Review Article

PolyADP-ribosylation and cancer

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The polyADP-ribosylation reaction results in a unique post-translational modification involved in various cellular processes and conditions, including DNA repair, transcriptional control, genomic stability, cell death and transformation. The existence of 17 members of the poly(ADP-ribose) polymerase (PARP) family has so far been documented, with overlapping functional consequences. PARP-1 is known to be involved in DNA base excision repair and this explains the susceptibility spectrum of PARP-1 knockout animals to genotoxic carcinogens. The fact that centrosome amplification is induced by a non-genotoxic inhibitor of PARP and in PARP-1 knockout mouse cells, is in line with aneuploidy, which is frequent in cancers. Genetically engineered animal models have revealed that PARP-1 and VPARP impact carcinogenesis. Furthermore, accumulating experimental evidence supports the utility of PARP and PARG inhibitors in cancer therapy and several clinical trials are now ongoing. Increasing NAD$^+$ levels by pharmacological supplementation with niacin has also been found to exert preventive effects against cancer. In the present review, recent research progress on polyADP-ribosylation related to neoplasia is summarized and discussed. (*Cancer Sci* 2007; 98: 1528–1535)

Cancer is a disease that is characterized by various genetic changes, with mutations occurring in many proto-oncogenes and tumor suppressor genes during a multistep process. These, together with epigenetic alterations, transcriptional deregulation, and aberrations in post-translational modification, are the forces driving carcinogenesis. Forty years have passed since poly(ADP-ribose), a biopolymer involved in a unique post-translational modification, the polyADP-ribosylation reaction, was first discovered.[1–3] PolyADP-ribosylation is an NAD$^+$-dependent enzymatic reaction resulting in covalent modification of acceptor proteins with repeating units of ADP-ribose residues (Fig. 1). The structure of the biopolymer was characterized some 30 years ago.[4] Originally poly(ADP-ribose) polymerase (PARP)-1 was found in the nuclei and shown to be activated by DNA strand breaks. The presence of a salvage pathway of NAD$^+$ synthesis in the nuclei points to the importance of the polyADP-ribosylation reaction. Subsequent to descriptions of monoADP-ribosylation of arginine residues of mammalian proteins, cyclic ADP-ribose formation from NAD$^+$ by ADP-ribosyl cyclase and NAD$^+$-dependent histone deacetylases, named sirtuins, is the other enzyme group that uses NAD$^+$ in an important regulatory system for gene transcription. Ironically, NAD$^+$ is also used as the substrate by various microbial toxins for monoADP-ribosylation reactions.[5] In clear contrast to many other post-translational modifications, poly(ADP-ribose) molecules covalently attached to acceptor proteins vary greatly in size, up to several hundred ADP-ribose residues with branching and large negative charges.[4,6] These underlie the unique structural and functional characteristics of polyADP-ribosylation.

The present review summarizes recent progress suggesting that polyADP-ribosylation is dynamic and important for the regulation of critical cell functions, including mechanisms suppressing carcinogenesis. Possible applications in cancer therapy and prevention are also discussed. A recent review of the molecular aspects of polyADP-ribosylation is helpful.[7]

PolyADP-ribosylation and related reactions

There are now 17 PARP members deduced from genome sequences[7] (Fig. 2). Their differences in the subcellular localizations of PARP and specific expression timing, in part associated with the mitotic apparatus including centrosomes and spindle body, suggest various functions[3,8] (Fig. 3, Table 1). Poly(ADP-ribose) (PAR) and poly(ADP-ribose) glycohydrolase (PARG) also localize in the spindle body and centrosomes during mitosis[9] (Fig. 3).

MonoADP-ribosylation reactions. Post-translational modification by single ADP-ribose residues is termed monoADP-ribosylation and is catalyzed by various viral and bacterial toxins,[5] and eukaryotic enzymes using NAD$^+$ as the substrate. Mammalian monoADP-ribosyl transferases (ART) 1–7 have already been reported and ART2 is located on cell surfaces and is able to catalyze autopolyADP-ribosylation.[10] Of note, pierisin, isolated from *Pierisis rapae*, is demonstrated to monoADP-ribosylate guanine residues of DNA and induce apoptosis of various cancer cells.[11]

DNA Repair

Single-strand breaks and base excision repair. PARP-1 is activated by single- and double-strand breaks (SSB and DSB, respectively) and binds to such DNA strand breaks with zinc finger motifs (Fig. 2). After hydrogen peroxide treatment or SSB induction, foci of poly(ADP-ribose) appear in nuclei within several minutes, followed by foci of X-ray repair cross-complementing I (XRCCI). In *PARP-1*$^{-/-}$ cells, these are not detected. XRCCI has a high affinity for poly(ADP-ribose), and polyADP-ribosylated PARP-1 is suggested to bind and recruit XRCCI to SSB.[12]

PARP-1 knockout cells show increased sensitivity to alkylating agents, topoisomerase (topo) I inhibitors and γ-irradiation.[13] Increased levels of SSB and DSB and a delay in DNA repair are observed in *PARP-1*$^{-/-}$ mouse embryonic fibroblast (MEF) after alkylating damage. When mutation frequency was measured in the *red*/*gam* gene using the *gpt*-Δ transgenic mouse system, the frequency of deletions, particularly those accompanying rearrangements, is increased in the livers of *PARP-1*$^{-/-}$ mice after treatment with *N*-bis(2-hydroxypropyl)nitrosamine (BHP).[14] After alkylation of bases in DNA, glycosylases active in base excision repair (BER) first remove alkylated DNA bases. During the BER process, strand breaks and gaps are produced, and PARP-1 is activated and

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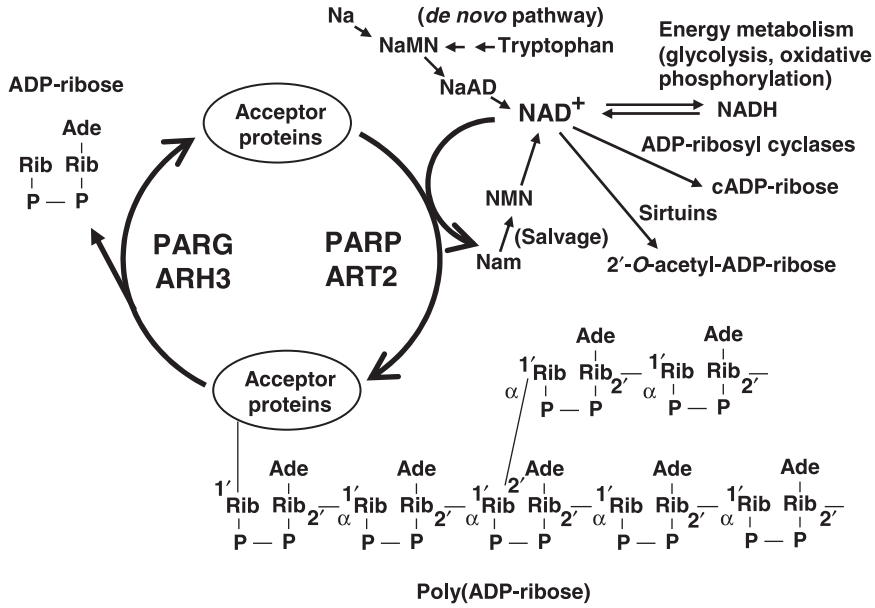

Fig. 1. PolyADP-ribosylation and related reactions. ARH3, ADP-ribose-(arginine) protein hydrolase 3; ART2, monoADP-ribosyl transferase 2; Na, nicotinic acid; NaAD, nicotinic acid adenine dinucleotide; NAD^+ (βNAD^+), nicotinamide adenine dinucleotide; Nam, nicotinamide; NaMN, nicotinic acid mononucleotide; NMN, nicotinamide mononucleotide; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerases.

Enzyme	Peptide structure	Gene locus	Function/property
PARP-1	DNA binding domain Zn fingers; Auto-modification domain; Z Z NLS BRCT; PARP domain; 1014; E; F54L; M129T; E251K; V762A; K940R	1q41-q42	DNArepair, genomic stability, cell death regulation, transcription, centrosome regulation
PARP-2	SAP; 570; E	14q11.2	DNA repair, genomic stability, telomere regulation, transcription
PARP-3	540; E	3p21	Centrosome regulation
VPARP (PARP-4)	BRCT IHRP IHRP NLS MVP-BD; 1724; E	13q11	Vault complex regulation, multidrug resistance
Tankyrase-1	HPS; 24 ankyrin repeats; SAM; 1327; E	8p23.1	Telomere regulation
Tankyrase-2	HPS; 16 ankyrin repeats; SAM; 1166; E	10q23.3	Golgi transport
PARP-9 (Bal-1)	macro-H2A macro-H2A; 854; T	3q13-q21	Overexpression in B cell agressive lymphoma
PARP-10	RRM; NES UIM; 1025; I	8q24.3	Suppression of transformation by c-MYC
PARG	NLS NES; NLS-like; catalytic domain; 976	10q11.23	DNA damage response, cell death regulation, transcription
ARH3	363; DD	1p34.3	PARG activity

Fig. 2. Poly(ADP-ribose) polymerases (PARP) family proteins relating to carcinogenesis, poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribose-(arginine) protein hydrolase 3 (ARH3). Peptide structures, domains, motifs, gene loci and functions/properties are shown. For PARP-1, positions of amino acids where single nucleotide polymorphisms (SNP) and mutations (italic) are shown. The caspase cleavage site is shown by the triangle. ARH, ADP-ribosyl protein hydrolase; BRCT, BRCA1 C-terminus; HPS, homopolymeric runs of His, Pro, and Ser; IHRP, inter-α-trypsin inhibitor family heavy chain-related protein motif; MVP-BD, major vault protein binding motif; NES, nuclear export signal; NLS, nuclear localization signal; RRM, RNA recognition motif; SAP, SAF-A/B, acinus, and PIAS motif; SAM, a sterile α motif; UIM, ubiquitin-interacting motif. The critical amino acid residue in the PARP domain of PARP-1 and the residues at the corresponding position for each PARP family protein are shown. The DD residues indicated in ARH3 are critical residues for PARG activity.

recruits XRCC1 and Ligase III-α complex in certain conditions, as illustrated in Fig. 4. PARP-1 may possibly protect the introduced DNA gaps (Fig. 4). In the absence of PARP-1, stalled BER may cause unligated SSB and may further induce DSB, and DNA fill-in reactions in short-patch or long-patch repair processes may be disturbed. The condensin I complex also supports BER through interactions with PARP-1 and XRCC1.[15] Furthermore, PARP-1 or PARP-2 can induce reactivation of

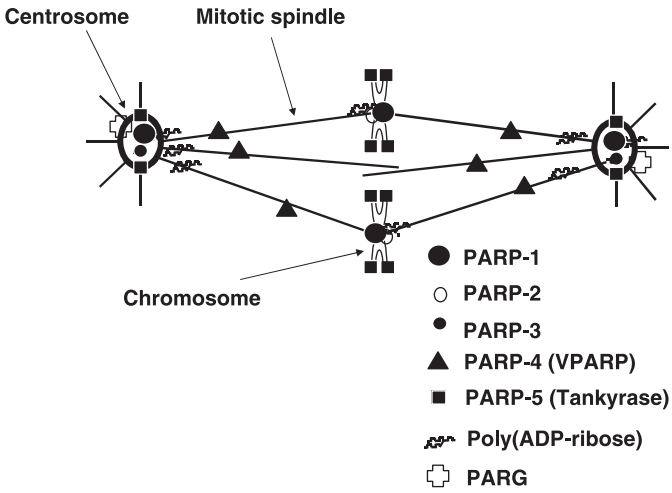

Fig. 3. Localization of poly(ADP-ribose) polymerase (PARP) family members, poly(ADP-ribose) glycohydrolase (PARG) and poly(ADP-ribose) at the mitotic apparatus.

stalled DNA topo I in covalent complexes and stimulate DNA strand break sealing.[16]

DSB and homologous recombination repair. DSB repair is mainly carried out by error-prone non-homologous end-joining (NHEJ) and error-free homologous recombination (HR) pathways (Fig. 4). Interactions of the DNA-dependent protein kinase (DNA-PK) complex, PARP-1 and Werner syndrome protein (WRN) seem to be involved in balancing these pathways.[17] Ku70/80 in the DNA-PK complex has a high affinity for DSB, and this affinity is reduced by polyADP-ribosylation.[17] In *PARP-1*$^{-/-}$ chicken DT 40 cells, the HR pathway is substantially inhibited by the Ku protein, indicating that PARP-1 functions in suppressing Ku protein blockage of HR repair.[18] WRN is recruited by interaction with Ku70/80 to DSB and is necessary for full activation of PARP-1.[17] The presence of an alternative DSB pathway involving PARP-1, PNK and DNA ligase III has also been suggested.

In HR repair, after bridging DSB by Rad50, Mre11 and the NBS-1 complex, the DSB terminus with a 3′-overhang structure is protected by Rad51 and BRCA2 (Fig. 4). In the absence of BRCA2, PARP-1 may possibly function to protect DSB ends from nuclease attack because *BRCA2*-deficient cancer cells are highly sensitive to PARP inhibitors and exhibit an increased frequency of DSB.[19,20] Some DSB-repair deficient cells, including examples that are *ATM*-deficient, also show hypersensitivity to PARP inhibitors.[21]

PARP-2 and DNA repair. PARP-2 is mainly present in centromeres during interphase and recognizes DNA loop structures. It is also activated by DNA damage.[7] After γ-irradiation, an increased level of DNA strand breaks was observed in the centromere regions of *PARP-2* knockout cells, these also demonstrating increased sensitivity to alkylating agents.[22] PARP-2 also supports BER and interacts with PARP-1, XRCC1, DNA polymerase-β and DNA ligase III. The functions of PARP-1 and PARP-2 in BER may be complementary only in a part and it remains to be determined whether their roles differ depending on the local chromatin structures.

PARG and DNA repair responses. PARP-1 and PARG are suggested to form a complex that breaks down poly(ADP-ribose) to ADP-ribose, resulting in regeneration of ATP molecules, which are required for DNA repair.[23] PARG has been found to re-localize at sites of DNA breaks induced by UV-A laser microirradiation in HeLa cells,[24] but further evidence of involvement in DNA repair needs to be obtained.

Transcriptional control

PARP-1 acts as a co-activator and co-repressor of transcription. In *PARP-1*$^{-/-}$ mice, NF-κB-dependent *inducible nitric oxide synthase* (*iNOS*) gene expression is substantially reduced.[25] Acetylation of lysine residues near the BRCT motif of PARP-1 is required for activation of transcription.[25] PARP-1 also acts as a co-activator of retinoic acid-inducible retinoic acid receptor (RAR)-dependent transcription of the *RARβ* gene, by binding to an inactive mediator that is then activated by RAR.[26] Subsequently the co-repressor complex is released, and recruited histone acetyltransferase complex activates transcription. PARP-1 also functions as a co-activator in β-catenin/TCF4-dependent transcription.[27] In estrogen receptor (ER)-dependent transcription of the *ER* gene, auto-polyADP-ribosylation stimulates formation of transcriptional complexes.[28] During transcriptional activation of the *ER* gene, PARP-1 interacts with topo II-β and transient DSB are induced, which is necessary for transcription.[29] The

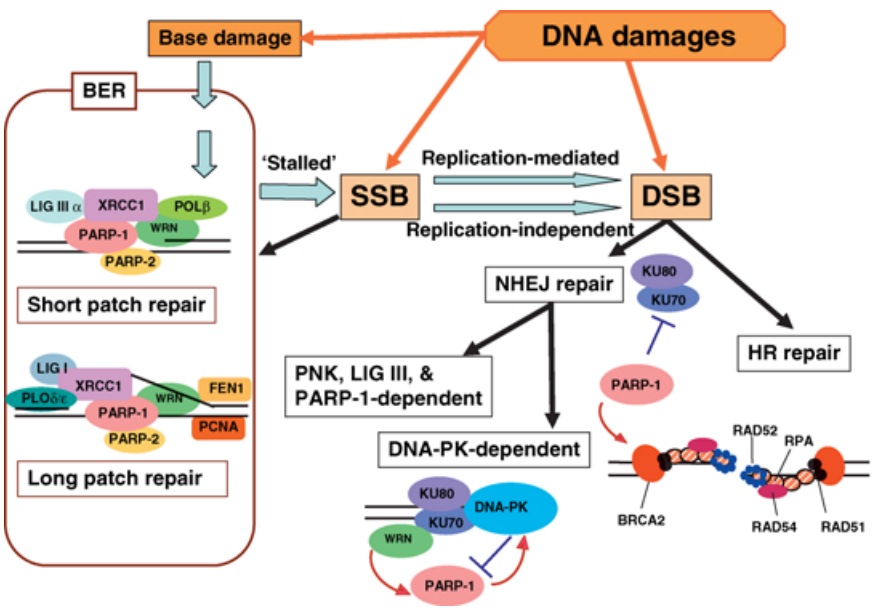

Fig. 4. Model for involvement of poly(ADP-ribose) polymerase (PARP) in DNA repair. DNA damage directly produces base damage, single-strand breaks (SSB) and double-strand breaks (DSB). SSB may be left unligated accidentally in the process of base excision repair (BER). SSB are possibly converted to DSB in a replication-mediated manner or replication-independently. Involvement of PARP in short-patch and long-patch repairs of BER, non-homologous end-joining (NHEJ) repair, and homologous recombination (HR) repair is shown. FEN1, flap endonuclease I; LIG, DNA ligase; PCNA, proliferating cell nuclear antigen; PNK, polynucleotide kinase; POL, DNA polymerase.

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Table 1. Phenotypic outcome of dysfunction in polyADP-ribosylation

Enzyme	Subject	Outcome	Method
PARP-1	Carcinogenesis	Susceptibility (induced by alkylating agents)↑	KO mice
		Susceptibility (in aged mice)↑	KO mice
	Genomic instability	SCE↑	KO mice
		Gene amplification↑	KO mice
		Micronuclei↑, chromosomal aberration↑	Antisense
		Centrosome amplification↑, Ploidy↑	KO mice
		Deletion mutation (after BHP treatment)↑	KO mice
		Transcriptional dysregulation↑	KO mice and *Drosophila*
	DNA damage response	DNA repair↓	KO mice, antisense
		Lethality of alkylating agents and γ-irradiation↑	KO mice
		Cell death induced by oxidative stress↓	KO mice
	Differentiation	Differentiation to trophoblast lineage↑	KO mice
PARP-2	Genomic instability	Chromosomal aberration↑	KO mice
		Ploidy↑	KO mice
		Aberration of spermatogenesis↑	KO mice
	DNA damage response	DNA repair↓	KO mice
		Lethality of alkylating agents and γ-irradiation↑	KO mice
	Differentiation	Adipocyte differentiation↓	KO mice
Tankyrase 1	Mitosis control	Aberration in chromosomal segregation↑	siRNA
PARG	DNA damage response	Lethality of alkylating agents and γ-irradiation↑	KO mice
	Neuronal dysregulation	Neuronal degeneration↑	KO *Drosophila*
VPARP	Carcinogenesis	Carcinogenesis induced by urethane↑	KO mice

KO, knock-out; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; SCE, sister chromatid exchanges; VPARP, vault-associated PARP.

polyADP-ribosylation reaction is required for transcriptional activation of wide regions of chromatin through 'puff formation'.[30]

DNA methylation, imprinting and PARP. Hypomethylation of the global genome and local DNA hypermethylation frequently occur from the early stages of carcinogenesis. In this context the finding that PARP inhibitors enhance DNA methylation of the *HTF9* gene promoter is of interest.[31] DNA methyltransferase (DNMT) 1 possesses two poly(ADP-ribose) binding motifs and DNMT activity is repressed after its binding to poly(ADP-ribose).[32]

In cancer cells, loss of imprinting is also observed. CTCF (CCCTC binding factor), which binds to the non-methylated maternal allele of the insulator domain in the *H19* imprinting control region (ICR), is preferentially polyADP-ribosylated.[33] More than 140 CTCF target sites have been found to be poly-ADP-ribosylated and chromatin insulator functions are sensitive to PARP inhibition. It remains to be clarified which members of the PARP family are involved in the regulation of imprinting.

Macrodomain and PARP-1. The release of histone from chromatin by polyADP-ribosylation, in the so-called 'histone shuttle model', may enable dynamic conversion of local chromatin structures. Besides histones, various proteins bind poly(ADP-ribose) in cellular extracts. PARP-9 (Fig. 2), PARP-14 and PARP-15 all contain macrodomains that consist of hydrophobic amino acids and a helix structure. Recently, the macrodomains of macroH2A and PARP-9 were demonstrated to bind monoADP-ribose and poly(ADP-ribose).[34] There is thus a possibility that the local or cellular ADP-ribose metabolic state is translated into transcriptional regulation through macrodomains.

Differentiation control

In early studies, PARP inhibitors were shown to modulate differentiation processes. In human promyelocytic leukemia, HL-60 cells, stimulation of differentiation into granulocytes was accompanied by loss of *c-myc* gene amplification.[35] In *H-ras*-transformed NIH3T3 cells, PARP inhibitors also caused loss of amplified *H-ras* and *c-myc* oncogenes and reversal of the transformed phenotype.[35]

During teratocarcinoma-like tumor formation from mouse embryonic stem (ES) cells, induction of the trophoblast lineage, including trophoblast giant cells (TGC), was observed in tumors derived from *PARP-1*$^{-/-}$ ES cells.[36] The properties of TGC are similar to those of the syncytiotrophoblastic giant cells (STGC) observed in human germ cell tumors. *PARP-1* deficiency may be related to induction of STGC during human germ cell tumor development.

Cell-cycle controls

PARP-1 is also involved in cell-cycle check-point control after DNA damage. After γ-irradiation, p53-dependent induction of the *p21* and *mdm2* genes is attenuated by PARP inhibitors and suppression of G1 arrest and enhancement of G2 arrest are observed.[2,37] After treatment with neocarzinostatin, an increased level of γ-H2AX, a marker of DSB, was observed, accompanied by augmented p53 phosphorylation at the ser 18 residue in *PARP-1*$^{-/-}$ MEF. This accompanied enhancement of kinase activity of the ATM protein.[38] In addition, S-phase entry from G0 phase was found to be delayed in several cell types by *PARP-1* deficiency.[2]

Role of PARP-1 in cell death regulation

During the course of carcinogenesis, various types of cell death stress, including oxidative stress induced by inflammation and energy depletion, may be operating. Survival may be associated with mutations or epigenetic alteration of genes responsible for cell death pathways. PARP-1 dependent cell death occurs after treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) or massive oxidative stress. After MNNG treatment, NAD^+ depletion and translocation of apoptosis-inducing factor-1 (AIF-1) from mitochondria to nuclei is normally observed, but this type of cell death is lacking in *PARP-1* knockout cells resistant to oxidative stress,[39] and streptozotocin-induced pancreatic β-cell death.[40] PARP inhibitors enhanced development of streptozotocin-induced pancreatic insulinomas in rats,

suggesting that PARP-1 dependent cell death is involved in the prevention of carcinogenesis.[41] Because more than half of cancers feature mutation in the p53-dependent apoptosis pathway, PARP-1 dependent cell death could be a good target for cancer therapy.

PARG$^{-/-}$ mice lacking the 110 kDa isoform are hypersensitive to alkylating agents and γ-irradiation, with reduced automodification activity of PARP-1.[42] *PARG*$^{-/-}$ ES cells also exhibit increased sensitivity to MMS treatment and γ-irradiation, apoptosis occurring within a much shorter period than in *PARG*$^{+/+}$ ES cells.[43] This was linked to a marked increase of polyADP-ribosylated proteins in nuclei and a reduction in NAD^+ levels. The cytotoxicity of MNNG and menadione is increased in *PARG*$^{-/-}$ cells.[44] These results suggest that PARG activity is involved in survival after DNA damage. The poly(ADP-ribose) polymer itself induces cell-death through induction of AIF release from mitochondria.[45] This is consistent with a large accumulation of polyADP-ribosylated compounds and neuronal cell death in *PARG* knockout *Drosophila*.[46] Thus, there is a possibility that Parg inhibitors might enhance chemo- or radiation therapy of cancers.

Chromosomal stability

It is well known that cancer cells are generally characterized by extensive genomic instability. Centrosome amplification is frequently observed and could be a cause of chromosomal missegregation between daughter cells.

A century ago, a hypothesis was proposed that malignant tumors arise through defects of centrosome functions that lead to improper cell divisions resulting in aneuploidy.[3] Many reports have appeared documenting that certain post-translational modifications, including phosphorylation and ubiquitylation, occur in centrosomes and regulate their function.[3] Because PARP-1, PARP-3, PARP-4 and PARP-5, as well as polyADP-ribosylated proteins and PARG, are found in the mitotic apparatus (Fig. 3), polyADP-ribosylation might be involved in the regulation of fidelity of correct separation of chromosomes during mitosis.[3,47] It is interesting to note that 3-aminobenzamide (3-AB), an inhibitor of PARP, seems to be not mutagenic (non-genotoxic) to *Salmonella*, but does cause centrosome amplification in mammalian cells.[47] They also may induce sister chromatid exchanges.[5] These data suggest that non-genotoxic compounds like PARP inhibitors might induce chromosomal instability, which could promote carcinogenesis.

Cellular transformation

PARP inhibitors like benzamide decrease *in vitro* transformation of human fibroblasts induced by various types of carcinogens, such as benzo[a]pyrene and MNNG.[48] In contrast, transformation of mouse C3H10T1/2 cells by ethylnitrosourea (ENU) or ethylmethanesulfonate was elevated by PARP inhibitors.[5] These effects are possibly related to transcriptional control by PARP family members. PARP-10 was recently identified as a c-myc interacting protein, suppressing cellular transformation induced by c-MYC and E1A protein.[49] PARP-10 polyADP-ribosylates itself, as well as core histones, and may be indirectly involved in the regulation of c-myc.

Animal models of tumorigenesis

PARP-1$^{-/-}$ mice show increased susceptibility to carcinogenesis induced by alkylating agents, including BHP, and azoxymethane.[2,50] In contrast, there is no such difference regarding carcinogenesis induced by a heterocyclic amine, IQ (2-amino-3-methylimidazo [4,5-*f*]quinoline) and 4-nitroqinoline 1-oxide (4NQO), both of which give rise to bulky DNA adducts.[51,52] Therefore, there are carcinogen specific effects concerning the involvement of PARP-1 in carcinogenesis. Alkylation damage to DNA bases may be repaired mainly by BER, while bulky DNA adducts induced by IQ and 4NQO may be targeted by nucleotide excision repair (NER). Susceptibility to carcinogenesis might thus be explained by the involvement of PARP-1 in the repair pathway for BER, but not for NER.

In *PARP-1*$^{-/-}$*p53*$^{-/-}$ mice, an increased frequency of spontaneous development of carcinomas and lymphomas has been observed. Medulloblastomas also develop at the age of 16 weeks, accompanied by activation of the hedgehog pathway through overexpression of the *GLI* (*Greig cephalopolysyndactyly syndrome*) gene.[53] It is notable that medulloblastomas are also observed in *Ligase IV*$^{-/-}$*p53*$^{-/-}$ mice, which are defective in NHEJ repair.[2] The combination of DNA-PKc mutations (SCID mutations) with *PARP-1* deficiency has resulted in increased incidence of T-cell lymphoma and partial recovery of V[D]J recombination in T cells.[54] *Ku80* heterozygous mutations with *PARP-1* homozygous mutations have caused enhanced frequency of hepatocellular carcinoma development in aged mice.[55] In addition, *WRN*$^{\Delta hel1/\Delta hel1}$ *PARP-1* null mice show an increased incidence of spontaneous tumors.[56] Furthermore, in aged *PARP-1*$^{-/-}$ mice, the incidence of spontaneous development of hepatocellular tumors is increased.[55] Recently, it was shown that *vault-associated PARP* (*VPARP*) knockout mice exhibit elevated susceptibility to carcinogenesis induced by urethane in the lungs and by dimethylhydrazine in the colon.[57]

Human cancer

In human cancers, increased expression of the *PARP-1* gene has been reported in Ewing's sarcomas,[2] and in malignant lymphomas.[2] In contrast, decreased expression has been observed in several gastric and colon cancer cell lines,[2] as well as in some breast cancers.[58]

Relations of genetic alterations in *PARP-1* gene with carcinogenesis have been reported by several authors. The heterologous *Met129Thr* mutation in the *PARP-1* gene has been reported in the germ cell tumor.[59] The *Val762Ala* single nucleotide polymorphism (SNP) was found to impact prostate cancer in Caucasians, the *Ala*/*Ala* allele being associated with a two-fold increase in susceptibility.[60] The 762Ala variant showed decreased PARP activity and reduced interaction with XRCC1 compared with the 762Val variant. With esophageal and lung cancers, a two-fold increase in risk with the *Ala*/*Ala* allele was observed in Chinese smokers.[61,62] It is also noteworthy that a combination effect of the *762Ala* allele of the *PARP-1* gene and the *399Gln* allele of the *XRCC-1* gene has been reported. It should be noted that the *762Ala* allele frequency is much higher in Asian compared to Caucasian populations. The relation of *Val762Ala* as well as *Lys940Arg* to the risk of lung cancer was investigated in Japan, but no associations were detected.[63] Genetic differences in the population or variation in the profile of environmental exposure to carcinogens may have exerted an influence.

PARP-9 contains two macroH2A domains that could repress transcription when localized sufficiently close to a promoter. This PARP was originally found as BAL1 (B-Aggressive Lymphoma 1) and is expressed at significantly higher levels in fatal high-risk diffuse large B-cell lymphomas (DLBCL) than in curable low-risk tumors.[64] Increased PARP-9 expression in DLBCL is associated with an activated peripheral B-cell phenotype and high rates of tumor cell migration.

Inhibition of PARP and PARG for potentiation of anticancer drugs

Earlier work in the 1980s was focused on the effects of PARP inhibitors, benzamide and 3-AB. 3-AB was demonstrated to enhance the cytotoxic effect of dimethylsulfate,[65] when used in

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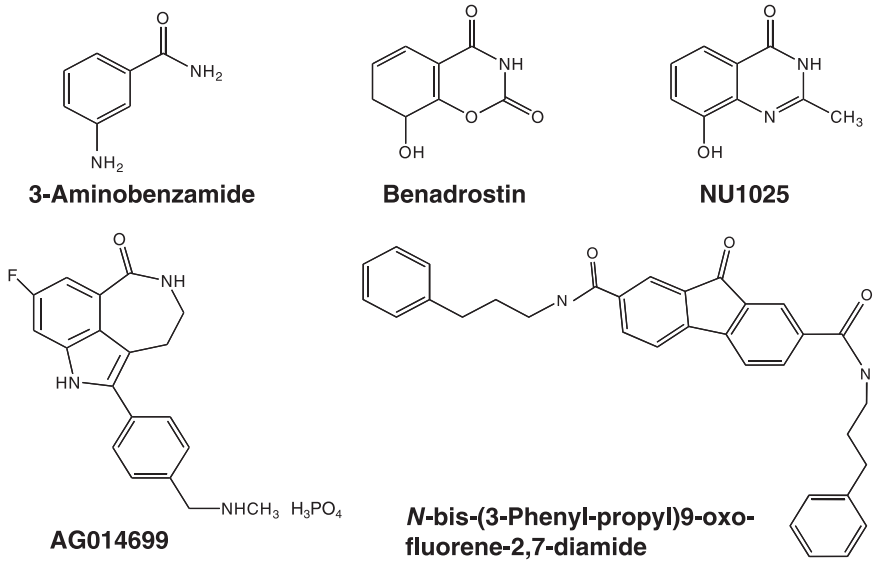

Fig. 5. Developed poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) inhibitors. Structures of PARP inhibitors, 3-aminobenzamide,[65] benadrostin,[2] NU1025, AG014699,[67] and PARG inhibitor *N*-bis(3-phenyl-propyl)9-oxo-fluorene-2,7-diamide[70] are shown.

combination with bleomycin as an anticancer drug in Ehrlich ascites mammary cancer cells.[66] Recently a more potent inhibitor, AG14361 and the AG014669 derivative (Fig. 5), were developed in England and are now undergoing Phase II clinical trials with the DNA methylating anticancer drug, temozolomide, for malignant melanomas.[67] The *BRCA2* gene required for HR repair is mutated in some cancer cells. Inhibitors of PARP that stall DNA replication forks and cause DSB might thus be expected to kill such mutated cells.[19,20] However, because the *BRCA2* deficient human cell line, CAPAN-1, was not found to be sensitive to PARP inhibition,[68] further studies are still necessary to understand what factors affect the consequences of PARP inhibition. A specific inhibitor for PARP5 (tankyrase 1) affecting telomerase function is also suggested to be a potential telomere-directed anticancer target.[69]

So far, PARG inhibitors have not been intensively studied as anticancer agents. Nobotanin B, adenosine diphosphate (hydroxymethyl)-pyrrolidinediol (ADP-HPD), as well as its cell-permeable derivative, 8-octylamino-ADP-HPD, have been reported. Pargamicin was also recently reported.[2] An approach to using a PARG inhibitor, *N*-bis-(3-phenyl-propyl)9-oxo-fluorene-2,7-diamide (Fig. 5), in combination with temozolomide to treat temozolomide-resistant cancers, has been published.[70] Therefore, PARG could be a new molecular target for cancer chemotherapy.

Chemoprevention

The substrate of PARP, NAD^+, is synthesized using nicotinic acid mononucleotide by the kynurenic pathway starting with L-tryptophan or using niacin (nicotinic acid, vitamin B3) supplied in the diet (Fig. 1). It has been reported that in rats maintained under a niacin-deficient diet, the level of NAD^+ in bone marrow is decreased, with even more extensive reduction in poly(ADP-ribose).[71] The animals were found to show greatly elevated susceptibility to ENU, particularly regarding induction of leukemias. In contrast, rats supplemented with niacin or nicotinamide in the diet had increased NAD^+ and basal and ENU-treated poly(ADP-ribose) levels in bone marrow. ENU-induced carcinogenesis was furthermore slowed.[71] Niacin deficiency was also shown to enhance skin cancer development with preventive effects of supplementation. Tashtoush *et al.* showed that myristyl nicotinate, a derivative of nicotinic acid, can be used as a topical agent increasing NAD^+ content by 40%.[72] Because NAD^+ is used in various physiological processes, the preventive effects of niacin could be due to a combinatory influence on ADP-ribosylation enzymes and other NAD-requiring reactions.

Concluding remarks

Carcinogenesis is multistage and may involve not only gene mutations but also abnormal dynamics of chromosomal organization, possibly also caused by non-genotoxic factors. For a better understanding of the entirety of neoplasia, more needs to be learned from basic biological as well as clinical features. Research on polyADP-ribosylation has progressed rapidly, as evidenced by the multitude of details available on the website, PARP link (http://parplink.u-strasbg.fr/index.html). Considering the fact that many post-translational modifications are actively involved in regulation of key reactions, interplay among processes like polyADP-ribosylation, monoADP-ribosylation, phosphorylation, acetylation, methylation, and ubiquitination of key proteins deserves greater attention. This research field might best be termed 'Proteomodificomics (PMM)'. Progress of PMM in today's post-genome era, with collaboration from various scientists and clinicians, should help establish new concepts for understanding the characteristics of clinical cancer that should lead to better diagnosis, treatment and prevention.

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