephropathy by preventing the activation of PARP. Phytother. Res. *23*, 72–77.

Chen, S.-H., and Yu, X. (2019). Targeting dePARylation selectively suppresses DNA repair-defective and PARP inhibitor-resistant malignancies. Sci. Adv. *5*, eaav4340.

Coulson-Gilmer, C., Littler, S., Barnes, B.M., et al. (2024). Intrinsic PARG inhibitor sensitivity is mimicked by TIMELESS haploinsufficiency and rescued by nucleoside supplementation. NAR Cancer *6*, zcae030.

Curtin, N.J., and Szabo, C. (2020). Poly(ADP-ribose) polymerase inhibition: past, present and future. Nat. Rev. Drug Discovery *19*, 711–736.

Cuzzocrea, S., Mazzon, E., Genovese, T., et al. (2007). Role of poly(ADP-ribose) glycohydrolase in the development of inflammatory bowel disease in mice. Free Radical Biol. Med. *42*, 90–105.

Cuzzocrea, S., and Wang, Z.Q. (2005). Role of poly(ADP-ribose) glycohydrolase (PARG) in shock, ischemia and reperfusion. Pharmacol. Res. *52*, 100–108.

Dai, W., Fu, Y., Deng, Y., et al. (2019). Regulation of Wnt singaling pathway by poly (ADP-ribose) glycohydrolase (PARG) silencing suppresses lung cancer in mice induced by benzo(a)pyrene inhalation exposure. Front. Pharmacol. *10*, 338.

D'Amours, D., Desnoyers, S., D'Silva, I., et al. (1999). Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J. *342 (Pt 2)*, 249–268.

Dillon, K.M., Bekele, R.T., Sztupinszki, Z., et al. (2022). PALB2 or BARD1 loss confers homologous recombination deficiency and PARP inhibitor sensitivity in prostate cancer. NPJ Precis. Oncol. *6*, 49.

Du, Y., Yamaguchi, H., Wei, Y., et al. (2016). Blocking c-met-mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. Nat. Med. *22*, 194–201.

Falsig, J., Christiansen, S.H., Feuerhahn, S., et al. (2004). Poly(ADP-ribose) glycohydrolase as a target for neuroprotective intervention: assessment of currently available pharmacological tools. Eur. J. Pharmacol. *497*, 7–16.

Finch, K.E., Knezevic, C.E., Nottbohm, A.C., et al. (2012). Selective small molecule inhibition of poly(ADP-ribose) glycohydrolase (PARG). ACS Chem. Biol. *7*, 563–570.

Fisher, A.E., Hochegger, H., Takeda, S., et al. (2007). Poly(ADP-ribose) polymerase 1 accelerates single-strand break repair in concert with poly(ADP-ribose) glycohydrolase. Mol. Cell. Biol. *27*, 5597–5605.

Frizzell, K.M., Gamble, M.J., Berrocal, J.G., et al. (2009). Global analysis of transcriptional regulation by poly(ADP-ribose) polymerase-1 and poly(ADP-ribose) glycohydrolase in MCF-7 human breast cancer cells. J. Biol. Chem. *284*, 33926–33938.

Fu, X., Li, P., Zhou, Q., et al. (2024). Mechanism of PARP inhibitor resistance and potential overcoming strategies. Genes Dis. *11*, 306–320.

Garg, V., and Oza, A.M. (2023). Treatment of ovarian cancer beyond PARP inhibition: current and future options. Drugs *83*, 1365–1385.

Geng, L., Sun, Y., Zhu, M., et al. (2023). The inhibition of PARG attenuates DNA repair in hepatocellular carcinoma. Mol. Biomed. *4*, 3.

Genovese, T., Di Paola, R., Catalano, P., et al. (2004). Treatment with a novel poly(ADP-ribose) glycohydrolase inhibitor reduces development of septic shock-like syndrome induced by zymosan in mice. Crit. Care Med. *32*, 1365–1374.

Gibson, B.A., and Kraus, W.L. (2012). New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat. Rev. Mol. Cell Biol. *13*, 411–424.

Gogola, E., Duarte, A.A., de Ruiter, J.R., et al. (2019). Selective loss of PARG restores PARylation and counteracts PARP inhibitor-mediated synthetic lethality. Cancer Cell *35*, 950–952.

Golan, T., Hammel, P., Reni, M., et al. (2019). Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. N. Engl. J. Med. *381*, 317–327.

González-Barrio, R., Borges, G., Mullen, W., et al. (2010). Bioavailability of anthocyanins and ellagitannins following consumption of raspberries by healthy humans and subjects with an ileostomy. J. Agric. Food Chem. *58*, 3933–3939.

Gravells, P., Grant, E., Smith, K.M., et al. (2017). Specific killing of DNA damage-response deficient cells with inhibitors of poly(ADP-ribose) glycohydrolase. DNA Repair *52*, 81–91.

Gravells, P., Neale, J., Grant, E., et al. (2018). Radiosensitization with an inhibitor of poly(ADP-ribose) glycohydrolase: a comparison with the PARP1/2/3 inhibitor olaparib. DNA Repair *61*, 25–36.

Groslambert, J., Prokhorova, E., Wondisford, A.R., et al. (2023). The interplay of TARG1 and PARG protects against genomic instability. Cell Rep. *42*, 113113.

Haince, J.F., Ouellet, M.E., McDonald, D., et al. (2006). Dynamic relocation of poly(ADP-ribose) glycohydrolase isoforms during radiation-induced DNA damage. Biochim. Biophys. Acta *1763*, 226–237.

Harrision, D., Gravells, P., Thompson, R., et al. (2020). Poly(ADP-ribose) glycohydrolase (PARG) vs. poly(ADP-ribose) polymerase (PARP)—function in genome maintenance and relevance of inhibitors for anti-cancer therapy. Front. Mol. Biosci. *7*, 191.

Hassa, P.O., and Hottiger, M.O. (2008). The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Front. Biosci. *13*, 3046–3082.

Holleran, J.P., Rodems, T.S., Sharma, S., et al. (2024). Abstract 2083: discovery of ETX-19477, a novel and selective PARG inhibitor with high potency against tumors with underlying replication stress. Cancer Res. *84 (6_Supplement)*, 2083.

Houl, J.H., Ye, Z., Brosey, C.A., et al. (2019). Selective small molecule PARG inhibitor causes replication fork stalling and cancer cell death. Nat. Commun. *10*, 5654.

Huang, D., and Kraus, W.L. (2022). The expanding universe of PARP1-mediated molecular and therapeutic mechanisms. Mol. Cell *82*, 2315–2334.

Hussain, G., Huang, J., Rasul, A., et al. (2019). Putative roles of plant-derived tannins in neurodegenerative and neuropsychiatry disorders: an updated review. Molecules *24*, 2213.

Illuzzi, G., Fouquerel, E., Amé, J.C., et al. (2014). PARG is dispensable for recovery from transient replicative stress but required to prevent detrimental accumulation of poly(ADP-ribose) upon prolonged replicative stress. Nucleic Acids Res. *42*, 7776–7792.

Jain, A., Agostini, L.C., McCarthy, G.A., et al. (2019). Poly (ADP) ribose glycohydrolase can be effectively targeted in pancreatic cancer. Cancer Res. *79*, 4491–4502.

Jaiswal, H., Singh, O.P., Chauhan, A.S., et al. (2018). A review on tannins. Eur. J. Biotechnol. Biosci. *6*, 16–17.

James, D.I., Smith, K.M., Jordan, A.M., et al. (2016). First-in-class chemical probes against poly(ADP-ribose) glycohydrolase (PARG) inhibit DNA repair with differential pharmacology to olaparib. ACS Chem. Biol. *11*, 3179–3190.

Janisiw, E., Raices, M., Balmir, F., et al. (2020). Poly(ADP-ribose) glycohydrolase coordinates meiotic DNA double-strand break induction and repair independent of its catalytic activity. Nat. Commun. *11*, 4869.

Kassab, M.A., Yu, L.L., and Yu, X. (2020). Targeting dePARylation for cancer therapy. Cell Biosci. *10*, 7.

Kaufmann, T., Grishkovskaya, I., Polyansky, A.A., et al. (2017). A novel non-canonical PIP-box mediates PARG interaction with PCNA. Nucleic Acids Res. *45*, 9741–9759.

Keil, C., Petermann, E., and Oei, S.L. (2004). Tannins elevate the level of poly(ADP-ribose) in HeLa cell extracts. Arch. Biochem. Biophys. *425*, 115–121.

Koh, D.W., Lawler, A.M., Poitras, M.F., et al. (2004). Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embryonic lethality. Proc. Natl Acad. Sci. USA *101*, 17699–17704.

Langelier, M.F., Eisemann, T., Riccio, A.A., et al. (2018). PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification. Curr. Opin. Struct. Biol. *53*, 187–198.

Le May, N., Iltis, I., Amé, J.C., et al. (2012). Poly (ADP-ribose) glycohydrolase regulates retinoic acid receptor-mediated gene expression. Mol. Cell *48*, 785–798.

Li, N., and Chen, J. (2014). ADP-ribosylation: activation, recognition, and removal. Mol. Cells *37*, 9–16.

Lin, K.K., Harrell, M.I., Oza, A.M., et al. (2019). BRCA reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. Cancer Discov. *9*, 210–219.

Litton, J.K., Rugo, H.S., Ettl, J., et al. (2018). Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N. Engl. J. Med. *379*, 753–763.

Lu, X.C., Massuda, E., Lin, Q., et al. (2003). Post-treatment with a novel PARG inhibitor reduces infarct in cerebral ischemia in the rat. Brain Res. *978*, 99–103.

Luo, X., Ryu, K.W., Kim, D.S., et al. (2017). PARP-1 controls the adipogenic transcriptional program by PARylating C/EBPβ and modulating its transcriptional activity. Mol. Cell *65*, 260–271.

Marques, M., Jangal, M., Wang, L.C., et al. (2019). Oncogenic activity of poly (ADP-ribose) glycohydrolase. Oncogene *38*, 2177–2191.

Martincuks, A., Zhang, C., Austria, T., et al. (2024). Targeting PARG induces tumor cell growth inhibition and antitumor immune response by reducing phosphorylated STAT3 in ovarian cancer. J. Immunother. Cancer *12*, e007716.

Marzio, A., Puccini, J., Kwon, Y., et al. (2019). The F-box domain-dependent activity of EMI1 regulates PARPi sensitivity in triple-negative breast cancers. Mol. Cell *73*, 224–237.e226.

Mashimo, M., Onishi, M., Uno, A., et al. (2021). The 89-kDa PARP1 cleavage fragment serves as a cytoplasmic PAR carrier to induce AIF-mediated apoptosis. J. Biol. Chem. *296*, 100046.

Matanes, E., López-Ozuna, V.M., Octeau, D., et al. (2021). Inhibition of poly ADP-ribose glycohydrolase sensitizes ovarian cancer cells to poly ADP-ribose polymerase inhibitors and platinum agents. Front. Oncol. *11*, 745981.

Maya-Mendoza, A., Moudry, P., Merchut-Maya, J.M., et al. (2018). High speed of fork progression induces DNA replication stress and genomic instability. Nature *559*, 279–284.

Meghani, K., Fuchs, W., Detappe, A., et al. (2018). Multifaceted impact of microRNA 493-5p on genome-stabilizing pathways induces platinum and PARP inhibitor resistance in BRCA2-mutated carcinomas. Cell Rep. *23*, 100–111.

Mertins, P., Mani, D.R., Ruggles, K.V., et al. (2016). Proteogenomics connects somatic mutations to signalling in breast cancer. Nature *534*, 55–62.

Messner, S., Schuermann, D., Altmeyer, M., et al. (2009). Sumoylation of poly(ADP-ribose) polymerase 1 inhibits its acetylation and restrains transcriptional coactivator function. FASEB J. *23*, 3978–3989.

Meyer, R.G., Meyer-Ficca, M.L., Whatcott, C.J., et al. (2007). Two small enzyme isoforms mediate mammalian mitochondrial poly(ADP-ribose) glycohydrolase (PARG) activity. Exp. Cell. Res. *313*, 2920–2936.

Meyer-Ficca, M.L., Meyer, R.G., Coyle, D.L., et al. (2004). Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. Exp. Cell. Res. *297*, 521–532.

Min, W., Cortes, U., Herceg, Z., et al. (2010). Deletion of the nuclear isoform of poly(ADP-ribose) glycohydrolase (PARG) reveals its function in DNA repair, genomic stability and tumorigenesis. Carcinogenesis *31*, 2058–2065.

Min, W., and Wang, Z.Q. (2009). Poly (ADP-ribose) glycohydrolase (PARG) and its therapeutic potential. Front. Biosci. *14*, 1619–1626.

Miwa, M., and Masutani, M. (2007). PolyADP-ribosylation and cancer. Cancer Sci. *98*, 1528–1535.

Mortusewicz, O., Fouquerel, E., Ame, J.-C., et al. (2011). PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms. Nucleic Acids Res. *39*, 5045–5056.

Nagashima, H., Lee, C.K., Tateishi, K., et al. (2020). Poly(ADP-ribose) glycohydrolase inhibition sequesters NAD^+ to potentiate the metabolic lethality of alkylating chemotherapy in IDH-mutant tumor cells. Cancer Discov. *10*, 1672–1689.

Niere, M., Mashimo, M., Agledal, L., et al. (2012). ADP-ribosylhydrolase 3 (ARH3), not poly(ADP-ribose) glycohydrolase (PARG) isoforms, is responsible for degradation of mitochondrial matrix-associated poly(ADP-ribose). J. Biol. Chem. *287*, 16088–16102.

O'Sullivan, J., Tedim Ferreira, M., Gagné, J.P., et al. (2019). Emerging roles of eraser enzymes in the dynamic control of protein ADP-ribosylation. Nat. Commun. *10*, 1182.

Patel, N.S., Cortes, U., Di Poala, R., et al. (2005). Mice lacking the 110-kD isoform of poly(ADP-ribose) glycohydrolase are protected against renal ischemia/reperfusion injury. J. Am. Soc. Nephrol. *16*, 712–719.

Patsouris, A., Diop, K., Tredan, O., et al. (2021). Rucaparib in patients presenting a metastatic breast cancer with homologous recombination deficiency, without germline BRCA1/2 mutation. Eur. J. Cancer *159*, 283–295.

Pettitt, S.J., Krastev, D.B., Brandsma, I., et al. (2018). Genome-wide and high-density CRISPR–Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. Nat. Commun. *9*, 1849.

Pillay, N., Brady, R.M., Dey, M., et al. (2021). DNA replication stress and emerging prospects for PARG inhibitors in ovarian cancer therapy. Prog. Biophys. Mol. Biol. *163*, 160–170.

Pillay, N., Tighe, A., Nelson, L., et al. (2019). DNA replication vulnerabilities render ovarian cancer cells sensitive to poly(ADP-ribose) glycohydrolase inhibitors. Cancer Cell *35*, 519–533.e8.

Pourfarjam, Y., Kasson, S., Tran, L., et al. (2020). PARG has a robust endoglycohydrolase activity that releases protein-free poly(ADP-ribose) chains. Biochem. Biophys. Res. Commun. *527*, 818–823.

Qi, H., Price, B.D., and Day, T.A. (2019). Multiple roles for mono- and poly(ADP-ribose) in regulating stress responses. Trends Genet. *35*, 159–172.

Rack, J.G.M., Ariza, A., Drown, B.S., et al. (2018). (ADP-ribosyl)hydrolases: structural basis for differential substrate recognition and inhibition. Cell Chem. Biol. *25*, 1533–1546.e12.

Rack, J.G.M., Liu, Q., Zorzini, V., et al. (2021). Mechanistic insights into the three steps of poly(ADP-ribosylation) reversal. Nat. Commun. *12*, 4581.

Rack, J.G.M., Palazzo, L., and Ahel, I. (2020). (ADP-ribosyl)hydrolases: structure, function, and biology. Genes Dev. *34*, 263–284.

Rapizzi, E., Fossati, S., Moroni, F., et al. (2004). Inhibition of poly(ADP-ribose) glycohydrolase by gallotannin selectively up-regulates expression of proinflammatory genes. Mol. Pharmacol. *66*, 890–898.

Ray Chaudhuri, A., Ahuja, A.K., Herrador, R., et al. (2015). Poly(ADP-ribosyl) glycohydrolase prevents the accumulation of unusual replication structures during unperturbed S phase. Mol. Cell. Biol. *35*, 856–865.

Ray Chaudhuri, A., Callen, E., Ding, X., et al. (2016). Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature *535*, 382–387.

Ray Chaudhuri, A., Hashimoto, Y., Herrador, R., et al. (2012). Topoisomerase I poisoning results in PARP-mediated replication fork reversal. Nat. Struct. Mol. Biol. *19*, 417–423.

Ray Chaudhuri, A., and Nussenzweig, A. (2017). The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat. Rev. Mol. Cell Biol. *18*, 610–621.

Richard, I.A., Burgess, J.T., O'Byrne, K.J., et al. (2021). Beyond PARP1: the potential of other members of the poly (ADP-ribose) polymerase family in DNA repair and cancer therapeutics. Front. Cell Dev. Biol. *9*, 801200.

Rose, M., Burgess, J.T., O'Byrne, K., et al. (2020). PARP inhibitors: clinical relevance, mechanisms of action and tumor resistance. Front. Cell Dev. Biol. *8*, 564601.

Rouleau-Turcotte, É., and Pascal, J.M. (2023). ADP-ribose contributions to genome stability and PARP enzyme trapping on sites of DNA damage; paradigm shifts for a coming-of-age modification. J. Biol. Chem. *299*, 105397.

Sasaki, Y., Fujimori, H., Hozumi, M., et al. (2019). Dysfunction of poly (ADP-ribose) glycohydrolase induces a synthetic lethal effect in dual specificity phosphatase 22-deficient lung cancer cells. Cancer Res. *79*, 3851–3861.

Schreiber, V., Dantzer, F., Ame, J.C., et al. (2006). Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. *7*, 517–528.

Sefer, A., Kallis, E., Eilert, T., et al. (2022). Structural dynamics of DNA strand break sensing by PARP-1 at a single-molecule level. Nat. Commun. *13*, 6569.

Shimokawa, T., Masutani, M., Nozaki, T., et al. (1998). The human poly(ADP-ribose) glycohydrolase maps to chromosome 10q11.23-21.1 by fluorescence in situ hybridization. Hum. Cell *11*, 243–246.

Slade, D. (2020). PARP and PARG inhibitors in cancer treatment. Genes Dev. *34*, 360–394.

Slama, J.T., Aboul-Ela, N., Goli, D.M., et al. (1995a). Specific inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol. J. Med. Chem. *38*, 389–393.

Slama, J.T., Aboul-Ela, N., and Jacobson, M.K. (1995b). Mechanism of inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol. J. Med. Chem. *38*, 4332–4336.

Sun, Y., Chen, J., Huang, S.N., et al. (2021). PARylation prevents the proteasomal degradation of topoisomerase I DNA-protein crosslinks and induces their deubiquitylation. Nat. Commun. *12*, 5010.

Tanuma, S.-i., Shibui, Y., Oyama, T., et al. (2019). Targeting poly(ADP-ribose) glycohydrolase to draw apoptosis codes in cancer. Biochem. Pharmacol. *167*, 163–172.

Tentori, L., Leonetti, C., Scarsella, M., et al. (2005). Poly(ADP-ribose) glycohydrolase inhibitor as chemosensitiser of malignant melanoma for temozolomide. Eur. J. Cancer *41*, 2948–2957.

Tsai, Y.J., Abe, H., Maruta, H., et al. (1991). Effects of chemically defined tannins on poly (ADP-ribose) glycohydrolase activity. Biochem. Int. *24*, 889–897.

Tulin, A., Naumova, N.M., Menon, A.K., et al. (2006). Drosophila poly(ADP-ribose) glycohydrolase mediates chromatin structure and SIR2-dependent silencing. Genetics *172*, 363–371.

Turner, N.C., Balmana, J., Poncet, C., et al. (2021). Niraparib for advanced breast cancer with germline BRCA1 and BRCA2 mutations: the EORTC 1307-BCG/BIG5-13/TESARO PR-30-50-10-C BRAVO Study. Clin. Cancer Res. *27*, 5482–5491.

van Beek, L., McClay, É., Patel, S., et al. (2021). PARP power: a structural perspective on PARP1, PARP2, and PARP3 in DNA damage repair and nucleosome remodelling. Int. J. Mol. Sci. *22*, 5112.

Wang, J.Q., Tang, Y., Li, Q.S., et al. (2019). PARG regulates the proliferation and differentiation of DCs and T cells via PARP/NF–κB in tumour metastases of colon carcinoma. Oncol. Rep. *41*, 2657–2666.

Wang, X., Mi, S., Zhao, M., et al. (2022). Quantitative analysis of the protein methylome reveals PARP1 methylation is involved in DNA damage response. Front. Mol. Biosci. *9*, 878646.

Waszkowycz, B., Smith, K.M., McGonagle, A.E., et al. (2018). Cell-active small molecule inhibitors of the DNA-damage repair enzyme poly(ADP-ribose) glycohydrolase (PARG): discovery and optimization of orally bioavailable quinazolinedione sulfonamides. J. Med. Chem. *61*, 10767–10792.

Wei, G., Wang, D., Lu, H., et al. (2007). Intranasal administration of a PARG inhibitor profoundly decreases ischemic brain injury. Front. Biosci. *12*, 4986–4996.

Wei, H., and Yu, X. (2016). Functions of PARylation in DNA damage repair pathways. Genomics Proteomics Bioinformatics *14*, 131–139.

Wei, L., Nakajima, S., Hsieh, C.-L., et al. (2013). Damage response of XRCC1 at sites of DNA single strand breaks is regulated by phosphorylation and ubiquitylation after degradation of poly(ADP-ribose). J. Cell Sci. *126*, 4414–4423.

Whatcott, C.J., Meyer-Ficca, M.L., Meyer, R.G., et al. (2009). A specific isoform of poly(ADP-ribose) glycohydrolase is targeted to the mitochondrial matrix by a N-terminal mitochondrial targeting sequence. Exp. Cell. Res. *315*, 3477–3485.

Yan, J., Zhou, Y., Wang, Y., et al. (2023). PARG promotes esophagus cancer cell metastasis by activation of the Wnt/β-catenin pathway. Biochem. Genet. *62*, 761–774.

Ying, W., and Swanson, R.A. (2000). The poly(ADP-ribose) glycohydrolase inhibitor gallotannin blocks oxidative astrocyte death. Neuroreport *11*, 1385–1388.

Yu, M., Chen, Z., Zhou, Q., et al. (2022). PARG inhibition limits HCC progression and potentiates the efficacy of immune checkpoint therapy. J. Hepatol. *77*, 140–151.

Žaja, R., Mikoč, A., Barkauskaite, E., et al. (2013). Molecular insights into poly(ADP-ribose) recognition and processing. Biomolecules *3*, 1–17.

Zhang, D., Hu, X., Li, J., et al. (2019). DNA damage-induced PARP1 activation confers cardiomyocyte dysfunction through NAD^+ depletion in experimental atrial fibrillation. Nat. Commun. *10*, 1307.

Zhang, D.Y., Zhu, Y., Wu, Q., et al. (2023). USP1 promotes cholangiocarcinoma progression by deubiquitinating PARP1 to prevent its proteasomal degradation. Cell Death. Dis. *14*, 669.

Zhang, M., Lai, Y., Vasquez, J.L., et al. (2020). Androgen receptor and poly(ADP-ribose) glycohydrolase inhibition increases efficiency of androgen ablation in prostate cancer cells. Sci. Rep. *10*, 3836.

Zhang, N., Zhang, Y., Wu, B., et al. (2021). Deacetylation-dependent regulation of PARP1 by SIRT2 dictates ubiquitination of PARP1 in oxidative stress-induced vascular injury. Redox. Biol. *47*, 102141.

Zhang, Z., Samsa, W.E., and Gong, Z. (2024). NUDT16 regulates CtIP PARylation to dictate homologous recombination repair. Nucleic Acids Res. *52*, 3761–3777.

Received August 27, 2024. Revised November 22, 2024. Accepted December 11, 2024.