

Chapter 2

Discovery of the PARP Superfamily and Focus on the Lesser Exhibited But Not Lesser Talented Members

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Abstract Poly(ADP-ribosyl)ation is a post-translational modification of proteins in which ADP-ribose units are sequentially transferred from the substrate NAD⁺ to acceptor proteins on glutamate, aspartate or lysine residues. The enzymes that catalyse this process are commonly called poly(ADP-ribose) polymerases or PARPs. In human, 17 proteins have been gathered in the PARP superfamily, based on their sequence homology with the catalytic domain of its founding member, PARP-1. In the first part of this chapter, we will recapitulate the history of the discovery of the PARP superfamily. Several excellent reviews have already presented biological processes involving PARP proteins, describing their involvement in DNA repair, transcription, post-transcriptional regulation, stress immunity and inflammation or (Feijs KL, Verheugd P, Luscher B (2013) Expanding functions of intracellular resident mono- ADP-ribosylation in cell physiology. FEBS J 280(15):3519–3529; Kleine H, Luscher B (2009) Learning how to read ADP-ribosylation. Cell 139(1):17–19; Gibson BA, Kraus WL (2012) New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat Rev Mol Cell Biol 13(7):411–424; Welsby I, Hutin D, Leo O (2012) Complex roles of members of the ADP-ribosyl transferase super family in immune defences: looking beyond PARP1. Biochem Pharmacol 84(1):11–20; Chambon P, Weill JD, Mandel P (1963) Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun 11:39–43). During the past decades, researchers' attention has mainly focused on the DNA-damage dependent PARPs and on tankyrases. In the second part of this chapter, we have chosen to present an exhaustive and thorough description of each PARP family member that has not been widely portrayed so far. For this reason, we will not describe the DNA-damage dependent PARPs, PARP-1, -2 and -3, reviewed in two other chapters of this book (Chaps. 7 and 8). We will also not detail the tankyrases TNKS1 and TNKS2, objects of a distinct chapter too (Chap. 4). We will

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highlight the possible therapeutic avenues opened by the new biological roles that emerged for these highly promising PARP family members but still rather poorly characterized.

Keywords PARP family • MART • Macro domain • Zinc finger • Cancer • Immunity • Transcription • Antiviral activity • RNA metabolism • Stress response

2.1 Discovery of the PARP Superfamily (History, Characteristics, MART/PARPs, Nomenclature)

The activity of poly(ADP-ribosyl)ation (PARylation) responsible for the synthesis of poly(ADP-ribose) was first described by Chambon et al. [1] as a “new DNA-dependent polyadenylic acid synthesising nuclear enzyme” in rat liver nuclear extracts. This new compound was then identified as being poly(ADP-ribose) or PAR [2]. The first description of PARP was made by Sugimura et al. [3] and for almost 30 years, PARP was thought to be the only enzymatic activity responsible for that post-translational modification reaction, and has been studied initially mostly related to the DNA damage response. In response to DNA breaks, PARP uses NAD⁺ to synthesise a linear or multibranched polymer of ADP-ribose onto various nuclear acceptor proteins or itself in an automodification reaction. The major benefit of this modification is that it facilitates DNA repair through the opening of the chromatin structure, by modifying the histones and the recruitment of DNA repair proteins complexes to the DNA damaged sites. The importance of PARP in this process has been clearly demonstrated by the independent generation of PARP-deficient mouse models [4–6]. These animals or their derived cells showed hypersensitivity to DNA damage treatments (ionizing radiation, alkylating agents) [7]. However, the extensive analyses made with the embryonic fibroblasts derived from PARP-deficient mice unexpectedly showed that some PAR was still able to accumulate following treatment with the DNA alkylating agent MNNG as demonstrated by the Jacobson’s lab in 1998 [8]. This result and other unpublished reports strongly suggested the existence of at least another enzymatic activity similar to that of PARP. In the plant *Arabidopsis thaliana* the first PARP-related polypeptide (APP) had a smaller molecular weight, 72 kDa, and displayed 60% similarity with the mammalian PARP [9]. Then a second gene was discovered with a molecular weight and a primary structure close to that of PARP and containing the classical Zn-fingers of the DNA binding site [10]. It became clear that PARP activity could exist as multiple forms but with representative sequence similarities at the level of the catalytic domain of the protein. A short time later, Tankyrase was identified and localized to human telomeres [11]. This protein of 142 kDa contains numerous ankyrin repeats with a C-terminal PARP catalytic domain capable to synthesise PAR independently from the presence of DNA. PARP-2 [12, 13] and PARP-3 were successively characterized, the first one responding to DNA damage in the nucleus, and the second, being localised to the centrosome [14]. The founding member of the PARP family was

therefore renamed PARP-1. Around the same time, vPARP (PARP-4) was identified in a two-hybrid screen as part of the vault particles, that are large ribonucleoprotein complexes [15].

Following 30 years of PARP-1 domination in the PARylation field, within 2 years four new members of the PARP family, with distinct primary structures, subcellular localizations and functions, were discovered by several research groups indicating that poly(ADP-ribosyl)ation is a more ubiquitous post-translational modification than first expected. Soon after, the fast accumulation of new sequences from human and mouse origins, provided by EST sequencing and human genome sequencing projects, has allowed to extensively search for new PARP related sequences. Finally a super family of 17 members has emerged [16]. These PARP domain-containing proteins are detailed in Table 2.1 and illustrated in Fig. 2.1.

An additional surprise was the variety of new domains associated with the PARP domain, suggesting their possible implication in many biological functions and likely in different subcellular locations (Table 2.1). Some domains or protein sequence motifs, like the WWE domain and the macro-domains are repeatedly found in a few of the PARPs. Another interesting aspect is that some similar functions, like the DNA binding function of the three DNA-dependent PARPs, are achieved with completely different protein domains, such as a combination of zinc fingers for PARP-1 and two very different N-terminal domains (in terms of primary sequence) for PARP-2 and PARP-3. Whereas the two tankyrases differ from each other only in their N-terminal HPS domain, absent in TNKS2, their functions seem to be very specific, as the knockdown of their expression revealed very different phenotypes with an essential regulatory function in mitotic segregation for TNKS1 [11, 17, 18] and a role in the basal metabolism for TNKS2 [19, 20].

The first structural studies have shown that the PARP catalytic domain binds NAD^+ via a unique protein fold similar to that of bacterial exotoxins (like diphtheria toxin) and different from the Rossmann fold of other NAD^+ -binding enzymes (like the dehydrogenases) [21]. In addition, the coordination mode of NAD^+ within the catalytic site is conserved from the bacterial exotoxins to eukaryotic PARPs [22]. Some very important amino acid residues have been defined to be essential for the catalytic function, the catalytic glutamate (E988) being essential for the elongation activity of PARP-1 [23]. The alignment of the sequences of the catalytic domain of the 17 PARPs reveals major conservation blocks that defined the “PARP signature” corresponding to key secondary structures constituting the active site [16]. Notably, the catalytic residue E988 is not conserved in all the PARPs. Of note, PARP-10, lacking this residue achieves catalysis through a substrate-assisted mechanism [24]. Based on structural homology with the diphtheria toxin and sequence analysis of active ADP-ribosyl transferases, it has been concluded that three amino acids within the PARP signature were crucial for NAD^+ recognition and the elongating activity: a histidine (H862 in PARP-1), a tyrosine (Y896 in PARP-1) and a glutamate (E988 in PARP-1) forming a triad motif ‘H-Y-E’ (see Table 2.1) that appears in PARylating PARPs (PARP-1, PARP-2, PARP-3, PARP-4 and the tankyrases (PARP-5a and 5b) [24–26]. In the other PARPs the E is replaced by either an I, Y, T, V, L predicting a mono(ADP-ribosyl)ating activity of the enzyme

Table 2.1 The PARP family: *ARTD* ADP-ribosyl transferase Diphtheria Toxin like; *BAL* B-aggressive lymphoma protein, *COAST6* collaborator of signal transducer and activator of transcription 6; *ND* not determined; *PARP* poly(ADP-ribose) polymerase; *vPARP* vault PARP; *ZAP1* zinc-finger antiviral protein 1; *ZC3H4/1* zinc-finger CCCH-type antiviral protein 1; *ZC3HDC1* zinc-finger CCCH domain-containing protein 1

PARP family member	Alternative name	Transferase name ^a	Subclass	Size ^b (aa)	Chromosome	Isoforms ^c	Uniprot Accession	Subcellular localization	Triad motif	Enzymatic activity ^d	Key functional motifs and domains
PARP1		ARTD1	DNA-dependent	1014	1q41-42	1	P09874	Nucleus	H-Y-E	P, B	WGR, zinc-fingers, BRCT
PARP2		ARTD2	DNA-dependent	583	14q11.2	2(1)	Q9UGN5	Nucleus	H-Y-E	P, B	DBD, WGR
PARP3		ARTD3	DNA-dependent	533	3p21.2	2(1)	Q9Y6F1	Nucleus, centrosomes	H-Y-E	P	WGR
PARP4	vPARP	ARTD4		1724	13q11	1	Q9UKK3	Cytoplasm, nucleus	H-Y-E	P(p)	BRCT
PARP5A	Tankyrase 1, TNKS1	ARTD5	Tankyrase	1327	8p23.1	2(1)	O95271	Nucleus, cytoplasm	H-Y-E	P, O	Ankyrin sequence repeats, SAM
PARP5B	Tankyrase2, TNKS2	ARTD6	Tankyrase	1166	10q23.3	1	Q9H2K2	Nucleus, cytoplasm	H-Y-E	P, O	Ankyrin sequence repeats, SAM
PARP6		ARTD17		630	15q22.3	3(1)	Q2NL67	ND	H-Y-Y	M(p)	
PARP7	TIPARP	ARTD14	CCCH PARP	657	3q25.31	1	Q7Z3E1	ND	H-Y-I	M	Zinc-fingers, WWE
PARP8		ARTD16		854	5q11.2	2(1)	Q8N3A8	ND	H-Y-I	M (p)	
PARP9	BAL1	ARTD9	macroPARP	854	3q21	3(1)	Q8IXQ6	Nucleus, cytoplasm	Q-Y-T	M (p)	Macro-domain
PARP10		ARTD10		1025	8q24.3	1	Q53GL7	Nuclear, cytoplasm	H-Y-I	M	

Table 2.1 (continued)

PARP family member	Alternative name	Transferase name ^a	Subclass	Size ^b (aa)	Chromosome	Isoforms ^c	Uniprot Accession	Subcellular localization	Triad motif	Enzymatic activity ^d	Key functional motifs and domains
PARP11		ARTD11		331	12p13.3	3(1)	Q9NR21	ND	H-Y-I	M (p)	WWE
PARP12	ZC3HDC1	ARTD12	CCCH PARP	701	7q34	1	Q9H0J9	Nucleus	H-Y-I	M (p)	Zinc-fingers, WWE
PARP13	ZC3HAV1, ZAP	ARTD13	CCCH PARP	902	7q34	5(1)	Q7Z2W4	Cytoplasm (nucleus)	H-Y-V	M (p)	Zinc-fingers, WWE
PARP14	BAL2, COAST6	ARTD8	macroPARP	1801	3q21.1	6(6)	Q460N5	Nucleus, cytoplasm	H-Y-L	M	Macro-domain, WWE
PARP15	BAL3	ARTD7	macroPARP	678	3q21.1	2(1)	Q460N3	Nucleus	H-Y-L	M (p)	Macro-domain
PARP16		ARTD15		322	15q22.2	3(1)	Q8N5Y8	Nucleus, RE membranes	H-Y-I	M (p)	

^a Based on the revised nomenclature of Hottiger et al. [26]

^b Size of the human protein in amino acids

^c Isoforms result of alternative splicing, and the reference isoform sequence is noted in parenthesis

^d Known or predicted enzymatic activity: mono- (M), oligo- (O) or poly(ADP-ribosylation)ation (P), or branching (B), predicted (p)

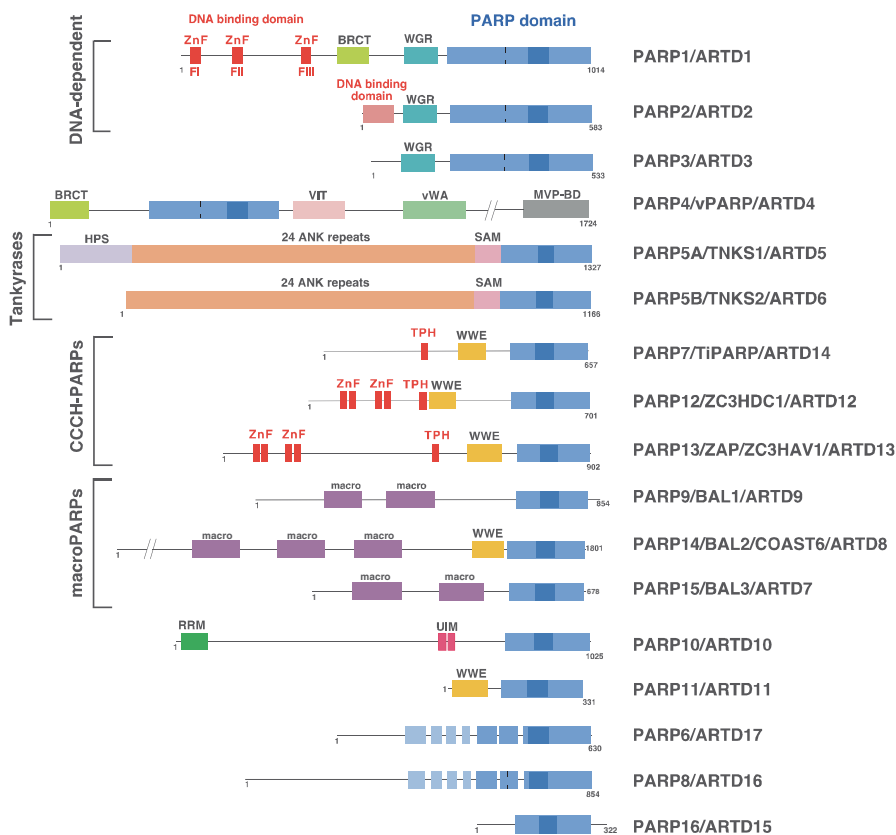


Fig. 2.1 Domain architecture of human poly(ADP-ribose) polymerase family members. Within each putative PARP domain, the region that is homologous to residues 859–908 of PARP-1—the PARP signature—is indicated by a *darker* colour. BRCT, SAM, UIM, MVP-BD, VWA and ANK are protein-interaction modules. *ANK* ankyrin; *BRCT* BRCA1-carboxy-terminus; *HPS* homopolymeric runs of His, Pro and Ser; macro, domain involved in ADP-ribose and poly(ADP-ribose) binding; *MVP-BD* MVP-binding; *PARP* poly(ADP-ribose) polymerase; *RRM* RNA-binding motif; *SAM* sterile α -motif; *TPH* TiPARP-homology; *UIM* ubiquitin-interacting motif; *VIT* vault inter- α -trypsin; *VWA* von Willebrand factor type A; *WGR* conserved W, G and R residues; *WWE* conserved W, W and E residues, domain involved in ADP-ribose and poly(ADP-ribose) binding; ZnF, DNA or RNA binding zinc fingers (except PARP-1 ZnFIII, which coordinates DNA-dependent enzyme activation)

and thus behaving like the mono-ADP-ribosyl transferases (mART). However it is still conceivable that these proteins could use alternative side chains in a slightly different geometry for catalysis. Indeed, PARP-14, -9, -10, -11, -13 and -7 display an aspartate at a position corresponding to the catalytic aspartate of a bacterial diphtheria toxin like ADP-ribosyl transferase, called rifampin ADP-ribosyltransferase (Arr). In this bacterial enzyme that shares three dimensional conformation similarities with PARP-1 NAD⁺-binding loop, the H and the Y of the triad were identified but not the conventional catalytic E residue. Instead this residue is replaced by

a D residue located in the neighbourhood within the substrate-binding loop and which fulfils the role of catalytic residue [26, 27]. To generalise and rationalise the nomenclature of the PARP family proteins, Hottiger et al. [26] have proposed to rename them with criteria based on the type of enzymatic reaction they catalyse, their structural features and on the rules for biochemical classifications, by removing the prefix “poly” and “mono” calling them ‘ADP–Ribosyl Transferases Diphtheria Toxin like’ or ARTDx, where x represents the specific number of the protein (see Table 2.1 for correspondence). The search of new members belonging to the PARP family, using the PARP signature domain of PARP-1, didn’t pick any other ADP-ribosyl transferases (ART family) or NAD⁺ binding proteins (dehydrogenases), indicating that the PARP signature sequence is an extremely well defined and unique functional domain. Its conservation during evolution, with PARP-1 and tankyrases as key players (almost always found in any multicellular organisms), makes the function of this domain of vital importance. The evolution of the mammalian (and plant) genome complexity has required that the number of genes coding for PARP proteins be augmented to fulfil important new cellular functions. This evolution occurred alongside sequence modifications, with substitution of some key amino acid (E to Y, I, etc.) that modifies the extent of the PARP activity towards a mART activity, but the structural domain of these new PARPs remains overall similar. For simplicity in the following text, all the different names for each protein will be mentioned at the beginning of each chapter, then the original “PARP” nomenclature, used at the gene level, will be used throughout.

2.2 The macroPARPs: PARP-9, PARP-14, PARP-15

The macroPARP subfamily is composed of three members defined by the presence of 1–3 macro domains: PARP-9/ARTD9/BAL1 (B-aggressive lymphoma 1), PARP-14/ARTD8/BAL2/CoaSt6 (B-aggressive lymphoma 2, Collaborator of Stat6) and PARP-15/ARTD7/BAL3 (B-aggressive lymphoma 3). The three macroPARP genes and the gene encoding a binding partner of PARP-9, BBAP (B-lymphoma and BAL-associated protein) are localized within ~200 kbp in the 3q21 human chromosomal region, an area of known abnormalities in multiple haematological malignancies [28–30]. This region is syntenically conserved in mouse chromosome region 16B3, with the exception of the PARP-15 gene, absent in rodents and many other species [31]. This suggests that the macroPARP genes may be evolutionarily and/or functionally related or have coordinated expression.

PARP-9 and PARP-14 are predominantly expressed in normal mouse lymphoid tissues, but PARP-9 transcripts were also detected in developing and adult brain, intestine and colon [32]. Looking at PARP-15 expression in human cell lines database revealed that it is restricted to cells with haematological origin (<http://www.broadinstitute.org/ccle/home>). This preferential lymphoid pattern of expression suggests that macroPARPs function predominantly in the immune system. Whereas accumulating data support this hypothesis for PARP-9 and PARP-14, no functional data have been reported so far for PARP-15.

Each macroPARP gene encodes for two or more isoforms generated by alternative splicing. A major (short or S) and a minor (long or L) form were identified for PARP-9 [29] as well as two isoforms for PARP-15 and at least six are reported in the databases for PARP-14, with one even lacking the C-terminal PARP domain. The functional relevance of these putative isoforms remains an open question, but suggests that the complexity might be even higher than expected for the functional characterization of these proteins.

2.2.1 *Structure/Domains of macroPARPs*

PARP-14 and PARP-15, possessing a “HYL” triad motif, exhibit auto- and hetero-mART activity [24, 28, 33, 34] whereas PARP-9, with a “QYT” motif, is inactive [24]. The 3D structure of PARP-14 and PARP-15 PARP domains were solved, as apo-enzymes or bound to PARP inhibitors [35]. Subsequently a virtual screening identified small ligands of PARP-14 and PARP-15 catalytic sites [36]. An activity-based assay was also developed for PARP-15 and validated by screening a small inhibitor library of known ARTD inhibitors [34]. These are all first steps opening the way to further optimization for increased potency and selectivity of PARP-14 and PARP-15 inhibitors.

The macro domains of macroPARPs were first depicted as transcriptional repressor modules, at least for PARP-9 and PARP-15 [28]. Macro domains were initially described as ADP-ribose, or for some of them, O-acetyl-ADP-ribose, binding modules, able to either bind MARYlated substrates or the last residue of PARYlated substrates [37–42]. More recently, a hydrolysing activity towards MAR has been uncovered for several macro-domain containing proteins such as TARG1/C6orf130/OARD1, MacroD1/LRP16 and MacroD2/C20orf133, defining these domains as readers and erasers of MARYlation [41, 43–45, 46]. In contrast to PARP-9 that can bind free PAR and PARYlated PARP-1 via its macro domain 2 [37, 47], PARP-14 macro domains are not able to bind PARYlated PARP-1 [42], despite being apparently recruited to laser-induced DNA damage sites [47]. However, PARP-14 macro domains 2 and 3, but not macro domain 1, can recognize MARYlated substrates such as automodified PARP-10 or PARP-10 substrates [42] (see below). Of note, macro domain 1 of both PARP-9 and PARP-14 can neither bind PAR nor MAR [41, 42, 47]. However, up to now no hydrolysing activity has been reported for any of the macroPARP family members.

PARP-14 is the sole macroPARP possessing a WWE domain. WWE was characterized as a PAR binding module, recognizing the iso-ADP-ribose motif, with the WWE of RNF146/Iduna E3 ligase recognizing the distal ADP-ribose and ribose-ribose glycosidic bond [40, 48, 49]. PARP-14 WWE motifs is however unable to bind PAR. The solution structure of PARP-14 WWE domain by NMR revealed similarity with the WWE domains of RNF146 and PARP-11, displaying however structural differences, such as an additional β -strand covering the hydrophobic pocket [49]. Together with the non-conservation of amino-acids playing a crucial role in the recognition of the adenine base of ADP-ribose, these specificities of PARP-14

WWE domain may explain the lack of binding to PAR and all ATP or ADP-ribose derivatives tested [48, 49]. The role of this non-functional WWE remains a mystery.

2.2.2 *PARP-9*

PARP-9 (experimentally identified as BAL1, B-aggressive lymphoma 1) was identified according to its differential expression in diffuse large B cell lymphomas (DLBCL), higher in some chemoresistant tumors with poor prognosis, particularly those associated with a brisk host inflammatory response [29, 30]. Overexpression of PARP-9 in a B-cell lymphoma cell line stimulates cell migration, suggesting a role for PARP-9 in the promotion of malignant B cell migration and dissemination in high risk DLBCL [29]. PARP-9 interacts with BBAP (B-lymphoma and BAL-associated protein), a ring finger E3 ligase of the DELTEX family, capable of heterodimerization with DELTEX members and self-ubiquitination [50]. DELTEX proteins participate in Notch signalling pathway that controls cell fate determination, notably in myogenesis, neurogenesis, lymphogenesis and intestinal homeostasis [51]. BBAP was proposed to regulate the subcellular localization of PARP-9, sequestering it within the cytoplasm [30]. PARP-9 was subsequently localized at the cell periphery where it colocalizes with actin filaments, but was also detected within the nucleus, at least in S-phase cells [52]. Of note, PARP-9 and BBAP genes are located head-to-head and partially overlapping, their mRNA are antisense through their respective 5'-extremities. The two genes are under the control of an IFN γ -responsive bidirectional promoter (see below) [30]. BBAP and PARP-9 are largely co-expressed in mouse during development and in adult animals [32]. However, some additional tissue-specific gene regulation may exist, with *PARP-9*, in contrast to *Bbap*, being expressed at higher levels in the developing gut than in brain, suggesting both common and independent tissue-specific regulations [32].

2.2.2.1 *PARP-9, a Transcription Co-Factor in IFN γ Signalling, Promoting Tumour Development*

PARP-9 and BBAP were highly expressed in primary host response (HR-)DLBCLs [30], tumours having increased expression of inflammatory mediators including interferon γ (IFN γ), mainly secreted by activated T lymphocytes and natural killer (NK) cells [53]. IFN γ regulates a variety of responses including antiviral state, inhibition of cellular proliferation, induction of apoptosis, activation of microbicidal effector functions and immunomodulation. The canonical Janus Kinase (JAK)/Signal Transducer and Activator of Transcription 1 (STAT1) pathway is the most common signalling route through which IFN γ potentiates its pleiotropic activity [54]. IFN γ modulates the host response to tumours in two opposite ways: at first, by preventing tumour development (immunosurveillance), but later by promoting the outgrowth of tumours with a reduced immunogenicity (immunoediting) [55].

Expression of PARP-9 and BBAP is induced by IFN γ in B-lymphoma cell lines, their bi-directional promoter containing functional STAT1 and IRF1 binding sites [30], thus defining PARP-9 and BBAP as IFN-stimulated genes (ISGs). PARP-9 itself acts as a transcriptional co-factor, its overexpression in a B-lymphoma cell lines modulating the expression of many type I and type II ISGs, or genes indirectly regulated by IFN γ [30]. Among these up-regulated genes were one of the masters regulators of type I and type II IFN γ response: IRF7 and STAT1 respectively, defining PARP-9 as an actor of the IFN signalling pathway. A recent study made a step forward in the elucidation of PARP-9's role in this process by examining the impact of the constitutive high expression of PARP-9 in HR-DLBCL cell lines [56]. Highly expressed PARP-9 is associated with intrinsic IFN γ signalling, with STAT1 being constitutively expressed and present in its activated phosphorylated form (STAT-Y701). PARP-9 stimulates the phosphorylation on Y701 of both STAT1 isoforms, the activating isoform STAT1 α and the antagonistically acting and transcriptionally repressive isoform STAT1 β , and interacted with both of them through its macro-domains in an ADP-ribosylation-dependent manner. PARP-9 promotes the nuclear accumulation of the repressive isoform STAT1 β and together with STAT1 β represses the expression of the tumour suppressor IRF1. Moreover, PARP-9 binds to the promoter of the STAT1-independent proto-oncogene BCL6 to enhance its expression. PARP-9 inhibition of the IRF1-mediated cell death and activation of the BCL6-mediated survival is associated with the increased expression of prosurvival factors PIM1, PIM2 and PARP-14 (see § 2.3), and decreased expression of the BCL6 antagonist BLIMP1 and of genes involved in cell cycle arrest or apoptosis such as p21, BAD, p53 and CASP3 [56]. Supporting these findings, PARP-9 knockdown strongly suppressed the proliferation of HR-DLBCL cell lines. Collectively, these results show that PARP-9 can promote proliferation, survival and chemoresistance in HR-DLBCL by suppressing the anti-proliferative and pro-apoptotic effects of IFN γ . The authors propose the appealing hypothesis that PARP-9 could induce a switch in STAT1 status, from tumour suppressor to oncogene in high-risk DLBCL. Therefore, in infiltrated DLBCL tumours, IFN γ production by dendritic cells could induce PARP-9 expression in tumour cells, leading to the up-regulation of genes involved in the inhibition of the anti-tumoural immune response, favouring tumour progression [56].

2.2.2.2 PARP-9 and the DNA Damage Response

The first indication that PARP-9 could be involved in the DNA damage response came with the discovery that its favourite partner BBAP was required for the efficient recruitment of the DNA damage response (DDR) factor 53BP1 to ionizing radiation (IR) or doxorubicin-induced DNA damages [57]. Histone H4 lysine 91 (H4K91) was identified as a substrate for BBAP E3 ligase activity, monoubiquitinated by BBAP in response to IR or doxorubicin, a prerequisite for histone H4K20 methylation. Since accumulation of the mediator protein 53BP1 at DSB depends on H4K20 methylation [58], this provides an explanation for the decreased 53BP1 recruitment to DNA damage sites in cells depleted in BBAP [57]. Next, the same

group studied the role of PARP-9 in the DSB repair [47]. Using laser microirradiation technology to locally introduce DNA damages, they revealed the fast but transient PARP-1 and PAR-dependent recruitment of PARP-9 to DNA breaks and demonstrated the critical role of the macro domain 2 in this process. Subsequently, PARP-9 recruits BBAP to the DNA damage sites. PARP-9-deficient cells showed increased sensitivity to doxorubicin and reduced repair of DNA breaks introduced by low-dose irradiation. The authors demonstrated that the PARP-1-BAL1-BBAP axis also favours the recruitment of BRCA1 and its binding partner RAP80, the ubiquitin-recognizing protein involved in DSB repair by homologous recombination [47]. In contrast, this axis is neither involved in the recruitment of ATM, MDC1 and RNF8 nor on H2AX phosphorylation at the damaged sites. The proposed model is that the PARP-1-BAL1-BBAP axis mediates ubiquitination of histone H4K91, increasing H4K20 methylation and thus favouring 53BP1 recruitment. Through its ubiquitin-interacting motifs (UIMs), RAP80 could also be recruited early to BBAP-mediated ubiquitinated targets, bringing BRCA1 with it. Next, the later accumulation or retention of RAP80/BRCA1 and 53BP1 at DNA breaks would rely on the RNF8/RNF168-mediated ubiquitination [59]. Moreover, BBAP can also ubiquitinate RAP80 on K43 and K48 for a yet unknown reason [47].

The mechanism proposed by Yan and colleagues [47] fits well with the current emerging view of a two-steps DSB repair process, in which initial recruitment of DDR factors occurs independently on H2AX phosphorylation, followed by sustained DDR factor retention or newly recruitment in a γ H2AX-dependent manner [59, 60]. A growing body of studies reports the PARP-dependent initial recruitment of DSB repair factors and the PARP-independent retention at the DNA damage site, as described recently for BRCA1 (via its partner BARD1) or NBS1 [61, 62]. Regarding the PAR-dependent early recruitment of BRCA1, the relative contribution of the PAR-PARP-9-BBAP-ubiquitin-RAP80 axis with the PAR-BARD axis remains to be determined [47, 62].

2.2.3 *PARP-14*

2.2.3.1 *PARP-14, a Transcription Co-Factor*

PARP-14 was first described as a transcriptional regulator, through its functional interaction with the Signal Transducer and Activator of Transcription 6 (STAT6), and thus originally named COAST6 (*Collaborator of STAT6*) [63]. PARP-14 was shown to potentiate interleukin 4 (IL4) induced transcription of STAT6-dependent genes [63]. IL4 is a key cytokine that regulates lymphocytes differentiation, proliferation and survival in thymus and spleen. PARP-14 expression and subcellular localization are not modified by IL4 treatment at least in lymphoma cells [63], suggesting that IL4-dependent PARP-14 co-factor activity on STAT6 transcription is regulated at the protein level. PARP-14 activity is required for the IL4 dependent STAT6 transcription, since a catalytically inactive PARP-14 mutant is devoid of this stimulating activity [33]. STAT6 is not PARylated by PARP-14 whereas the

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