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Opinion

Studying PAR-Dependent Chromatin Remodeling to Tackle PARPi Resistance

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Histone eviction and chromatin relaxation are important processes for efficient DNA repair. Poly(ADP) ribose (PAR) polymerase 1 (PARP1) is a key mediator of this process, and disruption of PARP1 activity has a direct impact on chromatin structure. PARP inhibitors (PARPis) have been established as a treatment for BRCA1- or BRCA2-deficient tumors. Unfortunately, PARPi resistance occurs in many patients and the underlying mechanisms are not fully understood. In particular, it remains unclear how chromatin remodelers and histone chaperones compensate for the loss of the PARylation signal. In this Opinion article, we summarize currently known mechanisms of PARPi resistance. We discuss how the study of PARP1-mediated chromatin remodeling may help in further understanding PARPi resistance and finding new therapeutic approaches to overcome it.

Highlights

Despite the success of PARP inhibitors (PARPis) in targeting BRCA-deficient tumors, the emerging PARPi resistance remains a major clinical hurdle.

Various PARPi resistance mechanisms have been unraveled to date, but their clinical relevance remains to be determined and they do not seem to explain all cases.

PARP1 mediates variable cellular processes through chromatin remodeling, which is crucial for replication, transcription, and the response to DNA-targeting chemotherapy.

It is important to study in more detail how PARP inhibition and subsequent PARPi resistance affect histone eviction and chromatin remodeling.

Targeting of PAR-regulated chromatin modifiers like ALC1, APLF, or the FACT complex may open a new route to understand and tackle PARPi resistance.

PARP1 Is an Important Player in the DDR

PARPs comprise a group of proteins with a key role in essential cellular processes [1]. Therefore, they have received great attention both for unraveling new molecular pathways and as therapeutic targets. PARPs participate in a plethora of cellular functions, such as the **DNA damage response (DDR)** (see Glossary), transcription, chromatin organization, and cell death [1]. PARP1 is the leading member of this superfamily of 17 proteins, which have been identified based on their homology to PARP1 [1]. PARP1 performs its function by catalyzing the formation of PAR chains on target proteins, using NAD^+ as a substrate [1]. Not all of the PARP family members are enzymatically active and some show mono(ADP-ribose) transferase activity only. Since they possess a conserved ADP-ribosyltransferase (ART) domain, they are also referred to as ARTs with diphtheria toxin homology (ARTDs). Reportedly, only four ARTDs, PARP1 (ARTD1), PARP2 (ARTD2), tankyrase PARP5a (ARTD5), and tankyrase PARP5b (ARTD6), possess intrinsic polymerase activity [2].

PARP1 is present throughout the nucleus, being the most abundant nuclear protein after histones [3]. Inactive PARP1 is associated with compact chromatin. Following DNA damage it becomes rapidly activated and catalyzes the formation of PAR chains on itself and on other proteins such as histones (PARylation) [4,5]. The catalytic function of PARP1 is important for the initiation of double-strand break (DSB) repair by both **homologous recombination (HR)** and **nonhomologous end joining (NHEJ)**. The auto-PARylated PARP1 and PARylated proteins at DSBs or single-strand DNA breaks (SSBs) serve as a platform for the recruitment of DDR proteins, which contain PAR-binding domains such as PAR-binding consensus motifs (PBMs), PAR-binding zinc-finger motifs (PBZs), macrodomain folds, or WWE domains [6]. The synthesized PAR molecules can attract a variety of proteins and serve as the glue between DNA repair and other molecular processes such as replication and transcription. PARP1 is also present in regulatory regions of actively transcribed genes as well as at Okazaki fragment maturation sites, emphasizing its multifaceted roles in cell biology [7,8]. Because it has such an

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important role in the orchestration of the molecular processes of the cell, PAR formation is tightly regulated. PAR is rapidly hydrolyzed in the cell, mainly by PAR glycohydrolase (PARG) and by the PAR hydrolase ARH3 [9,10].

Hence, PARylation, particularly autoPARylation of PARP1, is crucial among several post-translational modifications affecting the DDR. Surprisingly, the precise mechanism of how PARP1 is removed from the DNA has not been determined unambiguously. It was long believed that autoPARylation is necessary for the release of PARP1 from the DNA and it was suggested that the increased negative charge of PARylated PARP1 is responsible for its dissociation [11,12]. However, more recent observations have shown that PARylated PARP1 remains associated with chromatin after DNA damage induction using H_2O_2 [13]. It is possible that PARP1 recycling and reassociation with the DNA is so rapid that it does not allow the detection of PARP1-free chromatin. Still, PARylation alone may not be sufficient for PARP1 release [14]. Instead, evidence suggests that it requires the recruitment of the E3 ubiquitin ligase CHFR, which ubiquitinates PARylated but not un-PARylated PARP1 [14]. The CHFR-mediated ubiquitination of PARP1 seems to be important in the first wave of chromatin release, and more factors must have a role in this process [14]. These findings suggest further examination of CHFR function in the context of PARP or PARG inhibition.

PARPis and the Hurdle of PARPi Resistance

As a core component of the DDR process, PARP1 has gained increasing attention as a target for the treatment of tumors with defects in their HR machinery. PARP inhibition has become a prime example of adapting the concept of **synthetic lethality** to cancer therapy: tumors that have lost HR rely more heavily on PARP function, while normal tissues still have all DDR pathways available [15]. Inhibition of PARP in the HR-deficient cells will then cause lethality in tumor cells whereas the normal cells are not harmed. This resulted in the approval of several PARPis in breast (olaparib and talazoparib), ovarian (olaparib, niraparib, and rucaparib either alone or following platinum chemotherapy as maintenance therapy), and metastatic prostate cancer patients (olaparib) with a defect in homology-directed DNA repair [16,17]. This effect was initially discovered as a vulnerability of BRCA1- or BRCA2-deficient cells to PARPis. BRCA1/2 are key players in HR and cells lacking this DNA repair pathway are dependent on other PARP1-mediated repair pathways [18,19]. With PARPis, the catalytic activity of PARP1 is inhibited and PARP1 is also trapped on the chromatin. This leads to the stalling of replication forks and, if the forks collapse, DSBs will form, which cannot be fixed without HR, resulting in cell death [18,19]. PARP2 is also trapped by these inhibitors and both PARP1 and PARP2 will not be released from the DNA until the inhibitor comes off the active site allowing the utilization of NAD^+ [20].

Despite the efficacy and the clinical success of PARPis, BRCA1/2-mutated cancer patients are developing drug resistance [15]. Hence, intensive research has been performed in the past decade to unravel the nature of this resistance [15]. Multiple mechanisms have been identified to date; these are reviewed extensively [22–24] and the five major types are presented, in brief, in Table 1.

Studies in genetically engineered mouse models of BRCA1/2-mutated breast cancer indicate that there are more PARPi resistance mechanisms. In about half of the PARPi-resistant tumors, the mechanisms remain unclear [23,25]. Many of the unknown PARPi resistance mechanisms could potentially involve the restoration of PARP1 downstream pathways, circumventing the effect of PARP trapping and reinstalling a functional DDR. One of the most important regulatory

Glossary

Chromatin remodelers: a set of proteins that catalyze various chromatin-changing reactions including nucleosome sliding, conformational changes in the DNA, and histone variant exchange. To execute these functions, they commonly obtain energy from ATP hydrolysis; therefore, they are mostly characterized by a conserved ATPase domain.
DNA damage response (DDR): a set of signaling pathways for the detection and repair of DNA lesions. It comprises the activation of cell cycle checkpoints and DNA repair pathways, to prevent the generation of deleterious mutations.
Homologous recombination (HR): a DNA repair pathway that utilizes the sister chromatin of the homologous chromosome as a repair template to promote high-fidelity and error-free repair of DSBs. It occurs only in late S to G2 phase. BRCA1 and BRCA2 are critical members of this pathway and mutations in either of these genes lead to HR deficiency and increased levels of genomic alteration that can lead to cancer development.
Nonhomologous end joining (NHEJ): an error-prone DNA repair pathway that repairs DSBs throughout the cell cycle, including in S and G2 phase, without the need for a template. It can directly re-ligate the DNA ends without end resection, which can lead to small deletions.
Nucleosome: a section of DNA wrapped around a core of proteins, the fundamental subunit of chromatin. It comprises approximately 1.7 turns of DNA wrapped around a set of eight histones, the histone octamer. Each histone octamer comprises two copies of each of the H2A, H2B, H3, and H4 proteins. The cells need to use mechanisms to open the chromatin fibers and transiently remove these histones to permit DNA repair, replication, and transcription to proceed.
Synthetic lethality: occurs when the inactivation of either of two genes individually has little effect on cell viability whereas the loss of both genes simultaneously leads to cell death. In cancer, it is used when the inactivation of one gene by deletion or mutation and pharmacological inhibition of the other one leads to the death of cancer cells, whereas the normal, non-mutated cells remain intact.

Table 1. PARPi Resistance Mechanisms

Effect	Resistance mechanism	Refs
HR restoration	• Secondary BRCA1/2 mutations which give rise to a wild-type or hypomorphic functional protein	[93,94]
	• Loss of 53BP1 or its downstream REV7–RIF1–shieldin complex in BRCA1-deficient cells	[61,62,63,95–97]
Upregulation of drug efflux	• Overexpression of the drug efflux transporter MDR1/P-glycoprotein (P-gp), specific to some PARPis (e.g., olaparib)	[98,99]
Drug target alterations	• Downregulation of PARP1 expression in BRCA1/2-proficient cells	[100]
	• Point mutations in PARP1 that affect PARP1 trapping	[101]
Replication fork stabilization	• Impaired recruitment of MRE11 to stalled replication forks, which restores the BRCA1/2-mediated fork protection	[102]
	• Loss of EZH2/MUS81 axis of fork degradation	[103]
	• Loss of the histone acetyltransferase PCAF	[104]
Partial restoration of PARP1 signaling	• Loss of the PARP1 antagonist PARG, which restores PARylation and the subsequent recruitment of DDR proteins like XRCC1	[25]

functions of PARP1 is the rearrangement of chromatin structure. The PAR-dependent changes in chromatin structure are critical for the replication and transcription processes of the cell and are crucial for the initiation of DDR pathways, such as HR or NHEJ. It is evident that PARPi-resistant cells are able to rewire their DDR even in the absence of a functional PARP, but how these cells are able to restore the required chromatin remodeling is not exactly clear. Investigation of how PAR-dependent chromatin remodeling can affect the PARPi response of the cell may yield new insights into the mechanisms of PARPi resistance or the vulnerabilities of PARPi-resistant tumors.

Here, we briefly review the role of PARP1 in the regulation of chromatin structure and highlight some unresolved questions regarding the potential significance of PAR-mediated chromatin remodeling for the understanding and tackling of PARPi resistance.

PARylation of Histones Is a Critical Step in Response to DNA Damage

PARP1 in its inactive form is present in the nucleus and it is mainly associated with **nucleosomes**, where it contributes to the formation of inactive, condensed chromatin structures [26]. The association of PARP1 and PARP2 with the chromatin is important for the formation of heterochromatic regions in telomeres, centromeres, and silenced ribosomal DNA (rDNA) [3]. Although under normal conditions PARP1 also associates with active histone marks, it is mainly found in heterochromatic regions following DNA damage [7].

Following DNA damage, chromatin needs to be dynamically reorganized to orchestrate the DDR. It becomes evident that, in the presence of a DSB, the chromatin initially expands rapidly, followed by local compaction, which signals for HR or NHEJ. This is followed by chromatin relaxation, which is necessary for DDR protein accessibility at the DNA damage site [27,28]. Therefore, an efficient DDR requires a dynamic shift between decondensation and condensation events, which rely strongly on PARylation events [28,29].

Increased PARylation (i.e., the formation of PAR chains on PARP itself, histones, and other chromatin-associated factors) subsequently leads to changes in the chromatin structure that

allow or prevent the access of specific proteins [3]. On histones, serine residues are the main PARylation targets of PARP1, whereas PARP1 itself is PARylated on its aspartate and glutamate residues [30,31]. An important regulator of PARylation is histone PARylation factor 1 (HPF1), which was recently identified to be required as a cofactor for histone PARylation in the DDR [32–35]. PARylation of core histones, like H1, can then serve as a signal for their removal. Moreover, because of the negatively charged PAR, this modification can induce the nucleosomal disassembly and subsequent chromatin relaxation [4]. This loosened chromatin structure is then accessible for DNA repair, transcription, and replication factors [36]. It was suggested that PARP1 remains active for a longer period when attached to histone H4, resulting in larger amounts of PAR and the maintenance of an active, relaxed chromatin [37]. By contrast, when interacting with damaged DNA, PARP1 has short periods of activation since it is required to leave the chromatin to allow the access of DNA repair factors [37]. This PAR-mediated chromatin relaxation effect has been shown to be fully reversible following degradation of the PAR molecules with exogenous PARG administration, indicating that it is dependent on PARP activity [39]. Apparently, PARylation of histones creates a first wave of decondensation, which then facilitates the recruitment of additional mediators to enhance this process.

PARylation-Mediated Chromatin Remodeling in the Context of the DDR and PARPis

Following PARylation, chromatin relaxation is expanded by the recruitment of **chromatin remodelers**. PARylation of histones and PARP1 serves as a scaffold for the recruitment of chromatin remodelers carrying a PAR-binding domain [40]. The function of chromatin remodelers and chaperones is important for the disassembly and reassembly of the chromatin around the DNA damage sites. As a result, DDR proteins can gain access to the chromatin and transcription is stopped until the DNA damage is fixed [41]. Therefore, the PAR binding of chromatin remodelers and/or their PARylation by PARP1 can modify the chromatin structure and mediate the subsequent DDR. Some of the most important PAR-dependent chromatin remodelers are discussed here and listed in Table 2.

ALC1 May Act as a PARPi Resistance Mediator through PAR Chain Protection

ALC1/chromodomain-helicase-DNA-binding protein (CHD) 1-like (CHD1L) is an SNF2-like ATPase that promotes DDR-related nucleosome rearrangements for chromatin relaxation to

Table 2. Chromatin Remodeling Mediators with a PAR-Binding Domain

Name	Domain	Function	Refs
ALC1 /CHD1L	Macrodomain fold	DNA helicase: catalyzes nucleosome sliding in an ATP-dependent manner	[43]
SSRP1-FACT complex	CTR	Subunit of the histone chaperone: catalyzes dissociation of the H2A–H2B histone dimer from the nucleosome	[67]
CHD4-NuRD complex	HMG-box-like domain	Helicase/ATPase domain of the NuRD complex: facilitates the deacetylation of histone in controlling chromatin reorganization and transcriptional repression	[68,70]
macroH2A1.1	Macrodomain fold	Variant histone H2A: replaces H2A in nucleosomes to repress transcription	[52]
APLF	PBZ	Histone chaperone: chaperone activity for both the H2A–H2B dimer and the H3–H4 tetramer	[50,105]
CHD2	C terminus	Chromatin remodeler that acts as an ATPase to catalyze the assembly of chromatin into periodic nucleosome arrays	[57]

occur [43,83]. On DNA damage, ALC1 is rapidly recruited to SSBs or DSBs by PAR and it binds to PARylated PARP1 [42–44]. This PAR binding of ALC1 orchestrates bidirectional regulation. On the one hand, it abolishes the autoinhibitory state of ALC1 to become an active chromatin remodeler, and on the other hand it protects PAR from PARG hydrolysis [43–45]. Recent findings have shown that loss of ALC1 causes sensitivity to PARP inhibition by mediating enhanced trapping of PARP1 or PARP2 at the DNA damage sites [46–48]. The synthetic lethal interaction of ALC1 loss and PARP inhibition was also shown to be reversed by increased PARylation (i.e., by the inhibition of PARG) [47,48]. Based on these findings, we hypothesize that this synthetic lethality relies on ALC1-mediated PAR protection. In the absence of ALC1, PARG then removes the PAR chains from PARP1 and in this fashion the unPARylated PARP1 cannot be released from the chromatin.

When recruited to chromatin, ALC1 forms a complex that comprises XRCC1, core histone components, and the histone chaperone aprataxin-PNK-like factor (APLF) in addition to PARP1 [49,50]. APLF carries a PBZ domain and when binding to PAR, it serves as a regulatory link between PARylation and chromatin modulation. It has been shown to regulate ALC1 binding to histones and it is required for macroH2A1.1 recruitment and PAR binding at the DNA damage site [50]. This shows how PARP1 activity can build a recruitment cascade of chromatin remodelers, which will interact with each other, presumably to maintain an equilibrium between PARylation and chromatin rearrangements to define the choice of the DDR pathway.

macroH2A1 Loss May Indicate PARPi Sensitivity

Independent studies have shown that both splice variants of macroH2A1, macroH2A1.1 and macroH2A1.2, are recruited to DSBs and replication stress sites [28,51]. The macroH2A1.1 variant, which binds to PAR, was also implicated in the regulation of PAR metabolism and the underlying NAD^+ turnover [52]. Following DNA damage, binding of macroH2A1.1 to autoPARylated PARP1 inhibits PARP1 activity and prevents PAR hydrolysis. Hence, this interaction can promote chromatin recondensation events, affecting DDR and transcriptional processes [53]. In addition, macroH2A1.1 was shown to retain the 53BP1 recruitment at the DNA damage site [28]. In line with these findings, the alternative variant, macroH2A1.2, interacts with methyl transferase enzymes like PRDM2 to induce chromatin recondensation by the generation of repressive H3K9 methylation marks [28,54,55]. The compact chromatin marks were found to attract selectively BRCA1, possibly through the function of the H3K9me3 reader HP1, shifting the choice of the DDR pathway towards HR instead of NHEJ [28,54,55]. macroH2A1.1 is lost in many cancers [52] and this might lead to imbalances in PAR metabolism and BRCA1 recruitment to the DNA damage sites in these tumor cells. Therefore, there is a possibility that tumors lacking a functional macroH2A1 might also show increased sensitivity to PARP inhibition.

CHD2 and CHD7 Expression Changes May Promote HR Restoration

In contrast to macroH2A1, chromatin remodelers such as CHD2 and CHD7 stimulate chromatin changes that initiate NHEJ rather than HR [57,58]. On DNA damage, CHD2 is recruited to DSB by PARP1, where it binds PAR with its C terminus. CHD2 then interacts with the histone variant H3.3 and it incorporates it at DNA damage sites [57]. This creates an expanded chromatin structure, which supports the recruitment of NHEJ factors [57]. By contrast, the recruitment of CHD7 to the DNA damage site is not mediated by direct binding to PAR, but it seems to require a PAR-mediated, relaxed chromatin state resulting from the performance of other remodelers like

ALC1 or CHD2 [58]. Following localization at the DNA damage site, CHD7 participates in two important steps of chromatin reorganization. Initially, CHD7 contributes to the expansion of chromatin relaxation, supporting the recruitment of NHEJ repair factors. A re-compaction process mediated by CHD7 interaction with the deacetylase HDAC1/2 follows this [59]. The condensed chromatin formed from the deacetylation of H3 and H4 residues will then prevent further recruitment of NHEJ factors, putting a threshold on the process [58,59]. CHD7 activity also curtails 53BP1 accumulation at the DNA damage sites, which is known to restrain DNA end resection [60,61]. Loss of 53BP1 or its effector, the shieldin complex, is known to cause PARPi resistance by partially restoring HR in BRCA1-deficient tumor cells [61–63]. CHD7 overexpression is observed in various tumor types [64], which could have an effect on the ability of 53BP1 to be recruited to the DNA damage sites. Such a downstream effect, in a BRCA1-deficient context, might support the development of PARPi resistance.

Transcriptional Repression by Chromatin Remodeling Complexes as Key Targeting Event

Another important player in PARP1-mediated chromatin remodeling is the facilitates chromatin transcription (FACT) complex, which is a heterodimer of the hSpt16 and SSRP1 proteins. FACT binds and reorganizes the nucleosome to facilitate transcription. The hSpt16 subunit of the FACT complex is PARylated by PARP1 in response to DNA damage. This provokes the dissociation of the FACT complex from chromatin, inhibiting transcription at the DNA damaged sites [65,66]. In parallel, SSRP1 is proposed to recognize PARylated histones with its CTR domain and in that way mediate the early phase of histone eviction on DNA damage [67].

Another transcriptional repression complex, recruited to the DNA damage site by PAR, is the nucleosome remodeling and deacetylase (NuRD) complex [68]. The NuRD complex comprises histone deacetylase proteins HDAC1/2, CHD4, and metastasis associated 1 (MTA1), among others, which participate in chromatin rearrangements to halt transcription and favor DNA repair [68,69]. The CHD4 subunit was found to bind PAR with a high-mobility group (HMG) box-like domain located on its N-terminal region [70]. In the absence of CHD4, cells fail to recruit the HR proteins BRIT1, BRCA1, and replication protein A (RPA) at the DNA damage site and are therefore sensitive to PARPi [71].

Both the FACT and NuRD complexes are good examples of how PARP1 can indirectly alter the chromatin structure to prevent interference between DNA repair and transcription. The cell must require accurate coordination between these two processes; therefore, alterations in the subunits of either the FACT or the NuRD complex might lead to impaired HR initiation, which can determine the response of the cells to PARPi.

The PARP1 Cofactor HPF1 May Be Useful to Indicate PARPi Efficacy

Interestingly, the activity of the PARP1 cofactor HPF1 contributes to the recruitment of chromatin remodelers and DNA repair proteins and thereby regulates the subsequent pathway choice. HPF1 binds to PARP1 and this interaction shifts the PARP1 activity from autoPARylation to histone PARylation and the formation of shorter PAR chains [34,72]. In the absence of HPF1 activity, hyperautomodification of PARP1 results in an extended interaction with ALC1, while reducing the recruitment of proteins that require hypo-ADP-ribosylated PARP1, such as MacroD2 [34]. Based on these observations, changes in the expression of HPF1 in tumor cells may affect their PAR signature, result in chromatin alterations, and subsequently influence the PARPi response. Still, our understanding of the role of HPF1 in the PARPi response is limited: HPF1 increases the trapping effect of PARPis [73] and should thereby contribute to PARPi

efficacy. Surprisingly, HPF1-deficient cells are not resistant but even more sensitive to PARPis [34]. These reports point out the need for further investigations of HPF1 as a mediator of specific PAR signatures or a predictive biomarker of PARPi response.

Other Histone Modifications That May Determine PARP1 Activity and PARPi Resistance

PARPi treatment impairs the timing of the DDR, possibly as a consequence of its effect on histone eviction and the subsequent chromatin remodeling [74]. Phosphorylation and ubiquitination are modifications that are also critical for the regulation of chromatin remodeling. The most distinct histone modification relating to the DDR is the phosphorylation of H2AX (γH2AX) at Ser139, which serves as a platform for the recruitment of DNA repair factors at DSBs [75,76]. H2A is also ubiquitinated by RNF8 or RNF168, which are recruited to the DSBs by γH2AX [77,78]. However, compared with PARylation, these modifications occur at a later stage of the DDR and the expansion of the ubiquitin ligases on chromatin was shown to rely on PARP1 activity [67,79]. Other modifications like the acetylation of H3K9, which are also important for the spreading of γH2AX and the recruitment of SWI/SNF chromatin remodeling complexes, are mutually exclusive to PARylation [80–82]. These acetylation marks prevent the PARylation of their neighbor serine residues and the subsequent DNA repair protein recruitment. In parallel, DNA damage seems to provoke the deacetylation of these residues, which is reversed on PARPi treatment, indicating that PARylation takes over when it is needed. Similar observations were made for the phosphorylation mark H3S10, which is also associated with active transcription [81].

Together, these observations imply that PARylation has various roles in orchestrating chromatin alterations together with other post-translational modifications. The cell uses PARylation to rapidly rearrange the chromatin; for example, to shift from DNA replication/transcription to DNA repair or from NHEJ to HR. The initial PARylation appears to activate a chromatin remodeling cascade, where chromatin modifiers are activated or inhibited to produce a balanced and dynamic chromatin structure. Hence, if the PARylation status of the cell is altered by a PARPi or by PARPi resistance mechanisms such as loss of PARG, the subsequent changes in chromatin structure should have an impact on the downstream DDR pathways.

The Importance of Alterations of Chromatin Remodeling for PARPi Efficacy

Treatment of cells with PARPi abolishes the recruitment of chromatin remodelers like ALC1 or the FACT complex to the sites of DNA damage [67,83]. This indicates that the effects of these factors on chromatin structure are strongly controlled by PARP1 activity. Nevertheless, in PARPi-resistant cells in which PARP1 or PARP2 function is still substantially inhibited, the cells are able to bypass the PARPi effects through (partial) HR restoration or DNA repair protein recruitment. However, how PAR-mediated chromatin rearrangements are restored in these cells to allow these processes to restart has been overlooked. Currently, there are neither *in vitro* nor *in vivo* data showing how this critical step of chromatin remodeling is recovered or bypassed in PARPi-resistant tumors.

In the example of PARG loss-mediated PARPi resistance, PARylation is partially restored resulting in the recruitment of DNA repair factors [25]. In this case, we can assume that the PARylation signal also restores the recruitment of chromatin remodelers that are required for the regulation of the DDR. This raises the question of whether targeting PAR-dependent chromatin modifiers re-sensitizes these tumors to PARPi treatment. In addition, ALC1 and macroH2A1.1 have been reported to protect PAR from PARG hydrolysis [44,52]. This function might be important for the maintenance of the PAR signal required for an open chromatin structure but also for the

Table 3. Expression Levels of PBM-Containing Chromatin Remodelers in Cancer

Gene	Protein	Cancer type	Low/high level	Refs
CHD1L	ALC1	Hepatocellular carcinoma	High	[106]
		Intrahepatic cholangiocarcinoma	High	[107]
		Ovarian cancer	High	[108]
		Colorectal cancer	High	[109]
		Breast cancer	High	[110,111]
		Multiple myeloma	High	[112]
		Lung adenocarcinoma	High	[113]
APLF	APFL	Breast cancer	High	[114]
CHD4	NuRD complex	Non-small cell lung cancer	High	[115]
		Colorectal cancer	Low	[116]
		Gastric cancer	Low	[116]
		Endometrial cancer	Low	[117]
SSRP1	SSRP1: FACT complex subunit	Non-small-cell lung cancer	High	[118]
		Renal cell carcinoma	High	[87]
		Pancreatic ductal adenocarcinoma	High	[87]
		Breast cancer	High	[87]
		Colorectal adenocarcinoma	High	[87]
		Hepatocellular carcinoma	High	[119,120]
		Glioma	High	[121]
CHD2	CHD2	Chronic lymphocytic leukemia (CLL)	Low	[122]
		Breast implant-associated anaplastic large cell lymphoma	Low	[123]
		Gastric cancer	Low	[116]
		Colorectal cancer	Low	[116,124]
CHD7	CHD7	T cell lymphoma	High	[125]
		Breast cancer	High	[64]
		Colorectal carcinoma	High	[116,126]
H2AFY	macroH2A1	Lung cancer	Low	[86]
		Melanoma	Low	[127]
		TNBC	High	[128]
		Colon cancer	Low	[129]
		Prostate cancer	Low	[130]

Clinician's Corner

PARPi resistance represents a major hurdle for the treatment of BRCA-deficient tumors in the clinic.

Studying the genetic composition of tumors and how that may influence PARylation in response to DNA damage can be useful to further personalize PARPi treatment.

PAR-dependent chromatin relaxation is important in mediating the DDR, but it is unknown how the tumor is able to overcome the absence of this regulation in a PARPi-resistant setting.

Many PARPi resistance mechanisms remain to be discovered. Expression alterations of PAR-regulated chromatin remodelers can potentially predispose for resistance.

Understanding the intricacies of chromatin remodeling in PARPi-resistant tumors may help in finding new vulnerabilities to be targeted to overcome resistance.

recruitment of ubiquitin ligases, like CHFR, which will target PARP1 for degradation [14]. If such a hypothesis were true, one would assume that if PARP1 is trapped on chromatin, the protection of PAR chains might be advantageous for the development of PARPi resistance. In line with this and the recently identified role of ALC1 in PARP chromatin release [46–48], it is likely that overexpression of ALC1 results in PARPi resistance by reversing the toxic trapping effect of PARP inhibition. Such a hypothesis is also supported by the finding that loss of ALC1 sensitizes BRCA-mutated breast and ovarian tumor cells to PARPi [84].

Overall, overexpression or reduced expression of PAR-regulated chromatin modifiers is frequently observed in various kinds of tumors and it is possible to determine their response to

PARPi treatment. Table 3 depicts changes in the expression levels of chromatin modifiers, based on immunohistochemistry or RNA expression, in different types of cancers. Overexpression of ALC1, for example, has been shown to promote cancer progression and metastasis, whereas reduced expression of macroH2A is associated with poor lung cancer prognosis [85,86]. It is possible that tumors, where chromatin remodeling is not reliant on PARP1 activity, are more resistant to PARP inhibition. Therefore, checking whether this is valid for tumors with overexpressing chromatin remodeling genes may allow the already evaluated cytotoxic compounds against these tumors to be clinically exploited. For instance, the curaxin class of anticancer agents have already been characterized to eliminate aggressive tumors with high expression of the chromatin remodeling complex FACT [87–89]. Hence, in addition to PARP1, PARP2, and PARG, monitoring the activity of PARP-associated chromatin modifiers in tumor biopsies may also be useful to better predict the PARPi response of individual patients and to improve personalized treatments. Moreover, in line with the competitive relation between acetylation and PARylation, histone deacetylase inhibitors (HDACis) show a synergistic killing effect in combination with PARPi in triple-negative breast cancer (TNBC) *in vitro* and *in vivo* [90]. Six different HDACis have been FDA approved and it would be interesting to test whether they can reverse PARPi resistance.

Outstanding Questions

How do PARPi-resistant tumor cells, in which the inhibitor still blocks PARP function, compensate for the loss of PAR-mediated chromatin changes?

Does this compensation provide a new vulnerability that can be explored to treat PARPi-resistant tumors?

What is the chromatin remodeling landscape of PARPi-sensitive versus PARPi-resistant tumors?

Is the chromatin remodeling activity useful to predict the response to PARPis?

Concluding Remarks

Changes in chromatin structure are critical for the accomplishment of a DDR response and PARP1 is a major player mediating this link. PARP1 knockout mice are viable and PARP1 is not essential for most cell lines (https://depmap.org). This can obviously be explained by the redundant activity of PARP2. The current PARPis used in the clinic usually block both PARP1 and PARP2, with different efficacies. Although PAR-mediated chromatin remodeling is being extensively studied in the PARP biology field, little is known about the relevance of chromatin remodeling for the success of PARPi treatment and its implication in PARPi resistance (Figure 1). Chromatin remodelers might have a key role in the development of PARPi resistance and their targeting may be useful to re-sensitize resistant tumors. Certainly, we have to bear in mind that histone removal and chromatin remodeling do not rely only on PARylation. Other modifications including phosphorylation, acetylation, and ubiquitination also signal for the recruitment of histone chaperones and this might compensate for the loss of PARylation [80,91,92]. Nevertheless, DDR-related chromatin rearrangements largely rely on PARP1 activity and they serve as an important recycling regulator for PARP1 itself. Changes in the expression of PAR-regulated chromatin remodeling proteins is a common characteristic of many tumor types, indicating their significance in tumor development. Therefore, unraveling the chromatin remodeling landscape of PARPi-treated or -resistant tumors (see Outstanding Questions) might reveal hidden mechanisms that will help us obtain a complete understanding of PARPi resistance and find approaches to overcome it.

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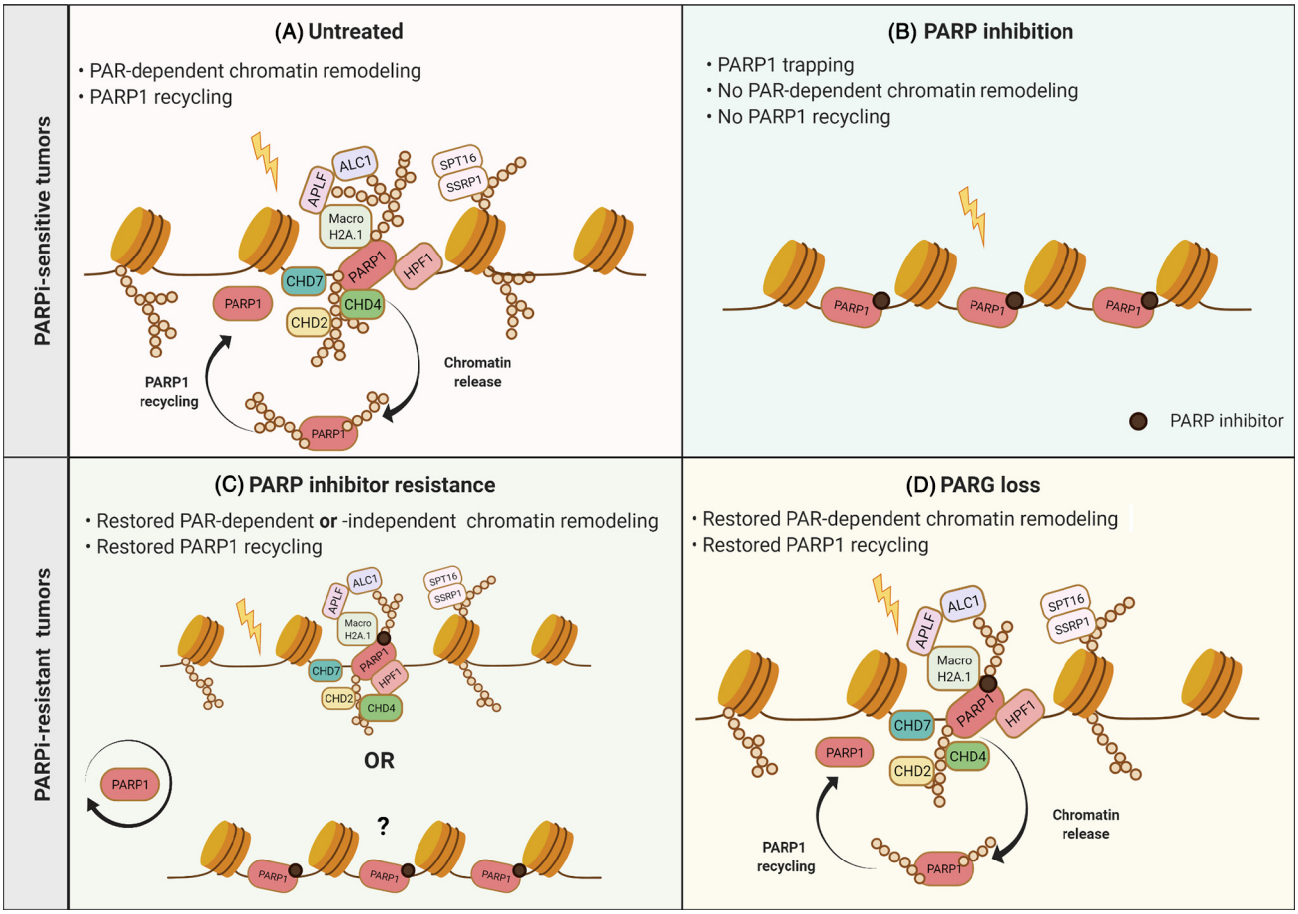
Declaration of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



Trends in Molecular Medicine

Figure 1. Chromatin Remodeling Following DNA Damage Is Poorly Characterized in Poly(ADP) Ribose (PAR) Polymerase Inhibitor (PARPi)-Sensitive versus -Resistant Tumors. (A,B) PARPi-sensitive tumors. In the presence of active PARP1, a PAR-mediated chromatin remodeling cascade begins and the various PAR-regulated chromatin modifiers are recruited to the DNA damage site to initiate the required relaxation and re-compaction events for DNA repair. These chromatin changes will eventually contribute to the chromatin release and recycling of PARP1 so that another DNA damage response cascade will follow. Following PARP inhibition, PARP1 is trapped on the chromatin and its inactivation leads to insufficient PARylation for the recruitment of chromatin remodelers and the initiation of chromatin changes. Under these conditions, PARP1 cannot be released and recycled. (C,D) PARPi-resistant tumors. It is unclear whether the PAR-mediated chromatin remodeling and accessibility and the following PARP1 recycling are restored in PARP1-resistant tumors. This may happen as a result of partial PAR signaling restoration on PAR glycohydrolase (PARG) loss or through the enhancement of another PARP-independent chromatin remodeling cascade, which will compensate for the absence of PARylation. Abbreviations: ALC1, amplified in liver cancer 1; APLF, aprataxin and PNK-like factor; CHD2/4/7, chromodomain helicase DNA binding protein 2/4/7; HPF1, histone PARylation factor 1; PAR, poly (ADP-ribose); PARP1, poly (ADP- ribose) polymerase 1; SPT16, suppressor of Ty 16 (facilitates chromatin remodeling-FACT subunit); SSRP1, structure specific recognition protein 1 (facilitates chromatin remodeling-FACT subunit).

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